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Usability Evaluation of an Admission, Discharge, and Transfer Information System: A Heuristic Evaluation

Zahra Ebnehoseini[1], Mahmood Tara[2], Marziyhe Meraji[3*], Kolsoum Deldar[4], Farnaz Khoshronezhad[5], Sanaz Khoshronezhad[6]

[1]Students Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [2]Department of Medical Informatics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [3]Department of Medical Records and Health Information Technology, Faculty of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran; [4]Faculty of Medicine, Department of Health Information Technology, Shahroud University of Medical Sciences, Shahroud, Iran; [5]Students Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [6]Students Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

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Keywords: Heuristic Evaluation, usability; Admission; Discharge and Transfer (ADT); Hospital Information Systems; Health information systems

***Correspondence:** Marziyhe Meraji. Department of Medical Records and Health Information Technology, Faculty of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran. E-mail: MerajiM1@mums.ac.ir

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Abstract

BACKGROUND: Admission, discharge and, transfer (ADT) process is one of the most important hospital workflows. ADT system is a part of a hospital information system (HIS).

AIM: The objective of this study was to evaluate the usability of the ADT system.

METHODS: The study performed at Mashhad University of Medical Sciences (MUMS) hospitals. Data collection instrument was a validated checklist of Pierotti heuristic evaluation. To determine the severity of usability problems, a hybrid of Nielson and Tampere unit for computer-human interaction (TAUCHI) severity scaling algorithm was used. Usability problems were divided into five categories (major, severe, minor, cosmetic, and technical). Six experts evaluated the ADT system independently. According to TAUCHI severity scale, if a feature has not yet been implemented in the ADT system, evaluators considered it a technical usability problem. Therefore, usability problems due to non-design feature in the ADT system were identified. Finally, the mean severity of each usability problems was calculated.

RESULTS: A total of 186 usability problems were identified. The frequency of major, sever, minor and cosmetic usability problems were 2, 65, 69 and 50, respectively. A total of 55 usability problems by the evaluators were recognised as technical problems. The highest mismatch with usability principles was related to the "recognition rather than recall". The range of the mean severity of usability problems was between 0-2.31.

CONCLUSIONS: Our result showed that although implementation of IHIS on a large scale, it still suffered from unresolved usability problems. Identification of usability problems and evaluation of their level of severity, which was simultaneously performed in this study, can be used as a guide to evaluate the usability of other HISs.

Introduction

Hospital information system (HIS) refers to a collection of integrated software systems used to collect, store, retrieve and present patients' data and information in a hospital. HIS has several components such as radiology, lab and nursing information system [1]. Although using health information technology potentially reduces serious damage to patients [2], some of the previous studies revealed the unintended adverse consequences of this technology, including increased documentation time, inconsistency with clinical workflow, increased error rising in patients' treatment [3].

Zheng states "Although health information systems promise healthcare improvement as well as medical error decrease, in the case where these systems are not used appropriately, it seems impossible to gain effective results. Factors, such as poorly designed user interface and inconsistency between system and work process, can cause unintended adverse consequences" [4].

Moreover, if an information system is unable to meet users' basic expectations, it will miss their trust gradually, and its efficient use will be deteriorated time by the time [5]. Therefore, it is highly significant to remove the usability problems of health information systems and to prevent their unexpected adverse consequences [6]. Usability is one of the key dimensions for the software quality, especially HIS. Usability evaluation supports a collection of parameters determining the system quality [7]. Usability evaluation generally includes two steps: first, usability problems identification and the the determination of their severity [8]. Heuristic evaluation is one of the most common methods to identify usability problems. These methods find usability problems with a minimum amount of time, cost and resources [9]. In this method, a small group of evaluators, based on a predetermined checklist and according to the usability evaluation principles, evaluates the system [10]. Severity rating can determine which serious usability problems require to be fixed immediately and can be used to allocate the relevant resources to repair them [8].

In this study, we evaluated the usability of the ADT system utilised in MUMS hospitals.

Methods

In 2002, MUMS implemented a customised HIS, namely IHIS (Iranian Hospital Information System), with its subsystems, including nursing, pharmacy and ADT system. Now, the IHIS is implemented in 26 hospitals of Khorasan Razavi Province and 10 healthcare centres. All these healthcare organisations used the same version of IHIS, and its update is carried out by the HIS Group of the MUMS IT Center.

This study was descriptive and cross-sectional. The study was conducted on April 2017 in the five selective MUMS hospitals (two general and three speciality hospitals). The heuristic method was used to detect the ADT system usability problems.

Data collection instrument was a validated checklist of Pierotti heuristic evaluation. This checklist includes 13 heuristic evaluation principles and 292 questions [11] (Table 1).

Table 1: The Pierotti 's Heuristic evaluation principles [11]

Visibility of system status (n = 29)	Flexibility and minimalist design (n = 16)
Match between system and the real world (n = 24)	Aesthetic and minimalist design (n = 12)
User control and freedom (n = 23)	Help and documentation (n = 23)
Consistency and standards (n = 51)	Skills (n = 21)
Help users recognize, diagnose, and recover from errors (n = 21)	Pleasurable and respectful interaction with the user (n = 14)
Error prevention (n = 15)	Privacy(n = 3)
Recognition rather than recall (n = 40)	

To determine the severity of usability problems, a hybrid of Nielson severity rating scale [12] and Tampere unit for computer-human interaction (TAUCHI) severity rating scale [13] was used (Table 2).

Table 2: Hybrid severity scaling algorithm for usability problems

Severity scale	Definition
Major	Imperative to fix this before the product can be released (Score = 4) (10)
Severe	Important to fix. Therefore it should be given high priority (Score = 3) (10)
Minor	Fixing this should be given low priority (Score = 2) (10)
Cosmetic	It should be fixed to use the system to be as pleasant as possible (Score = 1) (10)
No problem	Usability problem do not exist (Score = 0) (10)
Technical	These usability problems are most likely due to technical problems with the system. Features that have not been implemented yet (Score = 0-4 according to evaluators' opinion) (11)

Six experts were recruited as usability evaluators: One health information management specialist with two years of practical work experience with IHIS in a clinical setting, two master students in medical informatics with computer engineering background, one Ph.D. student in medical informatics with 7 years IHIS management experience and two information technology specialists with 15 years of IHIS management experience. All the usability evaluators had participated in the heuristic evaluation course. To improve the quality of the usability evaluations, several sessions were held to equalise the personal interpretations of the checklist items.

This study was conducted in two phases: First, each evaluator based on Pierotti checklist evaluated the user interface of the ADT system, and then evaluators determined the severity of each usability problem (according to hybrid severity scaling algorithm in Table 2). According to TAUCHI scale, if a feature has not been implemented in the ADT system yet, evaluators considered it a technical usability problem. Therefore, usability problems due to non-design or non-existence of a feature in the ADT system were identified, and the severity of them was determined. Second, the mean severity of each usability problem was calculated.

Results

Heuristic evaluation was conducted on the ADT system by 6 evaluators. A total of 210 usability problems were recognised. After eliminating the

duplicates problems, 186 unique usability problems remained. The severity of the identified problems included: 2 (1%) major problems, 65 (35%) severe problems, and 69 (37%) minor problems and 50 (27%) cosmetic problems. A total of 55 usability problems identified by the evaluators were recognised as technical problems.

The range of the mean severity of usability problems was between 0-2.31. The “help and documentation” principle had the highest level of problem severity. The “error prevention” and “flexibility and minimalist design” were ranked as the 2nd and 3rd principles, respectively.

Table 3: Frequency and the mean severity of identified usability problems

Heuristic evaluation principal	Number of usability problems	Mean (Range) of the severity of problems
Visibility of system status	Total= 19 (C=8, Mi=8, S=3, and Ma=0)	0.95 (0.00 - 2.31)
Match between system and the real world	Total=14 (C=1, Mi=6, S=7, and Ma=0)	1.16 (0.00 – 2.50)
User control and freedom	Total=11 (C=1, Mi=6, S=4, and Ma=0)	1.00 (0.00 – 3.00)
Consistency and standards	Total=21 (C=6, Mi=12, S=3, and Ma=0)	0.60 (0.00 – 2.50)
Help users recognize, diagnose, and recover from errors	Total=13 (C=2, Mi=5, S=6, and Ma=0)	1.25 (0.00 – 2.66)
Error prevention	Total=11 (C=0, Mi=7, S=3, and Ma=1)	1.46 (0.00 – 2.50)
Recognition rather than recall	Total=32 (C=20, Mi=5, S=6, and Ma=1)	1.08 (0.00 – 2.50)
Flexibility and minimalist design	Total=13 (C=2, Mi=4, S=7, and Ma=0)	1.74 (0.00 – 3.00)
Aesthetic and minimalist design	Total=7 (C=5, Mi=2, S=3, and Ma=0)	0.98 (0.00 – 2.50)
Help and documentation	Total=23 (C=2, Mi=7, S=14, and Ma=0)	2.31 (1.00 – 3.00)
Skills	Total=13 (C=1, Mi=4, S=8, and Ma=0)	1.42 (0.00 – 3.00)
Pleasurable and respectful interaction with the user	Total=8 (C=1, Mi=3, S=4, and Ma=0)	1.20 (0.00 – 3.00)
Privacy	Total=1 (C=1, Mi=0, S=0, and Ma=0)	0.22 (0.00 – 2.16)

Note: C = Cosmetic, Mi = Minor, S = Sever, and Ma = Major.

The “recognition rather than recall” principle with 32 problems and the mean severity of 1.08 had the highest number of usability problems. The “help and documentation” and “consistency and standards” were ranked as the 2nd and 3rd principles, respectively.

There was only one problem reported on the principle of “privacy” (Table 3). Table 4 showed a sample of the most important identified usability problems based on their severity.

Discussion

In this study, we used a heuristic evaluation checklist as well as a hybrid severity scaling algorithm to evaluate the ADT system interface. The study findings suggested many technical usability problems in the ADT system.

The results of this study showed that the "recognition rather than recall" and “consistency and standards” principles had the most usability problems, respectively. The findings of the study by Nabovati et al., on a heuristic evaluation of IHIS radiology and lab information subsystem [14] and Khajouei et al., on IHIS emergency subsystem [9] also reported the same problems. In the above two studies, these principles were reported to have the most frequent usability problems.

Table 4: Samples of the identified usability problems based on their severity

Samples of major usability problems
-The content of fields in the ADT did not match with the work process.
Samples of severing usability problems
-The ADT pages did not have appropriate titles.
-Extra data elements were displayed on the data entry pages.
-Data elements were not classified properly and did not have a logical sequence.
-Users did not have the choice of either clicking on menu items or using a keyboard shortcut
-The hand and eye movements between input devices were not minimised.
-When the users entered into a screen or dialogue box, the cursor was not positioned on fields and menus which users most likely to need.
-The origin of the system problems and their solutions was not demonstrated in the error messages.
-The ADT did not warn users if they made a potentially serious error.
Samples of minor usability problems
-Fields and menus were not visually distinct.
-If there were observable delays (greater than fifteen seconds) in the system’s response time, the user was not informed of the system's progress.
-Patients' information was not retrieved easily and correcting the mistakes was very difficult.
-Users could not customise the system colour coding.
-It was impossible to save patients’ information temporarily in the ADT.
Samples of cosmetic usability problems
-Various and distinctive colours and voices were not used in the ADT.
-Bold fonts were not used to attract users' attention.
-Visible symbols for active window were not used.
Note: ADT= Admonition, discharge, and transfer.

The findings of the study by Rezaei et al., [15] also revealed numerous usability problems in the principle of "recognition rather than recall". The findings of the usability evaluations of Agharezaee [6], Abedi and Khajouei [16], Thyvalikakath [17] and Verheul [18] on other HISs as well as electronic health records also showed numerous usability problems in the principle of "consistency and standards". As well, findings of Sadoghi [19], Meydani [20] and Asadi [21] illustrated that the standards are not adopted in HISs, while access to standard data in an organised format to provide appropriate and on-time healthcare service is highly significant [22]. It seems that the HISs have the same usability problems. Designers wishing to develop or update on HIS should pay particular attention to HIS's usability problems.

In this study, the smallest number of usability problems was related to the principle of “privacy”, while in the study by Nabovati et al., the smallest number of usability problems was reported in the principle of "help and documentation" [14]. The reason for the difference between the present study and their study is possibly concerned with detecting technical usability problems in the present study. System help was not designed in IHIS. Thus, Nabovati et al. did not evaluate usability problems in the principle of “help and documentation”. In this study, features that have not been implemented, were considered technical usability problems, and the level of their severity was determined. Therefore, in the present study, the number of problems related to the “system help” principle was ranked 2nd. This indicates that the hybrid methods used in this study have the potential to detect a larger number of usability problems.

The findings of our study on the Pierotti heuristic evaluation checklist also reveal that the principle of “help and documentation” has the highest level of problem severity. The findings of previous studies on other HISs in Iran [6] [9] [14] [15] [16] are also indicative of either the absence of system help design or design deficiency. The users in the study by Kimiafar et al. reported the use of guidelines, instructions and educational papers as a solution to address this problem [23].

One of the key findings in the present study detecting serious (major and severe) usability problems in the ADT system. A total of 36.5% of the identified usability problems were classified as major and severe. Although it was lower than the severity rating reported in the study by Nabovati et al., (%66 of the identified usability problems in their study were classified as severe and major) [14], it is still a considerable concern. This is because the high severity of usability problems can negatively impact the users' interaction with the system. This not only makes them dissatisfied but can also affect data entry and documentation quality. On the other hand, the studies by Khajouei et al., and Nabovati et al., were performed on other IHIS subsystems four and three years ago, respectively [9] [14]. Our study was conducted in 2017, which shows that IHIS still suffers from unresolved usability problems.

One of the major strengths of the current study was the use of professional evaluators, who had previous experience of practical work with IHIS in clinical settings. They were also able to recognise the system's problems as well. Moreover, our usability evaluation method was an easier and cheaper method to study numerous usability problems in a rather short period. This makes it superior to other available methods. As well, the findings of the present study can be generalised to other IHIS subsystems since many of the IHIS features are similar in various subsystems. Therefore, the findings of this study can help system designers to overcome IHIS usability problems in future editions. The designers of other HISs can also benefit from the findings of this study: they can be informed of the usability problems and their effects on the users' workflow. This can be used to prevent the same usability problems in others HISs. Identification of usability problems and evaluation of their level of severity, which was simultaneously performed in this study, can be used as a guide to evaluate the usability of other IHIS subsystems and other HISs.

In conclusion, in this study, we used a heuristic evaluation checklist as well as a hybrid severity scaling algorithm to evaluate the hospital information system interface. Our usability evaluation method was an easy and cheap method to study numerous usability problems in a rather short period. This makes it superior to other available methods. The findings of this study can help system designers to overcome hospital information system usability problems. As well, it can be used as a guide to evaluate the usability of other hospital information systems. As well, the current study performed at one the largest provinces in a developing country. Our result showed that although IHIS was implemented on a large scale in a developing country, it still suffered from unresolved usability problems.

According to the current study findings, it seems that designers wishing to develop or update on HIS should pay particular attention to HIS's to the principles of “consistency and standards" and "recognition and recall" and produce a better HIS. Moreover, the repetition of problems in the principle of “help and documentation” in this study and other previous studies reveals the importance of caring about HIS education and the creation of appropriate instruction for using the system.

References

1. Ismail NI, Abdullah NH, Shamsuddin A. Adoption of Hospital Information System (HIS) in Malaysian Public Hospitals. Procedia - Social and Behavioral Sciences. 2015; 172:336-43. https://doi.org/10.1016/j.sbspro.2015.01.373

2. Horsky J, McColgan K, Pang JE, Melnikas AJ, Linder JA, Schnipper JL, Middleton, B. Complementary methods of system usability evaluation: surveys and observations during software design and development cycles. Journal of biomedical informatics. 2010; 43(5):782-90. https://doi.org/10.1016/j.jbi.2010.05.010 PMid:20546936

3. Ologeanu-Taddei R, Morquin D, Bourret R. Understanding the Perceived Usefulness and the Ease of Use of a Hospital Information System: the case of a French University Hospital. Studies in health technology and informatics. 2015; 210:531. PMid:25991204

4. Zheng K, Padman R, Johnson MP, Diamond HS. An interface-driven analysis of user interactions with an electronic health records system. Journal of the American Medical Informatics Association. 2009; 16(2):228-37. https://doi.org/10.1197/jamia.M2852 PMid:19074301 PMCid:PMC2649313

5. Azizi Aa, Safari S, Mohammadi A, Kheirollahi J, Shojaei baghini M. A survey on the satisfaction rate of users about the quality of hospital information system in hospitals associated with Kermanshah university of medical sciences. Health Information Management. 2011; 8(4):566-71.

6. Agharezaei Z, Khajouei R, Ahmadian L, Agharezaei L. Usability evaluation of a laboratory information system. Director General. 2013; 10(2):1-12.

7. Karahoca A, Bayraktar E, Tatoglu E, Karahoca D. Information system design for a hospital emergency department: A usability analysis of software prototypes. Journal of biomedical informatics. 2010; 43(2):224-32. https://doi.org/10.1016/j.jbi.2009.09.002 PMid:19755173

8. Baker K. Heuristic Evaluation. 1997. http://grouplab.cpsc.ucalgary.ca/saul/681/1997/kevin/home.html. Accessed January 13, 2018.

9. Khajouei R, Azizi A, Atashi A. Usability evaluation of an emergency information system: a heuristic evaluation. Journal of Health Administration. 2013; 16(52):61-72.

10. Jaspers MW. A comparison of usability methods for testing interactive health technologies: methodological aspects and

empirical evidence. International journal of medical informatics. 2009; 78(5):340-53. https://doi.org/10.1016/j.ijmedinf.2008.10.002 PMid:19046928

11. Pierotti D. Usability techniques: heuristic evaluation a system checklist 1998. Available web.vu.lt/mif/k.lapin/files/2017/04/9_Heuristical_evaluation-2017.pdf. Accessed January 18, 2018.

12. Nielsen J. Severity ratings for usability problems. Papers and Essays. 1995; 54:1-2.

13. Li L, Helenius M. Usability evaluation of anti-phishing toolbars. Journal in Computer Virology. 2007; 3(2):163-84. https://doi.org/10.1007/s11416-007-0050-4

14. Nabovati E, Vakili-Arki H, Eslami S, Khajouei R. Usability evaluation of Laboratory and Radiology Information Systems integrated into a hospital information system. Journal of medical systems. 2014; 38(4):35. https://doi.org/10.1007/s10916-014-0035-z PMid:24682671

15. Rezaei-Hachesu P, Pesianian E, Mohammadian M. Evaluating Usability of Radiology Information Systems in Hospitals of Tabriz University of Medical Sciences. Acta Informatica Medica. 2016; 24(1):42. https://doi.org/10.5455/aim.2016.24.42-46 PMid:27041810 PMCid:PMC4789628

16. Abedi S, Khajouei R. Evaluating the Users' Interaction Problems with Physiotherapy Information System. Journal of Hospital. 2015; 14(3):83-92.

17. Thyvalikakath TP, Schleyer TK, Monaco V. Heuristic evaluation of clinical functions in four practice management systems: a pilot study. The Journal of the American Dental Association. 2007; 138(2):209-18. https://doi.org/10.14219/jada.archive.2007.0138 PMid:17272376

18. Van Engen-Verheul MM, Peute LW, de Keizer NF, Peek N, Jaspers MW. Optimizing the user interface of a data entry module for an electronic patient record for cardiac rehabilitation: A mixed method usability approach. International journal of medical informatics. 2016; 87:15-26. https://doi.org/10.1016/j.ijmedinf.2015.12.007 PMid:26806708

19. Sadoghi F, Shahi M, Davari dolatabadi N, Ebrahimi K. Hospital information systems interoperability in Iran. Bimonthly Journal of Hormozgan University of Medical Sciences. 2014; 18(3):235-41.

20. Meydani Z, Safdari R, Farshid far GR, Lak bala P. Comparative study of information management standards: an approach to implementing electronic health record. Medical Journal of Hormozgan University. 2006; 10(2):167-72.

21. Asadi F, Moghaddasi H, Rabiei R, Rahimi F, Mirshekarlou SJ. The Evaluation of SEPAS National Project Based on Electronic Health Record System (EHRS) Coordinates in Iran. Acta Informatica Medica. 2015; 23(6):369. https://doi.org/10.5455/aim.2015.23.369-373 PMid:26862248 PMCid:PMC4720822

22. Isfahani SS, Khajouei R, Jahanbakhsh M, Mirmohamadi M. The evaluation of hospital laboratory information management systems based on the standards of the American National Standard Institute. Journal of education and health promotion. 2014; 3(61). PMid:25077154 PMCid:PMC4113977

23. Kimiyafar K, Moradi G, Sadooghi F, Sarbaz M. Views of users towards the quality of hospital information system in training hospitals affiliated to Mashhad University of Medical Sciences-2006. Health Information Management. 2008; 4(1):43-50.

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Basic Science

The Effect of Vascular Graft and Human Umbilical Cord Blood-Derived CD34+ Stem Cell on Peripheral Nerve Healing

Ali Yilmaz[1], Abdullah Topcu[1], Cagdas Erdogan[2], Barbaros Sahin[3], Gulcin Abban[4], Erdal Coskun[5], Ayca Ozkul[1*],

[1]Adnan Menderes University, Neurosurgery Department, Aydın, Turkey; [2]Pamukkale University, Neurology Department, Denizli, Turkey; [3]University Medicine Faculty, Animal Laboratory, Denizli, Turkey; [4]Pamukkale University Medicine Faculty, Histology and Embryology Department, Denizli, Turkey; [5]Pamukkale University, Medicine Faculty, Neurosurgery Department, Denizli, Turkey

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***Correspondence:** Ayca Ozkul. Adnan Menderes University, Aydın, Turkey. E-mail: ozkulayca@hotmail.com

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Abstract

AIM: There are many trials concerning peripheral nerve damage causes and treatment options. Unfortunately, nerve damage is still a major problem regarding health, social and economic issues. On this study, we used vascular graft and human cord blood derived stem cells to find an alternative treatment solution to this problem.

MATERIAL AND METHODS: We used 21 female Wistar rats on our study. They were anesthetized with ketamine and we studied right hind limbs. On Group 1, we did a full layer cut on the right sciatic nerve. On Group 2, we did a full layer cut on the right sciatic nerve, and we covered synthetic vascular graft on cut area. On Group 3, we did a full layer cut on right sciatic nerve, and we covered the area with stem cell applied vascular graft.

RESULTS: At the end of postoperative 8. weeks, we performed EMG on the rats. When we compared healthy and degenerated areas as a result of EMG, we found significant amplitude differences between the groups on healthy areas whereas there was no significant difference on degenerated areas between the groups. Then we re-opened the operated area again to reveal the sciatic nerve cut area, and we performed electron microscope evaluation. On the stem cell group, we observed that both the axon and the myelin sheet prevented degeneration.

CONCLUSION: This study is a first on using synthetic vascular graft and cord blood derived CD34+ cells in peripheral nerve degeneration. On the tissues that were examined with electron microscope, we observed that CD34+ cells prevented both axonal and myelin sheath degeneration. Nerve tissue showed similar results to the control group, and the damage was minimal.

Introduction

Direct nerve repair with epineural micro sutures is still the gold Standard surgical treatment for severe axonotmesis and neurotmesis injuries. Epineural repair is performed when a tension free coaptation, otherwise authologus nerve grafts are mainly used [1] [2]. These autologous nerve grafts provide structural support to guide axonal regeneration, preventing neuroma formation and fibrous tissue invasion. However, there are some disadvantages for autologous nerve grafts as a limited source of the nerve grafts, permanent loss of the sensation at donor site [2]. Also, it may increase the donor site infection.

These problems bring out the need of new therapeutic approaches to nerve injury. An effective nervous construct may require some combination of three primary components. However, scaffolds' results are not better than an autograft [1].

Schwann cells (SCs) are peripheral glial cells that play a crucial role in the endogenous repair of the peripheral nervous system (PNS) by reconstructing myelin, which is necessary for functional recovery [3] [4]. For these reasons, SCs and Scwhann-like cells are one of the most widely studied cells for axonal regeneration and functional repair in PNS. In these

studies the stem cells obtained from many SCs'or Schwann-like cells' sources including human umblical cord blood-derivered stem cells (HUCB), adipose-derivered stem cells, bone marrow-derivered mesenchymal stem cells, and embriyonic stem cells [3] [5] [6] [7] [8]. Although adipose-derived stem cells have been mainly used for PNS regeneration in previous studies, we prefered using HUCB CD34 positive (CD34+) stem cell because of its greater availability, weak immunogenicity, lower risk of graft-versus host diseases and pluripotent differentiation, besides HUCBSC raises far less ethical controversies than ESC [9] [10] [11] [12].

In this study our aim was to examine and compare the effects of HUCB CD34+ stem cell and synthetic graft on peripheral nerve healing in an experimentally induced sciatic nerve injury.

Material and Method

The study was carried out in Experimental Research Laboratory of Faculty of Medicine at Pamukkale University. Approval forms were taken from parents of newborns from whom cord blood was collected. All experiments were performed in accordance with the guidelines for care and local ethical regulations established by the National Institutes of Health. The study used six-month-old 210 ± 30 gram (gr) Sprague-Dawley female rats. The rats were kept at room temperature of 22 ± 2ºC in 50% humid environment at 12:12 day/dark cycle during the experimental procedure. The subjects were divided into 3 groups. Group I: Control group (n = 7), sciatic nerve full thickness incision; Group II: vascular graft group (n = 7) sciatic nerve full thickness incision and covering lesion area with polytetrafluoroethylene (PTEF) type synthetic graft; Group III: stem cell group (n = 7) transplantation of HUCB-derived 3 X 10^4 $CD34^+$ stem cell into sciatic full thickness incision and covering the lesion with PTEF synthetic graft.

All surgical interventions were carried out by the same surgeon using standard microsurgery methods on the sciatic nerve. Surgical operations were performed under anaesthesia by 50 mg/kg Ketamine-HCL (Alfamine®-im) and 9 mg/kg Ksilazin HCL (Rompun®-im) mixture. The subjects were kept at appropriate positions on special fixing boards for surgical intervention, and the operations were carried out under operation microscope (107 Series, Seiler İnstrument, St. Louis, Missouri). Operation area (gluteal and femur region) was shaved and surgically cleaned with povidone-iodine. An oblique incision was made in the right lower extremity in such a way to follow hip joint movement removing the skin and reaching biceps femoralis muscle. Muscle tissue was opened by blunt dissection, and sciatic nerve was exposed. The right sciatic nerve was full thickness incised at 1 cm proximal of separation point of tibial and peroneal nerves using microscissors. In all groups, right hind extremity was used for the study, while un-operated left hind extremity was kept as control.

Collection of newborn umbilical cord blood, separation of $CD34^+$ stem cells and transplantation

A total of 50 ml of umbilical cord blood samples were collected from pregnant women who gave birth recently. It was seen that the umbilical cord taken was normal. Umbilical cord blood was taken from an umbilical vein using heparinised syringes immediately after leaving from newborn. Obtaining $CD34^+$ stem cells by positive selection from the cord blood via using of Magnet (Magnet EasSep, StemCell Technologies) and magnetic nanoparticles (EasySep Magnetic nanoparticles 1ml, StemCell Technologies) was performed in 7 steps. The cells selected by position selection were treated with trypan blue and counted by hemocytometer under a light microscope. It was found that there were 3 x 10^4 cells in 2µl.

Nerve tissue sections were kept in 5% glutaraldehyde solution prepared with Millonig's phosphate buffer for 1 hour and was dissected of 1 mm^3 on Petri covered with dental wax with glutaraldehyde on top using a razor. Tissue sections were again taken to glutaraldehyde solution and were fixed for 3 hours. The tissues were then shaken in Millonig's phosphate buffer for 10 minutes. After taking the tissues in Millonig's phosphate buffer for the second time, they were kept in the same tampon for a night. The next day, the tissues were fixed for a second time in 1% osmium tetraoxide solution prepared with Millonig'sphosphate buffer and were washed with phosphate tampon for 10 minutes 2 times. Dehydrated tissue sections were then immersed in propylene oxide+ embedding material for 30 minutes. Newly prepared embedding material in tissue sections was taken to tubes containing resin and was mixed in the rotator for a night. The next day, tissue sections were embedded into 00 polyethene capsules using freshly prepared embedding material and were polymerised at the 60ºC incubator for 48 hours. The blocks were then removed from the incubator and let to cool. Cross-sections of 500 Aº thickness were taken from the blocks using Reichert Ultracut ultramicrotome. The crosssections were collected in 200-300 meshed copper grids and stained with uranyl acetate saturated in 70% ethyl alcohol and Reynold's lead citrate solutions. Stained cross-sections were analyzed in Zeiss E.M. 10 B electron microscope.

In postoperative 8. Week, all rats were electrophysiologically evaluated using TecaMedelec Premiere Plus device. Active electrode was placed on gastrocnemius muscle of subjects, while the reference electrode was placed on Achilles tendon. Similar to

the first surgical operation, the sciatic nerve was freed from surrounding tissues, and sciatic nerve incision line was found. Nerve body was directly stimulated with monopolar Teflon-coated electrodes. During stimulation procedure, 10 mm distance was kept between active and reference stimulation electrodes. As stimulation points, the best possible distal and proximal points were selected in such a way to leave the repair line of nerve. The distance between proximal and distal stimulation points was measured with a caliper. Supra-maximal voltage was used for stimulation. Following the stimulation, the point where the first deviation from isoelectric line was marked as initial latency [millisecond (msn)]; peak point of axon was marked as peak latency [millisecond (msn)]; distance between positive and negative highest peaks was marked as amplitude [microvolt (μV)]. Compound muscle action potentials recorded after stimulation first in distal than in proximal were overlapped. The transmission rate of action potentials between proximal and distal points; peak-peak amplitude and area in compound muscle action potential were calculated. Values in experimented right and healthy left (control) legs were statistically compared by computing experimental leg/control leg ratios.

SPSS for Windows (version 13.00) computer package program was used for statistical analysis. P value ≤ 0.05 was considered as significant. One-Sample Kolmogorov-Smirnov Test was used to test the suitability of data for normal distribution. It was found that data was fit for normal distribution. One Way ANOVA Test was used to compare data.

Results

Comparison of degenerate side latency and amplitude values in the three groups showed no statistically significant difference (Table 1 and 2). Comparison of intra-group health and degenerated side latency and amplitude values showed a statistically significant difference.

Table 1: EMG findings showing amplitude decrease in groups II and III with peripheral nerve injury

Groups	Healthy side amplitude values			Degenerated side amplitude values		
	X ± SD	f	*p	X ± SD	f	*p
Group 1	26.42 ± 7.11			23.14± 7.92		
Group 2	25.85 ± 7.33	0.012	0.988	12.28 ± 9.06	3.723	0.044*
Group 3	26.28 ± 7.06			16.14 ± 5.08		

* Oneway ANOVA Test; SD: standard deviation.

Axon and myelin sheath degeneration was significant in lesion and untreated group (Group 1). In the majority of myelinated nerve fibres, the integration between axon and myelin sheath was lost, and gaps appeared. Myelin sheath was observed to be thicker than normal in some fibres; while the sheath was observed to be quite thin in others.

In nerve fibres with thick myelin, oedema between the fibres was increased creating gaps in the myelin sheath. In degenerated nerve fibres, myelin sheath lost its normal order parallel array and took a lamellar shape in some regions.

Table 2: EMG findings showing increased latency values in groups 2 and 3 with peripheral nerve injury without any statistically significancyroup

Groups	Healthy side Latency values			Degenerated side Latency Values		
	X ± SD	f	*p	X ± SD	f	*p
Group 1	1.22 ± 0.94			1.33 ± 0.55		
Group 2	1.20 ± 0.67	0.256	0.777	1.55 ± 0.20	2.461	0.114
Group 3	1.22 ± 0.04			1.49 ± 0.26		

* Oneway ANOVA Test; SD: standard deviation.

Nerve fibres with no myelin were significant in some regions Collagen fibres observed in latitudinal and longitudinal sections between the axons indicated an increase in connective tissue (Figure 1).

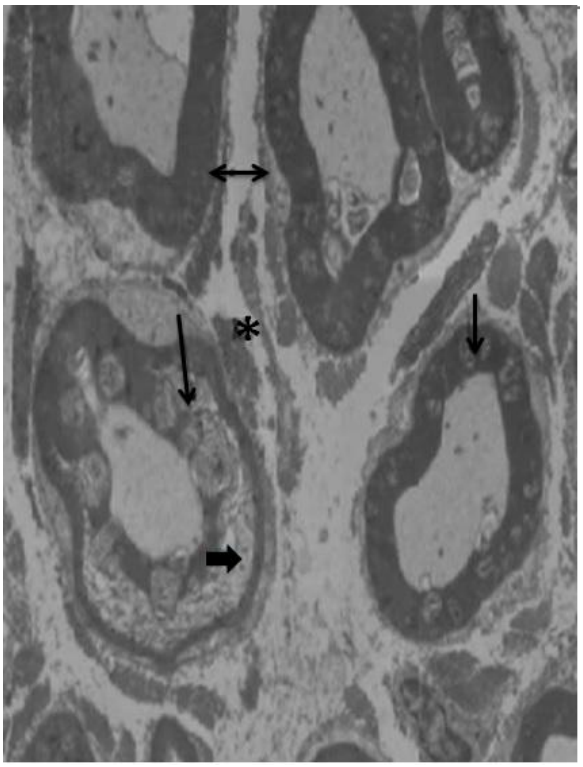

Figure 1: Electron microscopic image of the untreated group. Arrow: the abnormal appearance of the myelin sheath, thick arrow: myelin sheath openings star: astrocytes, double-headed arrow: a cross-section of collagen fibrils

In-lesion group with PTEF graft application (Group 2), it was observed that degeneration continued in myelin sheath and axons, however significantly decreased when compared to the untreated group. Gaps between the myelin sheath in the untreated group were found to decrease in group 3. Similarly, nerve fibres with thick myelin sheath observed in the untreated group were not observed in this group. On the other hand, connective tissue fibres and demonised nerve fibres were significant also in

this group. However they were relatively decreased (Figure 2).

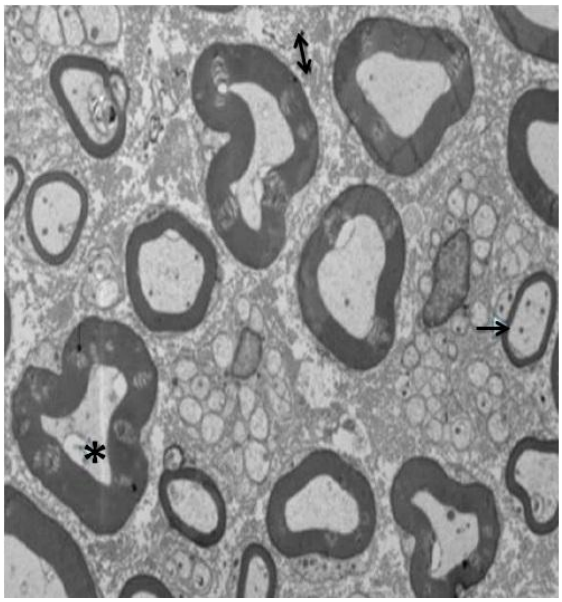

Figure 2: Electron microscopic image of vascular graft group. Arrow: the abnormal appearance of the myelin sheath, star: astrocytes, double-headed arrow: a cross-section of collagen fibrils

In the group with the lesion, PTEF graft and human cord blood-derived $CD34^+$ stem cell (Group 3), general outlook was significantly positive than the group which was treated with vascular graft only. In this group, gaps observed between myelin sheath were lost. Nerve fibres began to take their normal look. Connective tissue fibres were very low in amount. Myelin coated nerve fibres and demyelinated nerve fibers were also visible in this group. Axonal degeneration was still present in some fibers, however pathological findings were significantly less found (Figure 3 and 4).

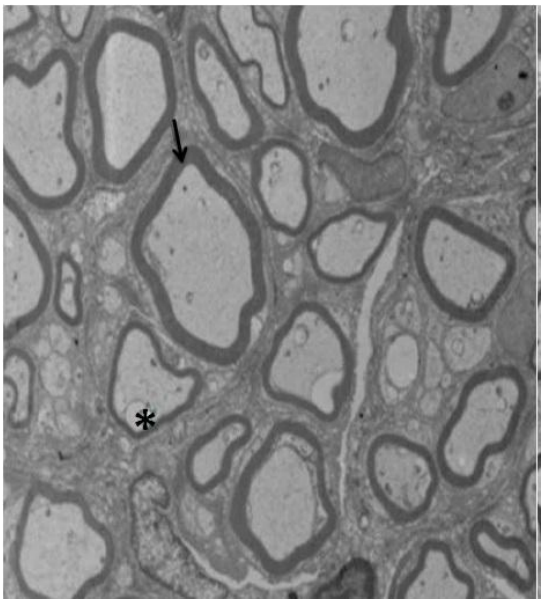

Figure 3: Electron microscopic image of vascular grafts and cord blood-derived stem cells group. Arrow: myelin sheath, star, astrocytes

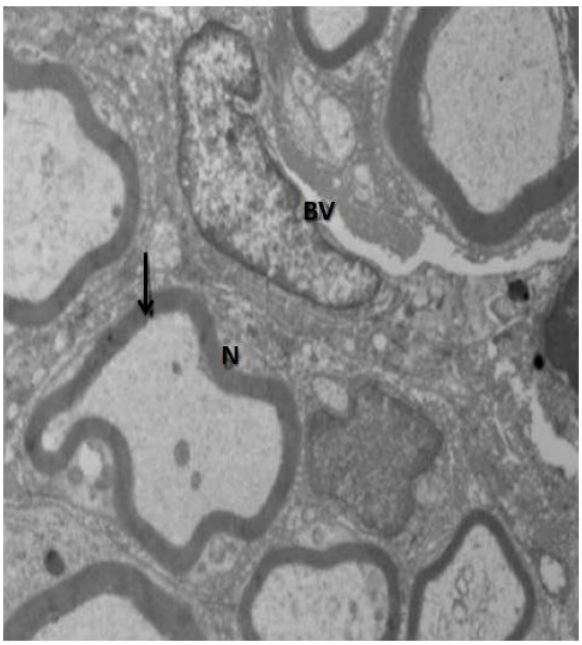

Figure 4: Electron microscopic image of the control group. Arrow: myelin sheath, NE: blood vessel, S; cross-sectional nerve

Discussion

Analysis of electron microscope images of the groups revealed that axon and myelin sheath degeneration was significant in injured peripheral nerve in the control group, with disintegration between axon and myelin sheath; gaps in majority of myelinous nerve fibers; edema between thick myelinous nerve fibers with lost of myelinous sheath in degenerated nerve fibers. These findings indicate that axon integration was damaged and endogenous repair mechanisms were inadequate in peripheral nerve injuries. In group 2 where synthetic vascular graft was used as treatment, it was found that degeneration in myelin sheath and axons continued, however all these findings were significantly decreased when compared to the control group. Gaps observed in myelin sheath in the control group were decreased in this group. In group 3 which was treated with vascular graft with CD34+ cells, we found that both axon and myelin sheath degeneration were prevented. It was observed that nerve tissue looked like the original tissue and damage decreased to a minimum. These findings support the view that umbilical cord blood-derived $CD34^+$ stem cell transplantation can have positive effects on peripheral nevre injuries.

In our study, the results obtained in the Control Group and the Synthetic Graft Group are actually not a surprise;because when the literature is examined it is observed that, when the axons are separated from the peripheric tissue with vascular

autograft or synthetic neural tubes, it facilitates the cleaning of the debris tissue which is formed due to the injury via SCs and macrophages in a coordination; then the neurotropic factors, which support the axonal growth,are accumulated in the tube intensely, and supports the healing [1] [2] [4].

The situation in our study that creates the difference is that the $CD34^+$ hemopoietic stem cells obtained from HUCB have been used in vivo in neurotmesis type injuries for the first time and the results are positive. When the stem cell sources used in the repair of peripheral nerves are examined, it is observed that these sources are mostly in the form of adipose tissue, skin, bone marrow, cells that are similar to SCs produced with the activity of various growth factors in culture medium stemming from mesenchymal stem cells of the HUCB or the mesenchymal cells that contain SCs [13] [14] [15] [16] [17]. Recently mesenchymal stromal cells (MSC), have become the focus of attention for the treatment of nerve injury, owing to their positive effects in the regeneration process [18] [19] In a recent study Sanchez et al. showed that murine and canine Ad-MSC associated with decellularized vein scaffold had positive effects on sciatic nerve regeneration in a rat model [18]. The most proper time for repairing the damage after peripheric nerve injuries are the shortest time possible after the injury [1] [20]. The conversion of stem cells obtained from various sources into Schwann-like stem cells in culture medium requires time, and obtaining stem cell from bone marrow originating from mesenchyme is an extremely invasive method posing another disadvantage [2] [3] [9]. Also, to repair the injury in peripheral nerve injuries as soon as possible, the stem cell to be obtained must be quick to obtain, must be able to be used freshly, must be easily available in big amounts and not pose any risks in the donor during or after the process. The stem cells obtained from the HUCB blood may be considered as the most suitable source for the above mentioned criteria.

$CD34^+$ hemopoietic stem cells obtained from the HUCB were examined both in vitro and in vivo media many times regarding their central system stroke models and spinal cord trauma models. In such study conducted by Nishino et al., [21]. It was reported that the $CD34^+$ stem cells obtained from HUCB were used in spinal cord trauma models and that these transplanted cells lived for at least 3 weeks; however, disappeared completely after 5 weeks. Also, it was also reported in the same study that the transplanted $CD34^+$ stem cells increased functional healing in rats. In addition to these findings, the transplanted $CD34^+$ stem cells did not express the neuronal lineage markers. Kao et al., [12]. Conducted another study with spinal cord trauma and reported that when the $CD34^+$ stem cells stemming from HUCB were given systemically, the Glial delivered neurotropic factor (GDNF) and the Vascular Endothelial Growth Factor (VEGF) amount in the cord; and that when the opposite practice was applied with $CD34^-$ stem cells obtained from the HUCB and saline,there were no significant increase observed in the GDNF and VEGF in the injured cord. In the light of these findings, they reported that the $CD34^+$ stem cells obtained from HUCB increased the hind leg functions of the rats with the restoration of the injured cord and with the GDNF and VEGF production. It is known that the GDNF has a potent survival factor characteristics for mesencephalic dopaminergic neurons and motor neurons; and that the VEGF increases the neovascularization in the ischemic zone [12].

There have been many in vitro studies conducted to examine the effects of the $CD34^+$ stem cells on the recovery of nerves. The $CD34^+$ hemopoietic stem cells stemming from the HUCB were used in these studies. In one of these in vitro studies conducted by Fan et al., [22], $CD34^+$ stem cells obtained from the HUCB were used, and it was reported that the fibronectin (Fn) accelerated the endothelial cell (EC) differentiation which has the Flk-1, vWF surface markers of the $CD34^+$ stem cells. Bracci-Laudiero et al. [23]. Conducted a two-color flow cytometry study and reported that $CD34^+$ stem cells obtained from the HUCB expressed the NGF and the TrkA which is its specific receptor; however, they also reported that these stem cells did not express the P75 which is known as the pan-neurotrophin receptor and which is a member of the Tumour Necrosis Factor (TNF) receptor superfamily. However, the P75 receptor binds with all neurotrophins in a similar affinity but in different kinetics. On the contrary, the Trk family receptors are highly specific [24].

In our study, the $CD34^+$ hemopoietic stem cells obtained from the HUCB protected the axon and myelin sheath from degeneration. We consider that this regeneration occurs with the stem cells producing various neurotrophic factors and neurotrophins, minimizing the effect of the injury in the PNS, and supporting the SCs, and this situation is not contrary to the literature, because it is known that after neurotmesis type injury, in order to repair the wound, the SCs express neurotrophin and extracellular matrix protein such as NGF, BDNF, Neurotrophin-3 (NT-3), Neurotrophin 4/5 (NT-4/5), CTNF, TGF-1b, and bFGF; provided extracellular matrix products such as fibronectin,laminin, tenascin-C, type IV and type V collagen, heparin sulfate and chondroitin sulfate proteoglycan and also express adhesion molecules such as NCAM, L1 and N-cadherin [2] [4] [24] . Also, in a study conducted by Fan et al., [25]., it was reported that the mononuclear cells obtained from HUCB expressed BDNF, NGF, GDNF, NT-3 and NT-5 mRNA. In addition to this, the fact that the $CD34^+$ stem cells obtained from the HUCB including many different subgroup cells like multi-potent and lineage-committed progenitor cell type support this viewpoint.

Another interesting situation is that although the end-to-end anastomosis or SCs obtained from different sources or stem cells are planted to the injury

area after PNS injury, the growth speed in the injured nerves does not change. Although this situation seems like a paradox, in fact, it coincides with the hypothesis that the stem cells express various growth factors and support the SCs, because the injury in peripheric nerve injuries occur not in the nerve body but the axon in the nerve extension; and when it is considered that the growth of the axons is under the control of the genes which are in the cell nucleus, the situation which seems like a paradox is in fact not contrary to the literature. This situation supports the hypothesis that the stem cells support the SCs.

In our study, it was observed that this healing effect was not reflected in the amplitude and latency values in the EMG. The results of the EMG having been obtained like this in our study may be due to one reason. The EMG having been performed without reaching the end organ after the recovery of the nerve. We believe EMG findings may reveal the effect of healing process in the chronic phase. Further studies about stem cell treatment both in acute and chronic phase of peripheral nerve injury may also show better EMG findings suggesting lower axonal loss.

There are limitations of our study. First of all we didn't study any biochemical markers and neurotrophic factors to examine the effect of stem cell and underlying mechanism of the healing process. Additionally not only the acute but also the chronic phase EMG findings may be helpfull.

In conclusion, although how the $CD34^+$ stem cells obtained from the HUCB support the regeneration and prevent the degeneration is not clear much, the findings makes us consider that the $CD34^+$ stem cells obtained from the HUCB support this via various neurotrophic factors. To determine the mechanisms clearly, further studies are necessary. Obtaining stem cells from HUCB in a fast and easy way may provide an advantage in repairing the PNS injuries in the early period.

References

1. Grinsell D, Keating CP. Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. BioMed research international. 2014; 2014.

2. Faroni A, Mobasseri SA, Kingham PJ, Reid AJ. Peripheral nerve regeneration: experimental strategies and future perspectives. Advanced drug delivery reviews. 2015; 82:160-7. https://doi.org/10.1016/j.addr.2014.11.010 PMid:25446133

3. Matsuse D, Kitida M, Kohama M, Nishikawa K, Makinoshima H, et all. Human umbilical cord-derived mesenchymal stromal cells differentiate into functional Schwann cells that sustain peripheral nerve regeneration. J Neuropathol Exp Neurol. 2010; 69(9):973-85. https://doi.org/10.1097/NEN.0b013e3181eff6dc PMid:20720501

4. Pabari A, Lloyd-Hughes H, Seifalian AM, Mosahebi A. Nerve conduits for peripheral nerve surgery. Plast Reconstr Surg. 2014; 133(6):1420-30. https://doi.org/10.1097/PRS.0000000000000226 PMid:24867724

5. Faroni A, Smith RJ, Reid AJ. Adipose derived stem cells and nerve regeneration. Neural Regen Res. 2014; 9(14):1341-6. https://doi.org/10.4103/1673-5374.137585 PMid:25221589 PMCid:PMC4160863

6. Dezawa M, Takahashi I, Esaki M, Takano M, Sawada H. Sciatic nerve regeneration in rats induced by transplantation of in vitro differentiated bone-marrow stromal cells. European Journal of Neuroscience. 2001; 14(11):1771-6. https://doi.org/10.1046/j.0953-816x.2001.01814.x PMid:11860471

7. Schäfer S, Berger JV, Deumens R, Goursaud S, Hanisch UK, Hermans E. Influence of intrathecal delivery of bone marrow-derived mesenchymal stem cells on spinal inflammation and pain hypersensitivity in a rat model of peripheral nerve injury. Journal of neuroinflammation. 2014; 11(1):157. https://doi.org/10.1186/s12974-014-0157-8 PMid:25212534 PMCid:PMC4172959

8. Lee EJ, Xu L, Kim GH, Kang SK, Lee SW, Park SH, Kim S, Choi TH, Kim HS. Regeneration of peripheral nerves by transplanted sphere of human mesenchymal stem cells derived from embryonic stem cells. Biomaterials. 2012; 33(29):7039-46. https://doi.org/10.1016/j.biomaterials.2012.06.047 PMid:22795857

9. McGuckin CP, Forraz N, Allouard Q, Pettengell R. Umbilical cord blood stem cells can expand hematopoietic and neuroglial progenitors in vitro. Experimental cell research. 2004; 295(2):350-9. https://doi.org/10.1016/j.yexcr.2003.12.028 PMid:15093735

10. Domanska-Janik K, Buzanska L, Lukomska B. A novel, neural potential of non-hematopoietic human umbilical cord blood stem cells. International Journal of Developmental Biology. 2003; 52(2-3):237-48. https://doi.org/10.1387/ijdb.072315kd PMid:18311714

11. Sanberg PR, Willing AE, Garbuzova-Davis SV, Saporta S, Liu G, Sanberg CD, Bickford PC, Klasko SK, El-Badri NS. Umbilical cord blood-derived stem cells and brain repair. Annals of the New York Academy of Sciences. 2005; 1049(1):67-83. https://doi.org/10.1196/annals.1334.008 PMid:15965108

12. Kao CH, Chen SH, Chio CC, Lin MT. Human umbilical cord blood-derived CD34+ cells may attenuate spinal cord injury by stimulating vascular endothelial and neurotrophic factors. Shock. 2008; 29(1):49-55. PMid:17666954 PMid:17666954

13. Kingham PJ, Kolar MK, Novikova LN, Novikov LN, Wiberg M. Stimulating the neurotrophic and angiogenic properties of human adipose-derived stem cells enhances nerve repair. Stem cells and development. 2013; 23(7):741-54. https://doi.org/10.1089/scd.2013.0396 PMid:24124760

14. McKenzie IA, Biernaskie J, Toma JG, Midha R, Miller FD. Skin-derived precursors generate myelinating Schwann cells for the injured and dysmyelinated nervous system. Journal of Neuroscience. 2006; 26(24):6651-60. https://doi.org/10.1523/JNEUROSCI.1007-06.2006 PMid:16775154

15. Dezawa M, Takahashi I, Esaki M, Takano M, Sawada H. Sciatic nerve regeneration in rats induced by transplantation of in vitro differentiated bone-marrow stromal cells. European Journal of Neuroscience. 2001; 14(11):1771-6. https://doi.org/10.1046/j.0953-816x.2001.01814.x PMid:11860471

16. Matsuse D, Kitada M, Kohama M, Nishikawa K, Makinoshima H, Wakao S, Fujiyoshi Y, Heike T, Nakahata T, Akutsu H, Umezawa A. Human umbilical cord-derived mesenchymal stromal cells differentiate into functional Schwann cells that sustain peripheral nerve regeneration. Journal of Neuropathology & Experimental Neurology. 2010; 69(9):973-85. https://doi.org/10.1097/NEN.0b013e3181eff6dc PMid:20720501

17. Kizilay Z, Aktas S, Kahraman Cetin N, Bakay Ilhan D, Ersoy G, Erken HA. Effect of systemic application of bone marrow-derived mesenchymal stem cells on healing of peripheral nerve injury in an experimental sciatic nerve injury model. Turk Neurosurg. 2017. https://doi.org/10.5137/1019-5149.JTN.20811-17.1

18. Sanchez DNR, Bertanha M, Fernandes TD, Resende LAL, Deffune E, Amorim RM.Effects of Canine and Murine Mesenchymal Stromal Cell Transplantation on Peripheral Nerve Regeneration. Int J Stem Cells. 2017; 10(1):83-92. https://doi.org/10.15283/ijsc16037 PMid:28446003 PMCid:PMC5488780

19. Caseiro AR, Pereira T, Ivanova G, Luís AL, Maurício AC. Neuromuscular Regeneration: Perspective on the Application of Mesenchymal Stem Cells and Their Secretion Products. Stem Cells

Int. 2016; 2016:9756973. https://doi.org/10.1155/2016/9756973 PMid:26880998 PMCid:PMC4736584

20. Hart AM, Terenghi M, Wiberg M. Neural death after peripheral nerve injury and experimental strategies for neuroprotection. Neurol Res. 2008; 30(10):999-1011. https://doi.org/10.1179/174313208X362479 PMid:19079974

21. Nishio Y, KodaM, Kamada T, Someya Y, Yoshinaga K, Okada S, Harada H, Okawa A, Moriya H, Yamazaki M. The use of hemopoietic stem cells derived from human umbilical cord blood topromote restoration of spinal cord tissue and recovery of hindlimb function in adult rats. J Neurosurg Spine 2006; 5(5):424-433. https://doi.org/10.3171/spi.2006.5.5.424 PMid:17120892

22. Fan CL, Li Y, Gao PJ, Liu JJ, Zhang XJ, Zhu DL. Differentiation of endothelial progenitor cells from human umbilical cord blood CD 34+cells in vitro. Acta Pharmacol Sin. 2003; 24(3):212-8. PMid:12617768 PMid:12617768

23. Bracci-Laudiero L, Celestino D, Starace G, Antonelli A, Lambiase A, Procoli A, Rumi C, Lai M, Picardi A, Ballatore G, Bonini S. CD34 positive cells in human umbilical cord blood expres nerve growth factor and its specific receptor TrkA. Journal of neuroimmunology. 2003; 136(1-2):130-9. https://doi.org/10.1016/S0165-5728(03)00007-9

24. Boyd JG, Gordon T. Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. Molecular neurobiology 2003; 27(3):277-323. https://doi.org/10.1385/MN:27:3:277

25. Fan CG, Zhang QJ, Tang FW, HanZB, Wang GS, Han ZC. Human umbilical cord blood cells express neurotrophic factors. Neuroscience letters. 2005; 380(3):322-325. https://doi.org/10.1016/j.neulet.2005.01.070 PMid:15862910

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Turmeric Extract Supplementation Reduces Tau Protein Level in Repetitive Traumatic Brain Injury Model

Andre Marolop Pangihutan Siahaan[1*], Iskandar Japardi[1], Aldy Safruddin Rambe[2], Rr Suzy Indharty[1], Muhammad Ichwan[3]

[1]Department of Neurosurgery, Medical Faculty Universitas Sumatera Utara, Medan, Indonesia; [2]Department of Neurology, Medical Faculty Universitas Sumatera Utara, Medan, Indonesia; [3]Department of Pharmacology and Therapeutic, Medical Faculty Universitas Sumatera Utara, Medan, Indonesia

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***Correspondence:** Andre Marolop Pangihutan Siahaan. Department of Neurosurgery, Medical Faculty Universitas Sumatera Utara, Medan, Indonesia. E-mail: andremarolop@usu.ac.id

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Abstract

BACKGROUND: Repetitive traumatic brain injury (RTBI) has gained much attention in this decade, especially in contact sports athletes and military personals. This injury is correlated with early neurodegenerative changes that are marked with the increased of tau protein. Turmeric extract (TE) is a well-known anti-inflammation and antioxidant that decreases tau protein expression in neurodegenerative disease.

AIM: This study aimed to prove the effect of TE on tau protein level after RTBI.

METHODS: Forty Sprague Dawley mice were divided into four groups, i.e. negative sham control group, the control group, and two treatment groups. A weight drop model was used by applying a 40-gram mass that was dropped from a 1-meter height onto the vertex of the head, with a total frequency of 12 times, divided into 4 days (day 0, 1, 3, and 7; 3 traumas on each day). TE was given to all treatment groups with 500 mg/kg BW doses once daily. The first treatment group had TE for seven days along the trauma. The second treatment group had pretreatment TE extract, given from seven days before first trauma and continued along the trauma protocol days. Tau protein level was measured on brain and serum using ELISA method.

RESULTS: There was a significant reduction of tau protein level in both treatment groups compared to trauma group, either in serum or brain, but we also found significant differences regarding brain tau level between the treatment and pretreatment group.

CONCLUSION: This study might provide evidence of with the role of pretreatment TE in RTBI.

Introduction

Repetitive traumatic brain injury (RTBI), particularly concussion, is frequently happened in some populations, like contact sports athletes and military personals. In a survey involving 222 professional football players, the incident of head impact is very diverse, from four times to 125 times in two weeks. Another report said that around 30% of football players do heading for more than 1000 times annually [1]. In the military, it is estimated that up to 35% of American service personnel in Iraq and Afghanistan have sustained a concussion [2]. Almost all sports-related traumatic brain injury is minor, with spontaneous recovery within days to weeks, even a small number of individuals develop long-lasting and progressive symptoms.

Following traumatic brain injury, even in mildest form, ionic flux, hyper glycolysis, and metabolic uncoupling will result in an energy crisis that can be lasted until 7 to 10 days [3]. The time needed for recover may render the brain vulnerable to the second event. RTBI has been shown they exacerbate the impairment of the brain's cellular oxidative metabolism similar to severe traumatic brain injury [4]. RTBI itself will result in progressive white matter injury

and behavioural dysfunction [5]. Around 17% of individuals with RTBI will develop a constellation of several dysfunctions in behaviour, mood, cognitive, and motoric [6]. Over the last several decades, it is known that these symptoms are correlated with progressive neurodegeneration process that happens in this population. This early neurodegenerative process is named chronic traumatic encephalopathy (CTE). The epidemiology of CTE is unknown since microscopic examination after an autopsy is the only widely accepted way to diagnose this condition [7].

As in another neurodegenerative process, there are several proteinopathies that can be found in CTE. Protein tau is the main proteinopathy. Hyperphosphorylation will dissociate tau from tubulin and expose new phosphorylation site. In this state, protein tau will be insoluble, accumulated into oligomers and neurofibrillary tangles [8]. This condition resembles Alzheimer's disease (AD), with different in tau distribution. In CTE, tau is most commonly found in the superficial layer, compared to layers III and V in AD [9] [10]. Factors that initiate the development of tau pathology are still unknown but associated with chronic neuroinflammation. Neuroinflammation is a common finding after TBI, especially in moderate and severe TBI. In mild TBI, neuroinflammation is usually short-lived, but RTBI may lead to chronic neuroinflammation due to self-perpetuating inflammatory cycle, including the sustained release of inflammatory mediators. It is shown that microglial neuroinflammation contributes to tau accumulation in CTE [11].

To this date, there is no approved therapy for CTE. Rest and symptomatic therapy are the main treatment approaches. The answer may lie in understanding the principle of tau proteins, where tau proteins aggregate through nucleation, templating, growth, multiplication, and spread. Each of these mechanisms represents a potential target for therapeutic intervention. Therapies that target tau phosphorylation, tau aggregation, and microtubule stabilisation are already in clinical trials [12].

For a long time, practitioners of Ayurvedic, traditional Chinese medicine, and Southeast Asian medicine have used turmeric root extract (TE) for the culinary, religious, and medical condition. Curcumin, the major active compound of TE, is a strong anti-inflammatory and antioxidant properties [13]. The use of curcumin in the AD has been studied extensively. Curcumin reduces accumulation of soluble tau aggregates via inhibition the kinase [14]. Even so, all human clinical trials fail to prove the role of curcumin in the AD, regarding cognitive function [15]. The main problem is curcumin's low bioavailability [16].

The present study was undertaken to evaluate the effects of turmeric extract in RTBI concerning protein tau level, both in brain and plasma.

Materials and Methods

The subjects of this experiment were 6-8 weeks old Sprague Dawley rats weighing 300-350 g (n = 40). This experimental protocol was approved by an Institutional Ethics Committee in Universitas Sumatera Utara, Medan, Indonesia. The animals were housed with 12 h light/12 h cycle and given access to food and water ad libitum.

The animals were exposed to the weight-drop model of TBI that had been described before [17]. A 40 g mass was dropped from 1 m high unto the vertex. A 2.5 cm metal plate was placed on the vertex to prevent skull fracture. The total cumulative traumas were 12 times, divided 3 times each day on day 0, 1, 3, and 7. We did not use anaesthesia in this protocol.

The rats were randomly allocated into four groups (n = 10) as follow: a control (sham-operated) group, a trauma group, and two group treatments, i.e., treatment 1 (after trauma), and treatment 2 (pretreatment) groups. The control group underwent neither trauma nor TE. The trauma group underwent trauma only, but no TE. The first treatment group received TE for seven days along the trauma and the second treatment group received TE started from seven days before the trauma and along the trauma.

Turmeric extract (Sido Muncul, Indonesia, 18% curcumin) was suspended in distilled water and administered to animals via oral gavage in 2 cc solution. Turmeric extract was given per oral with dose 500 mg/kgBW once daily. The animals were weighed after the acclimatisation. Turmeric extract was administered in the morning, 1 hour before the first trauma.

As a marker of neurodegenerative process, we investigated AT-8 expression. The expression of all markers was investigated on paraffin-embedded sections using the avidin-biotin-peroxidase complex method. Four-millimetre-thick paraffin sections were dewaxed, rehydrated, and microwave for 10 minutes. The endogenous peroxidase activity of the investigated specimens was blocked with 3 per cent H_2O_2 for 10 minutes, followed by 25 minutes washing with phosphate-buffered saline (PBS). The tissue sections were incubated with normal rabbit serum for 10 minutes, and then the slides were incubated at room temperature with monoclonal mouse AT-8 (Santa Cruz). Sections were washed with PBS and incubated with a secondary antibody for 30 minutes. Sections were washed twice with PBS, developed with 0.05% 3, 3 diamino-benzinetetrahydrochloride for five minutes, and slightly counterstained.

All samples were evaluated by the first author (not blinded to specimen). A positive signal for AT-8 in brain tissue was quantitatively estimated by the distribution of positively stained cells in the cortical brain. Cell counts were carried out using a light

binocular microscope with 1000 times magnification in 20 high power fields.

Protein tau level was measured in plasma and brain. Animals were sacrificed one day after the last protocol day. After anaesthesia, rats were decapitated, and brains were dissected. Brain tissues were homogenised in ice-cold homogenization buffer (50 m*M* Trizma base/HCl buffer, pH 7.4 containing protease inhibitors and 2 Ì*M* of the phosphatase inhibitor okadaic acid), these brain homogenates were placed in -20°C.

Blood was taken from the left ventricle. After collection of the whole blood, blood was left undisturbed at room temperature for 15-30 minutes. The clot was removed by centrifuging at 1,000-2,000 x g for 10 minutes. The plasma then was maintained at -20°C. Blood and plasma levels of total tau were determined using a commercially available sandwich ELISA system (Cusabio).

Values were expressed as means ± SD. The results were computed statistically using one-way analysis of variance for each group. The Tuckey post hoc testing was performed for intergroup comparisons. A difference was considered significant at the $p < 0.05$ level.

Results

There was no mortality rate in this protocol. We also did not find a decrease in body weight (Table 1). There was no significant difference regarding body weight on all three groups (Table 1), either in negative sham control group (377.22 ± 29.72 gr vs. 378.44 ± 29.66 gr), trauma group (351.78 ± 29.89 gr vs. 349.33 ± 38.90 gr), or in treatment group (367.89 ± 36.70 gr vs. 357.89 ± 39.89 gr).

Table 1: Weight changes in subjects (Mean ± SD)

Group	Day 0 (gr)	Day 7 (gr)	p
Negative sham	377.22 ± 29.72	378.44 ± 29.66	0.910
Trauma	351.78 ± 29.89	349.33 ± 38.90	0.482
Treatment	367.89 ± 36.70	357.89 ± 39.89	0.950

Paired t-test. Significant if $p < 0.05$

There was significantly difference between tau level between the negative sham group and trauma group, either in the brain (Figure 1A) or plasma (Figure 1B). In the negative sham control group, brain protein tau level was 4.21 ± 0.22 pg/ml while plasma tau protein level was 4.36 ± 0.13 pg/ml). There was significant increase of tau protein level following RTBI, either in brain (17.76 ± 2.06 ng/ml, $p < 0.05$) or plasma (6.16 ± 1.11 ng/ml, $p < 0.05$).

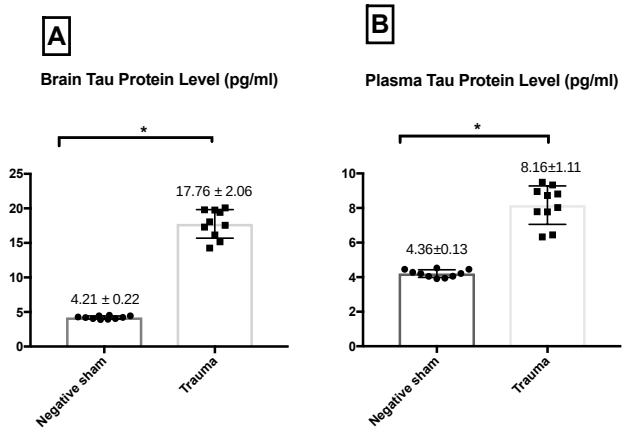

Figure 1: Change in protein tau level following RTBI in the brain (A) and plasma (B). There was significant protein tau level difference between trauma and negative sham group both in the brain and in plasma. Data was shown as Mean ± SD. T-test; significant if $p < 0.05$

There was a significant decrease of tau protein level in both treatment groups compared to the trauma group, either in the brain (Figure 2A) or in plasma (Figure 2B). In treatment 1 group, the brain tau protein level was 13.31 ± 1.45 pg/ml and the plasma tau protein level was 5.25 ± 0.77 pg/ml.

These were significantly different compared to in trauma group ($p < 0.05$). In treatment 2 group, either brain tau protein level (3.60 ± 0.23 pg/ml) or plasma tau protein level (4.99 ± 0.53 pg/ml) was also significantly different compared to trauma group ($p < 0.05$). There was significant brain tau protein level between treatment 1 group and treatment 2 group, which was not seen at the plasma.

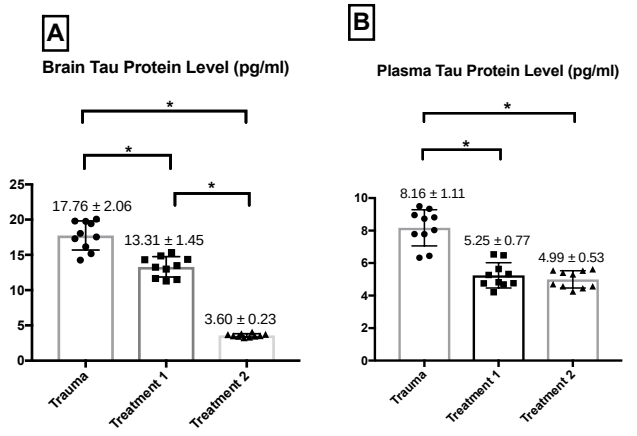

Figure 2: Change in protein tau level after TE supplementation on the brain (A) and plasma (B). There was significant protein tau level difference among either treatment 1 or treatment 2 group with trauma group in brain and plasma. There was also a significant difference regarding protein tau level between treatment 1 and treatment 2 group in bran. The significant difference was not found in plasma. Data was shown as Mean ± SD. T-test; significant if $p < 0.05$

Discussion

We reported no mortality rate in this protocol. Xu *et al.,* (2006) report the same finding. The mortality rate is increased along the increased weight and height. With the 50 g-1 m impact, mortality is 18.2%, and with the 60 g-1 m impact, mortality is increased up to 40% [17].

We also did not find a decrease in body weight (Table 1). In this RTBI model, the animals underwent injury schedule at days 0, 1, 3, and 7, with three hits daily. It made a total of 12 TBI events. The use of this model was meant to replicate the pattern of multiple concussion in contact sports athlete. This model did not show evidence of weight loss, compared to the more "severe" model. Samini *et al.,* (2013) find significant weight decrease following a weight-drop model of TBI. The body weight decrease was probably due to injury affecting feeding behaviour and injury to the anterior hypothalamus, even though multi-factorial mechanisms are involved in TBI [18].

Tau is a soluble intracellular protein with little secondary structure. One of protein tau's functions is to modulate the stability of microtubules. Tau is localised in neuronal cells, but it is not released. Therefore, CSF and blood tau levels in healthy subjects are expected to be low or non-existent.

In pathologic conditions, associated with tauopathy, the soluble protein tau becomes hyperphosphorylated at specific site forming an insoluble form. The brains of some individuals who experience RTBI and develop CTE display tauopathy, predominantly perivascular and in the cortex [19]. Several pathogenic mechanisms have been suggested to contribute to this tau-specific pathology profile, include an imbalance in the activity levels of the tau-specific kinase, dysfunction in protein clearance mechanism, prolonged neuroinflammation, and increased genetic susceptibility (ApoE4 allele) [20]. Increased of protein tau's level in the brain is reported in neurodegenerative diseases, such as AD (21). High protein tau level is also found in the brain's interstitial fluid [22], CSF [23], and blood [24].

Neselius *et al.,* (2013) reported that tau plasma levels were significantly increased in Olympic boxers, even without a symptom of concussion [25]. In mild TBI, tau is a poor predictor of CT lesions and post-concussion syndrome [26]. There are inconsistencies regarding the evaluation of protein tau across the literature, i.e. in the form of cleaved-tau, total-tau, and phosphorylated tau. This inconsistency may also be a result of many factors, including the sensitivity and specificity of the tau assays used and the timing of the sample [27].

Penetration of protein tau into the blood suggested that this protein might enter the circulation through the blood-brain barrier (BBB) disruption. Although the integrity of BBB was not assessed, the disruption of BBB in the previous report with the same trauma protocol was minimal [17]. Kane *et al.,* (2012) also reported minimal BBB disruption when using 95-g mass dropped from a 1.5-m height [28]. Other hypotheses that could be proposed are release via glymphatic system or a more complex intracellular transport via macrophages of phagocytic microglia (24). However, these hypotheses require further studies.

However, the mechanism behind tau pathology in repetitive concussive head injury is unknown. Although protein tau is widely used as a biomarker for the diagnosis of CTE, it is not yet clear that tau is the principal mediator of pathogenesis. Protein tau deposition could be just a result of cumulative biochemical changes that happen after RTBI [29].

In this study, treatment groups were divided into two groups. The first treatment group received TE along the trauma for seven days. The second group was pretreatment group that received TE seven days before the trauma was started and continued along the trauma. Protein tau level was significantly lower in either first or second treatment group compared to the trauma group. Nevertheless, protein tau level on the brain was significantly lower on the second treatment group compared to the first. The same difference was not found in plasma (Figure 2A and 2B).

The potential role of TE in degenerative disease is supported by epidemiological evidence. It is reported that people age 70-79 years old in India has a 4.4-fold less prevalence of AD compared to the same population age in the US. The regular intake of turmeric in common Indian diet has been thought as the primary reason for this finding [30]. In Singapore, it was also found that people who regularly ate curry have better cognitive performance than people who rarely or never ate curry [31].

Curcumin was shown to affect protein kinase modulation, such as Akt [32], JNK [33], and GSK-3β [34]. Since protein kinase has a crucial role in protein tau phosphorylation, it may explain why tau protein level was significantly decreased in both treatment group. In neurodegenerative disease, there is also dysfunction in molecular chaperone. Molecular chaperones, such as heat shock proteins (HSPs), play a significant role in the removal of misfolded or mutant proteins. Curcumin affects augmenting expression or function of HSPs in the cell [35]. The anti-inflammatory properties of curcumin also play a significant role in this effect. Curcumin also induces M2 macrophage polarisation in experimental autoimmune myocarditis [36]. M2 macrophages are associated with the production of anti-inflammatory cytokines, increased phagocytic activity, as well as regulation of tissue repair and remodelling [37].

In this research, we found significant protein tau level difference between the first and second treatment group. The second treatment group was a

pre-treatment group that received TE for one week before TBI. Curcumin retreatment will induce Nrf2 that is a regulator of cellular resistance to oxidants [38]. In a model of traumatic brain injury, curcumin pre-treatment also decreases malondialdehyde expression, decreases the lesion size, and improves neurological function [18]. This finding might also support the role of curcumin as a neuroprotective agent, not as a treatment agent. It also could explain the reason behind the failure of curcumin in a human study regarding Alzheimer's disease [15].

There was no significant difference regarding total plasma tau protein level between the two treatment groups. The expression of tau is predominantly in brain, but tau can also be found at both mRNA and protein level in salivary glands and kidney. This is a potential confounder that may help explain the difference of total tau protein level between brain and plasma. In plasma, the half-life of tau appears to be shorter (hours) than in cerebrospinal fluid (weeks) [39].

In conclusion, turmeric extract supplementation reduces tau protein level in brain and plasma following repetitive traumatic brain injury. The most significant effect was found in the pre-treatment group. Further studies to demonstrate the effect of turmeric extract on the clinical outcome will be needed.

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References

1. Hawkes N. Professional football may be linked to brain injuries usually seen in boxing. 2017: j811.

2. Peskind ER, Brody D, Cernak I, McKee A, Ruff RL. Military- and sports-related mild traumatic brain injury: clinical presentation, management, and long-term consequences. J Clin Psychiatry. 2013; 74(2):180–8–quiz188.

3. Giza CC, Hovda DA. The New Neurometabolic Cascade of Concussion. Neurosurgery. 2014; 75:S24–S33. https://doi.org/10.1227/NEU.0000000000000505 PMid:25232881 PMCid:PMC4479139

4. Vagnozzi R, Signoretti S, Cristofori L, Alessandrini F, Floris R, Isgro E, et al. Assessment of metabolic brain damage and recovery following mild traumatic brain injury: a multicentre, proton magnetic resonance spectroscopic study in concussed patients. Brain. 2010; 133(11):3232–42. https://doi.org/10.1093/brain/awq200 PMid:20736189

5. Longhi L, Saatman KE, Fujimoto S, Raghupathi R, Meaney DF, Davis J, et al. Temporal Window of Vulnerability to Repetitive Experimental Concussive Brain Injury. Neurosurgery. 2005; 56(2):364–74. https://doi.org/10.1227/01.NEU.0000149008.73513.44 PMid:15670384

6. Montenigro PH, Baugh CM, Daneshvar DH, Mez J, Budson AE, Au R, et al. Clinical subtypes of chronic traumatic encephalopathy: literature review and proposed research diagnostic criteria for traumatic encephalopathy syndrome. Alz Res Therapy. 2014; 6(5-8):709–17. https://doi.org/10.1186/s13195-014-0068-z PMid:25580160 PMCid:PMC4288217

7. McKee AC, Cairns NJ, Dickson DW, Folkerth RD, Keene CD, Litvan I, et al. The first NINDS/NIBIB consensus meeting to define neuropathological criteria for the diagnosis of chronic traumatic encephalopathy. Acta Neuropathol. 2017; 131(1):75–86. https://doi.org/10.1007/s00401-015-1515-z PMid:26667418 PMCid:PMC4698281

8. Cowan CM, Mudher A. Are tau aggregates toxic or protective in tauopathies? Front Neurol. 2013; 4:114. https://doi.org/10.3389/fneur.2013.00114 PMid:23964266 PMCid:PMC3741634

9. Schmidt ML, Zhukareva V, Newell KL, Lee VM, Trojanowski JQ. Tau isoform profile and phosphorylation state in dementia pugilistica recapitulate Alzheimer's disease. Acta Neuropathol. 2001; 101(5):518–24. PMid:11484824

10. McKee AC, Cantu RC, Nowinski CJ, Hedley-Whyte ET, Gavett BE, Budson AE, et al. Chronic Traumatic Encephalopathy in Athletes: Progressive Tauopathy After Repetitive Head Injury. Journal of Neuropathology & Experimental Neurology. 2009; 68(7):709–35. https://doi.org/10.1097/NEN.0b013e3181a9d503 PMid:19535999 PMCid:PMC2945234

11. Cherry JD, Tripodis Y, Alvarez VE, Huber B, Kiernan PT, Daneshvar DH, Mez J, Montenigro PH, Solomon TM, Alosco ML, Stern RA. Microglial neuroinflammation contributes to tau accumulation in chronic traumatic encephalopathy. Acta neuropathologica communications. 2016; 4(1):112. https://doi.org/10.1186/s40478-016-0382-8 PMid:27793189 PMCid:PMC5084333

12. van den Bedem H, Kuhl E. Molecular mechanisms of chronic traumatic encephalopathy. Current Opinion in Biomedical Engineering. 2017; 1:23-30. https://doi.org/10.1016/j.cobme.2017.02.003

13. Curcumin: an orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. British Journal of Pharmacology. 2013; 169(8):1–21. PMid:23072488 PMCid:PMC3632233

14. Darvesh AS, Carroll RT, Bishayee A, Novotny NA, Geldenhuys WJ, Van der Schyf CJ. Curcumin and neurodegenerative diseases: a perspective. Expert Opinion on Investigational Drugs. 2012; 21(8):1123–40. https://doi.org/10.1517/13543784.2012.693479 PMid:22668065

15. Hamaguchi T, Ono K, Yamada M. REVIEW: Curcumin and Alzheimer's Disease. CNS Neuroscience & Therapeutics. 2010; 16(5):285–97. https://doi.org/10.1111/j.1755-5949.2010.00147.x PMid:20406252

16. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of Curcumin: Problems and Promises. Mol Pharmaceutics. 2007; 4(6):807–18. https://doi.org/10.1021/mp700113r PMid:17999464

17. Xu L, Nguyen JV, Lehar M, Menon A, Rha E, Arena J, et al. Repetitive mild traumatic brain injury with impact acceleration in the mouse: Multifocal axonopathy, neuroinflammation, and neurodegeneration in the visual system. Experimental Neurology. 2016; 275(Part 3):436–49. https://doi.org/10.1016/j.expneurol.2014.11.004 PMid:25450468

18. Samini F, Samarghandian S, Borji A, Mohammadi G, bakaian M. Curcumin pretreatment attenuates brain lesion size and

improves neurological function following traumatic brain injury in the rat. Pharmacology, Biochemistry and Behavior. 2013; 110(C):238–44. https://doi.org/10.1016/j.pbb.2013.07.019 PMid:23932920

19. McKee AC, Alosco ML, Huber BR. Repetitive Head Impacts and Chronic Traumatic Encephalopathy. Neurosurg Clin N Am. 2016; 27(4):529–35. https://doi.org/10.1016/j.nec.2016.05.009 PMid:27637402 PMCid:PMC5028120

20. Ojo J-O, Mouzon B, Greenberg MB, Bachmeier C, Mullan M, Crawford F. Repetitive mild traumatic brain injury augments tau pathology and glial activation in aged hTau mice. Journal of Neuropathology & Experimental Neurology. 2013; 72(2):137–51. https://doi.org/10.1097/NEN.0b013e3182814cdf PMid:23334597

21. Scheltens P, Blennow K, Breteler MMB, de Strooper B, Frisoni GB, Salloway S, et al. Alzheimer's disease. Lancet. 2016; 388(10043):505–17. https://doi.org/10.1016/S0140-6736(15)01124-1

22. Marklund N, Blennow K, Zetterberg H, Ronne-Engström E, Enblad P, Hillered L. Monitoring of brain interstitial total tau and beta amyloid proteins by microdialysis in patients with traumatic brain injury. Journal of Neurosurgery. 2009; 110(6):1227–37. https://doi.org/10.3171/2008.9.JNS08584 PMid:19216653

23. Franz G, Beer R, Kampfl A, Engelhardt K, Schmutzhard E, Ulmer H, et al. Amyloid beta 1-42 and tau in cerebrospinal fluid after severe traumatic brain injury. Neurology. 2003; 60(9):1457–61. https://doi.org/10.1212/01.WNL.0000063313.57292.00 PMid:12743231

24. Olczak M, Niderla-Bielińska J, Kwiatkowska M, Samojłowicz D, Tarka S, Wierzba-Bobrowicz T. Tau protein (MAPT) as a possible biochemical marker of traumatic brain injury in postmortem examination. Forensic science international. 2017; 280:1-7. https://doi.org/10.1016/j.forsciint.2017.09.008 PMid:28942078

25. Neselius S, Zetterberg H, Blennow K, Randall J, Wilson D, Marcusson J, et al. Olympic boxing is associated with elevated levels of the neuronal protein tau in plasma. Brain Injury. 2013; 27(4):425–33. https://doi.org/10.3109/02699052.2012.750752 PMid:23473386

26. Ma M, Lindsell CJ, Rosenberry CM, Shaw GJ, Zemlan FP. Serum cleaved tau does not predict postconcussion syndrome after mild traumatic brain injury. The American Journal of Emergency Medicine. 2008; 26(7):763–8. https://doi.org/10.1016/j.ajem.2007.10.029 PMid:18774039 PMCid:PMC2576476

27. Papa L, Ramia MM, Edwards D, Johnson BD, Slobounov SM. Systematic Review of Clinical Studies Examining Biomarkers of Brain Injury in Athletes after Sports-Related Concussion. J Neurotrauma. 2015; 32(10):661–73. https://doi.org/10.1089/neu.2014.3655 PMid:25254425 PMCid:PMC4426313

28. Kane MJ, Angoa-Pérez M, Briggs DI, Viano DC, Kreipke CW, Kuhn DM. A mouse model of human repetitive mild traumatic brain injury. Journal of Neuroscience Methods. 2012; 203(1):41–9. https://doi.org/10.1016/j.jneumeth.2011.09.003 PMid:21930157 PMCid:PMC3221913

29. Ojo JO, Mouzon BC, Crawford F. Repetitive head trauma, chronic traumatic encephalopathy and tau: Challenges in translating from mice to men. Experimental Neurology. 2016; 275(Part 3):389–404. https://doi.org/10.1016/j.expneurol.2015.06.003 PMid:26054886

30. Chandra V, Pandav R, Dodge HH, Johnston JM, Belle SH, DeKosky ST, et al. Incidence of Alzheimer's disease in a rural community in India The Indo–US Study. Neurology. Lippincott Williams & Wilkins; 2001; 57(6):985–9. https://doi.org/10.1212/WNL.57.6.985

31. Ng TP, Chiam PC, Lee T, Chua HC, Lim L, Kua EH. Curry Consumption and Cognitive Function in the Elderly. American Journal of Epidemiology. 2006; 164(9):898–906. https://doi.org/10.1093/aje/kwj267 PMid:16870699

32. Gao Y, Li J, Wu L, Zhou C, Wang Q, Li X, Zhou M, Wang H. Tetrahydrocurcumin provides neuroprotection in rats after traumatic brain injury: autophagy and the PI3K/AKT pathways as a potential mechanism. Journal of surgical research. 2016; 206(1):67-76. https://doi.org/10.1016/j.jss.2016.07.014 PMid:27916377

33. Wang YL, Li JF, Wang YT, Xu CY, Hua LL, Yang XP, Geng S, Wang SS, Wang Z, Yin HL. Curcumin reduces hippocampal neuron apoptosis and JNK-3 phosphorylation in rats with Aβ-induced Alzheimer's disease: protecting spatial learning and memory. Journal of Neurorestoratology. 2017; 5:117-23. https://doi.org/10.2147/JN.S125567

34. Sun J, Zhang X, Wang C, Teng Z, Li Y. Curcumin Decreases Hyperphosphorylation of Tau by Down-Regulating Caveolin-1/GSK-3β in N2a/APP695swe Cells and APP/PS1 Double Transgenic Alzheimer's Disease Mice. Am J Chin Med. 2017; 45(08):1667–82. https://doi.org/10.1142/S0192415X17500902 PMid:29132216

35. Maiti P, Manna J, Veleri S, Frautschy S. Molecular chaperone dysfunction in neurodegenerative diseases and effects of curcumin. BioMed Research International. 2014; 2014(1):495091–14. https://doi.org/10.1155/2014/495091

36. Gao S, Zhou J, Liu N, Wang L, Gao Q, Wu Y, Zhao Q, Liu P, Wang S, Liu Y, Guo N. Curcumin induces M2 macrophage polarization by secretion IL-4 and/or IL-13. Journal of molecular and cellular cardiology. 2015; 85:131-9. https://doi.org/10.1016/j.yjmcc.2015.04.025 PMid:25944087

37. Simon DW, McGeachy MJ, Bayır H, Clark RSB, Loane DJ, Kochanek PM. The far-reaching scope of neuroinflammation after traumatic brain injury. Nature Reviews Neurology. 2017; 13(3):171–91. https://doi.org/10.1038/nrneurol.2017.13 PMid:28186177 PMCid:PMC5675525

38. González-Reyes S, Guzmán-Beltrán S, Medina-Campos ON, Pedraza-Chaverri J. Curcumin pretreatment induces Nrf2 and an antioxidant response and prevents hemin-induced toxicity in primary cultures of cerebellar granule neurons of rats. Oxidative Medicine and Cellular Longevity. Hindawi; 2013; 2013(22):801418–14.

39. Zetterberg H. Review: Tau in biofluids - relation to pathology, imaging and clinical features. Neuropathol Appl Neurobiol. 2017; 43(3):194–9. https://doi.org/10.1111/nan.12378 PMid:28054371

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Neuroprotective Effects of Purple Sweet Potato Balinese Cultivar in Wistar Rats With Ischemic Stroke

I Made Oka Adnyana[1*], AA Raka Sudewi[1], DPG Purwa Samatra[1], DN Suprapta[2]

[1]Neurology Department, Sanglah Hospital, Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia; [2]Biopesticide Laboratory, Faculty of Agriculture, Udayana University, Denpasar, Bali, Indonesia

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***Correspondence:** I Made Oka Adnyana. Neurology Department, Sanglah Hospital/Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia. E-mail: oka.adnyana.sps@gmail.com

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Abstract

BACKGROUND: Purple sweet potato (*Ipomoea Batatas L.*) is one of the sources for anthocyanin, which promotes the health through antioxidant, anti-inflammatory, anti-cancer, neuroprotection, and anti-apoptosis activities. Oxidative stress has been shown to be the cause of apoptosis in ischemic stroke.

AIM: The objective of this research was to delineate the pleiotropic effects of anthocyanin for neuroprotection during an acute stroke event.

METHODS: Anthocyanin was extracted from Balinese cultivar of purple sweet potato and subsequently administered to rat models of induced ischemic stroke (labelled as treatment group), as well as a placebo (labelled as a control group). Several parameters were in turn evaluated, i.e. the activities of anti-apoptotic (Bcl-2) as well as pro-apoptotic (cytochrome c, caspase-3) molecules, and apoptosis rate. Bcl-2 levels were determined using the histochemical method, cytochrome c and caspase-3 via ELISA method, while apoptosis rate was measured by TdT-medicated Dutp-Nick End Labeling (TUNEL) assay.

RESULTS: Bcl-2 expression demonstrated significantly higher Bcl-2 expression in the treatment compared with control group (median 31.2 vs. 1.1; p = 0.001). Accordingly, pro-apoptotic cytochrome c and caspase-3 levels were also found significantly lower in the treatment as opposed to control group (mean 4.17 vs. 8.06; p = 0.001; mean 3.81 vs. 8.02; p = 0.001). Ultimately, apoptosis rate was found markedly lower among treatment than control groups (mean 3.81 vs. control 21.97; p = 0.003).

CONCLUSION: The results of this study indicated a significant neuroprotective effect of anthocyanin derived from Balinese cultivar of PSP. Anthocyanin was able to increase and reduce anti-apoptotic and pro-apoptotic protein levels, respectively, resulting in lesser cellular apoptotic rate when compared with placebo. The potential mechanism was thought mainly due to its anti-oxidant properties.

Introduction

Purple sweet potato (*Ipomoea Batatas* L.), has different coloured tubers, comprising purple, white, red or yellow. Its roots and skin contain a lot of polyphenols, including anthocyanin and phenolic acids which are a source of vitamins A, B, C, Fe, Ca and phosphorus [1], [2]. Purple colour in the purple sweet potato (PSP) is due to high levels of anthocyanins. Higher anthocyanin in the PSP cause higher stability than other sources of anthocyanin [1], [3]. Anthocyanin possesses antioxidative properties [3], [4], maintains calcium ion homeostasis [5] [6], anti-apoptosis [7], [8], protective against cerebral ischemia [9], anti-inflammatory [10] and neuroprotective. Anthocyanin derived from Balinese cultivar have antioxidant effects by suppressing the production of malondialdehyde (MDA) *in vivo* and inducing endogenous antioxidants [11], [12].

Ischemic stroke happens because of blockage of blood flow to the brain, failing energy formation, cellular homeostatic disorders, acidosis and binding of calcium ions, excitotoxicity, reactive-oxygen species-mediated toxicity, glial cell activation, activation of complement, impaired blood brain barrier integrity and white blood cells infiltration [13]. Reduced cerebral blood circulation results in a decrease in ATP production, which is necessary for all brain cell activities. The failure of ATP formation will lead to depolarisation of cell membranes and excessive release of glutamate to extracellular space, thus activating NMDA and AMPA receptors, resulting in increased calcium ions in the cells, which in turn increases the formation of free radicals. Free radicals will cause cell death through necrosis and apoptosis

mechanisms [13], [14].

Apoptosis in ischemic stroke can occur via extrinsic and intrinsic mechanisms. The intrinsic mechanism is induced by oxidative stress. Mitochondria are critical in the occurrence of intrinsic apoptosis due to oxidative stress. Increased ROS (reactive oxygen species) and intracellular calcium ions stimulate the release of pro-apoptotic family protein B cell lymphoma-2 protein (Bcl-2), such as Bcl-2 associated X protein (Bax), Bcl-2 antagonist killer (Bak), a Bcl-2 antagonist of death (Bad) and P_{53} from mitochondria. In mitochondria, there are also anti-apoptotic molecules such as Bcl-2 itself, Bcl-2 extra-long (Bcl-el), Bcl-2 homology of the ovary (Boo), protein kinase and extracellular signal-regulated kinase (ERK) that inhibit the activity of the pro-apoptotic protein. Under normal circumstances, there is a balance between anti- and pro-apoptotic proteins. When ischemic stroke occurs, this balance changes wherein there is an increase in the number of pro-apoptotic molecules, leaving open pores at the outer membrane of the mitochondria and outgoing pro-apoptotic proteins and will result in apoptotic cascades beginning with the cytochrome c secretion from mitochondria that will join apoptotic protease activating factor (APAF) and creating an apoptosome which triggers pro-caspase 9 into caspase 9 which further activates caspase-3 as the executor caspase, resulting in apoptosis [15], [16], [17].

Based on the mechanism of the occurrence of brain cell death (apoptosis) in ischemic stroke due to oxidative stress, and the antioxidant properties of Balinese cultivar PSP to neutralize oxidative stress, the researchers wanted to examine anthocyanin's benefit obtained from PSP Balinese cultivar against oxidative stress in Wistar rats with ischemic stroke, by investigating bcl-2 anti-apoptotic molecules, pro-apoptotic molecules such as cytochrome c, caspase-3 and apoptosis rate.

Material and Methods

Three-month-old male Wistar rats 200-250 g in weight, obtained from Bio Farma Laboratory, Bandung, Indonesia was kept for a week in the cage at the place of research in Bioscience Laboratory Brawijaya University Malang for habituation. All animals used in this study were treated accordingly by adhering to National Institutes of Health guide for the care and use of laboratory animals. Food was provided according to a standardised protocol to ensure those rats were healthy. Rats were fed regularly, a night before the ischemic stroke induction protocol. In the morning, ischemic stroke was inducted according to previous methods [18]. A total of 20 rats were used for the study, each of which was 10 for the control and treatment group. The anthocyanin dosage used was 3 mL/day as previously used [19] intragastric only in the treatment group for 7 days, while all rats were decapitated under anaesthesia on the 8^{th} day for further analyses.

PSP was extracted as follows: PSPs were rinsed under clear water before further processed. After peeled these sweet potatoes were cut transversely with 2-2.5 cm in thickness. Slices were subsequently added with water in 1:1 ratio, and then filtered with three layered-gauze. The liquid obtained from filtration was in turn heated until it boiled for 30 minutes for sterilisation purpose before filled into a bottle.

The tissue to be examined was reorganised with xylol, and 90%, 80%, and 70% of absolute alcohol, respectively were dropped for 5 minutes, then dropped with PBS (phosphate buffer saline) with pH of 7.4 for 10 minutes. It was subsequently blocked with BSA (bovine serum albumin), then mixed with primary antibody (monoclonal mouse anti-human Bcl-2). The preparation was then mixed with secondary IgG biotin antibody in PBS for one hour. The tissue then added with streptavidin HRP (horseradish peroxidase) at room temperature for 30 minutes. Afterwards, DAB was dropped for 20-40 minutes. Counterstain was subsequently performed using Meyer hematoxyline. The brownish colour is seen during histochemical analysis was derived from the bcl-2 protein cells. Cell counting was in turn performed by the axiovision ratio method, which can be downloaded at http://153.1.200:8080/immunoratio.

Wells of ELISA plate had been previously filled with cytochrome c antibody and caspase-3. The sample (serum) was filled into the well, then incubated at room temperature for 2 hours and washed three times. Then biotin was added and incubated for an hour. The phosphate buffered saline teonin (PBST) and HRP-avidin enzyme were added and incubated for an hour, then rinsed four times. TBM substrate was subsequently added and incubated for 5-10 minutes at room temperature. Lastly, 100 µL of stop solution was added, which turned the blue colored wells into yellow. The wells were then read with ELISA reader at 450 nm wavelength.

The rat's cortical neuron cells were added with DNA fragmentation assay kit inserted into coplin jar, then washed with PBS for 5 minutes, and covered with protein kinase solution. After washed twice with PBS, samples were covered with the buffer derived from the kit then added with TdT incubation buffer. The samples were then placed in a dark room at $37^{o}C$ in the incubator for an hour. It was soaked with anti-Brdu-biotin antibody for an hour at room temperature. It was in turn, washed with PBS then soaked with strevtavidin HRP conjugate. Cells which underwent apoptosis were visualized by adding the DAB subsrate and countered with HE before calculated by axiovision ratio.

Baseline data of Bcl-2 expression,

cytochrome c, caspase 3 and number of cells undergoing apoptosis were tested for normality using Shapiro Wilk. If the data was normally distributed, independent t-tests were conducted to find the difference in Bcl-2, cytochrome c, and caspase-3 expressions, as well as the number of cells undergoing apoptosis between the control and treatment group. In contrast, when the data was abnormally distributed, data transformation was performed. When the transformation results were normally distributed then the independent t-test was conducted, otherwise Mann-Whitney test was conducted to identify the difference of Bcl-2, cytochrome c, and caspase 3 expressions, as well as the number of cells undergoing apoptosis with 95% confidence interval ($p < 0.05$). All data were processed by using SPSS 20 for Windows.

Results

The Mann-Whitney test of Balinese cultivar PSP extract effect on Bcl-2 expression demonstrated significantly higher Bcl-2 expression in the treatment group ($p < 0.05$) compared with the control group (Table 1)

Table 1: Analysis result of the Mann-Whitney test for Bcl-2 expression

	n	Median (minimum-maximum)	p*
Control	10	1.1 (0.9-4.1)	0.001
Treatment	10	31.2 (7.6-65.9)	

*signicant at $p < 0.05$.

To clarify Bcl-2 expression in the control and treatment groups, the histochemical features are presented in Figure 1.

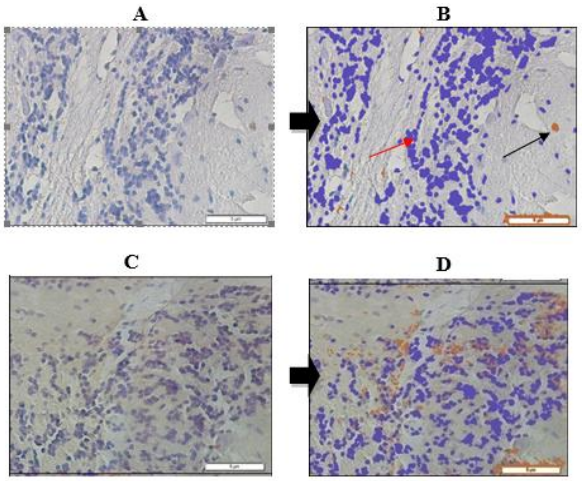

Figure 1: Bcl-2 expression in Wistar rats' cerebral neuron. A) Bcl-2 expression before analyzed with Axio Vision Ratio (control); B) Bcl-2 expression after analyzed with Axio Vision Ratio (3.6%) (control); C) Bcl-2 expression before analyzed with Axio Vision Ratio (treatment); D) Bcl-2 expression after analyzed with Axio Vision Ratio (13.4%) (treatment); →Bcl-2 expression; →Pre-stained nucleus

Table 2 demonstrated cytochrome c levels among treatment group were markedly lower than control ($p < 0.05$).

Table 2: Independent t-test of cytochrome c levels

	n	Mean ± SD	Mean difference (95% CI)	p*
Control	10	8.06 ± 0.07	3.89 (3.84-3.94)	0.001
Treatment	10	4.17 ± 0.02		

*significant at $p < 0.05$.

Effect of purple sweet potato Balinese cultivar extract on caspase-3 level

To determine the difference in caspase-3, the Mann-Whitney test is presented in Table 3.

Table 3: Mann-Whitney test result for caspase-3 level

	N	Median (minimum-maximum)	p*
Control	10	8.02 (7.99-8.16)	0.001
Treatment	10	3.81 (3.75-3.84)	

*significant at $p < 0.05$.

Mann-Whitney test showed that caspase-3 levels among treatment group were markedly lower than control ($p = 0.001$).

The effect of the purple sweet potato Balinese cultivar on apoptosis

TUNEL test results from Wistar rat on day 8 showed a lower number of apoptosis events in the treatment group when compared with control ($p <0.05$) as presented in Table 4.

Table 4. Independent t-test of anthocyanin administration on apoptosis among Wistar rats with ischemic stroke between treatment and control group

Group	N	Mean ± SD	Mean difference (95% CI)	p*
Control	10	21.97 ± 13.92	18.16 (8.17-28.15)	0.003
Treatment	10	3.81 ± 1.79		

*significant at $p < 0.05$.

To clarify the results of the apoptosis between control and treatment groups, the apoptosis was presented in Figure 2. Expression of apoptotic cells in the control group (A and B) and treatment group (C and D).

Discussion

The apoptotic cascade occurs due to an imbalance of anti-apoptotic protein (Bcl-2) with pro-apoptotic proteins (Bax and Bak). Oxidative stress has been demonstrated to cause apoptosis in ischemic strokes (apoptosis) [20]. Anthocyanin has been shown to exert antioxidant effects by increasing the production of endogenous antioxidants and as radical

scavengers. Balinese cultivar of PSP also possesses antioxidant properties by increasing endogenous antioxidant production to counteract the effects of oxidative stress on ischemic stroke [12], [19], [21].

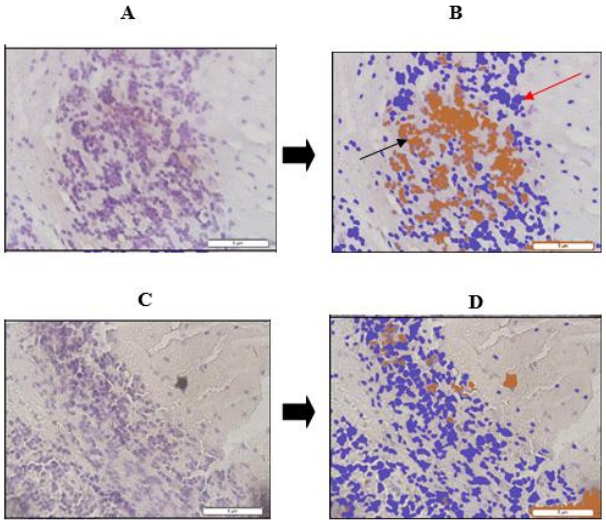

Figure 2: Expression of cerebral cortex neurons in Wistar rats undergoing apoptosis. A) Cellular expression undergoing apoptosis before analyzed with Axio Vision Ratio (control); B) Cellular expression undergoing apoptosis after analyzed with Axio Vision Ratio (28.8%) (control); C) Cellular expression undergoing apoptosis before analyzed with Axio Vision Ratio (treatment); D) Cellular expression undergoing apoptosis after analyzed with Axio Vision Ratio (6.8%) (treatment); →Pre-stained cells underwent DNA fragmentation; →Post-stained cells underwent DNA fragmentation

Consequently, apoptosis was inhibited. Inhibition of apoptotic cascade occurs when the amount of anti-apoptotic protein is in equilibrium or higher than pro-apoptotic protein, which in this study is evidenced by significantly higher Bcl-2 levels ($p < 0.05$) in the treatment group. A similar study was found in anthocyanins extracted from *Ipomoea Batatas* cultivar Ayamurasaki which has been shown to neutralise the effects of ROS and decrease p53 protein activity, so that apoptosis was lower by increasing Bcl-2 expression and decreasing Bak expression, thus increasing the Bcl2/Bak ratio (Han et al., [22]). The study by Yao et al., [23] also found that anthocyanin (quercetin) administered at a dosage of 10 and 20 mg/kg/day results in higher Bcl-2 expression ($p < 0.05$ and $p < 0.01$) in the treatment versus that in control group.

Cytochrome c is a protein consisting of 104 amino acids and has many functions such as electron transfer in the respiratory chain to form energy. Another function is to play a role in cardiolipin peroxide, ROS scavenger and apoptosis. Various cytochrome c functions are governed by their association with specific tissues. Under normal circumstances of bonding with Tyr97 and Tyr48, cytochrome c phosphorylation will form ATP. In a state of oxidative stress, bonding occurs with $p66^{she}$and cytochrome c dephosphorylation occurs, which obviates its role from the respiratory chains of cytochrome c, so that binding with cardiolipin is released and cytochrome c exits from the mitochondria. The cytochrome c released from the mitochondria will bind to APAF forming an apoptosome, which will activate the subsequent apoptotic cascade (Gamdo et al., [24]). The PSP extract of Balinese cultivars has been shown to suppress oxidative stress and induce endogenous antioxidants [12], [19], [21], resulting in lower cytochrome c levels in the treatment than control ($p < 0.05$). These outcomes were similar to the research conducted by Lu et al., [7], which found that PSP colour (PSPC) can suppress cytochrome c elevation in the cytoplasm in older rats of Kunming strain induced by D-galactose. The suppression occurs because PSPC has the effect of increasing endogenous antioxidant expression such as Cu and Zn SOD and catalase. The study by Ullah et al., [26] also obtained the same result that on mouse-induced hippocampus neurons induced by kainic acid for 12 hours there was markedly elevated cytochrome c levels. In hippocampal neurons treated with anthocyanins before induction with kainic acid, there was markedly reduced cytochrome c levels ($p < 0.05$).

Caspase is an amino-terminal prodomain carboxy-terminal protease domain. Based on its role, caspase is classified as the initiator and executor. Caspase is generally in an inactive state called pro-caspase and will become active when there is a death signal which will then be followed by cell death (apoptosis). Caspase 3, 6 and 7 act as the executor's caspases and are inactive in the form of a procaspase dimer. This procaspase will become active when there is a split between large and small subunits, resulting in a conformational change so that it becomes mature caspase. Activation of caspase 3 occurs by a chain reaction through the release of cytochrome c which binds to APAF to form apoptosome. Apoptosome then activates procaspase 9 into caspase 9. Caspase 9 then activates caspase 3 as the executor's caspase. Caspase 3 will destroy and degrade cell components such as structural proteins in the cytoskeleton, cell nucleus proteins and enzymes involved in cellular repair and activate DNAase enzymes, thus detaching from their association with the caspase DNAse (ICAD) inhibitor [26], [27].

The effect of Balinese cultivar of PSP on Bcl-2 in this study was markedly different between those two groups, wherein the treatment group was higher in the treatment group, resulting in the inhibition of proapoptotic protein release such as cytochrome c, so that its level was lower in the treatment group which leads to a lower apoptotic activity. Consequently, apoptosis cascade will be reduced so that the level of caspase-3 in the treatment group in this study was also lower ($p < 0.05$) than in the control group (Table 3). A similar study was obtained by Yao et al., [23] in which caspase-3 expression on the cerebral cortex cells of rats ligated in the medial cerebral arteries was found to be lower in the anthocyanin-treated group

(quecertin). A study conducted by Shah et al., [28], obtained similar results, i.e. prenatal rat neurons induced with ethanol demonstrated significantly higher levels of caspase-3 in the sham group as opposed to those treated with anthocyanin with a dose of 0.1 mg/mL ($p < 0.05$). Similar results were obtained by Badshah et al., [29], which examined the effect of anthocyanin against the toxic beta-amyloid effects injected intraventricularly in the hippocampus, in which caspase-9 and -3 were significantly higher among those rats without anthocyanin treatment ($p < 0.05$) as opposed to shame.

Apoptosis or programmed cell death is a normal life process. Disorders in apoptosis will trigger the onset of various diseases such as cancer, Parkinson's, Alzheimer's and stroke. Apoptosis occurs due to the formation of pores in the outer membrane of the mitochondria. The outer membranes of the mitochondria are formed as a response to the increased permeability of small molecule solution in the intermembrane of the mitochondria. Antiapoptotic proteins such as Bcl-2, Bcl-XL and Mcl inhibit the cascade of proapoptotic signals such as Bax. It migrates to the outer layer of the mitochondrial membrane to form pores, which induces cytochrome c release into the cytoplasm to stimulate the subsequent apoptotic cascade [17], [29], [30], [31].

In this study, we found that Bcl-2 expression was higher in the treatment than in the control group. The cytochrome c and caspase-3 levels were found lower in the treatment group thus resulting in lower apoptosis rate ($p < 0.05$).

Similar results have also been found in other investigators such as Shin et al., [32] who examined the anthocyanin's neuroprotective effect against rat's middle cerebral artery occlusion and reperfusion. In anthocyanin-treated rats, infarct volume and the number of apoptotic cells were significantly lower than placebo ($p < 0.05$). Anthocyanin exerts its neuroprotective effect by significantly inhibiting JNK and p53 activities. In mice treated with anthocyanin, JNK and p53 levels were significantly lower ($p < 0.05$). A study by Ye et al., [6] investigated the effect of PSPC on pheochromocytoma cells induced by beta-amyloid. Cells induced with beta-amyloid previously treated with PSPC have shown decreased calcium ion levels, improved mitochondrial membrane potential, and reduced caspase-3 expression and DNA fragmentation, all of which ameliorate apoptosis. Lu et al., [7] investigated the PSPC effect of Korean black soybean on apoptosis among older rats induced with D galactose. D-galactose administration induced a higher apoptosis rate in cerebral cortex neurons and hippocampus as opposed to the control group ($p < 0.01$). Conversely, simultaneous administration of PSPC and D-galactose decreased the apoptosis rate of cerebral cortex and hippocampus neurons. The effect of PSPC here is by increasing the concentration of antioxidants such as Cu, and ZN-SOD and catalase.

In conclusion, this study proved that the PSP Balinese cultivar extract exerted neuroprotection on Wistar rats with ischemic stroke reflected by higher Bcl-2, cytochrome c, and caspase-3 levels, while lowering the apoptosis rate.

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References

1. Suprapta, DN., Antara, M, Arya, N., Sudana, M., Duniaji, AS., Sudarma, M. Review of cultivation and consumption of various yam types as an alternative food source. Report of Research Results BAPEDA Bali and Faculty of Agriculture Udayana University, 2004.

2. Pasca-Panda V, Sonkambe M. Phytochemical constituents and pharmacological activities of Ipomoea batatas L. Int J Res Phytochem Pharmacol. 2012; 2(1):25-34.

3. Montilla EC, Hillebrand S, Winterhalter P. Anthocyanins in purple sweet potato (Ipomoea batatas L.) varieties. Fruit, Vegetable and Cereal Science and Biotechnology. 2011; 5(2):19-23.

4. de Pascual-Teresa S. Molecular mechanisms involved in the cardiovascular and neuroprotective effects of anthocyanins. Archives of biochemistry and biophysics. 2014; 559:68-74. https://doi.org/10.1016/j.abb.2014.04.012 PMid:24791600

5. Shih PH, Wu CH, Yeh CT, Yen GC. Protective effects of anthocyanins against amyloid β-peptide-induced damage in Neuro-2A cells. Journal of agricultural and food chemistry. 2011; 59(5):1683-9. https://doi.org/10.1021/jf103822h PMid:21302893

6. Ye J, Meng X, Yan C, Wang C. Effect of purple sweet potato anthocyanins on β-amyloid-mediated PC-12 cells death by inhibition of oxidative stress. Neurochemical research. 2010; 35(3):357-65. https://doi.org/10.1007/s11064-009-0063-0 PMid:19771514

7. Lu J, Wu DM, Zheng YL, Hu B, Zhang ZF. Purple sweet potato colour alleviates d-galactose-induced brain aging in old mice by promoting survival of neurons via PI3K pathway and inhibiting cytochrome c-mediated apoptosis. Brain Pathology. 2010; 20(3):598-612. https://doi.org/10.1111/j.1750-3639.2009.00339.x PMid:19863544

8. Kim HG, Ju MS, Shim JS, Kim MC, Lee SH, Huh Y, Kim SY, Oh MS. Mulberry fruit protects dopaminergic neurons in toxin-induced Parkinson's disease models. British Journal of Nutrition. 2010; 104(1):8-16. https://doi.org/10.1017/S0007114510000218 PMid:20187987

9. Bhuiyan MI, Kim JY, Ha TJ, Kim SY, Cho KO. Anthocyanins extracted from black soybean seed coat protect primary cortical neurons against in vitro ischemia. Biological and Pharmaceutical Bulletin. 2012; 35(7):999-1008. https://doi.org/10.1248/bpb.b110628 PMid:22791144

10. Wang D, Wei X, Yan X, Jin T, Ling W. Protecatechuic acid, a metabolite of anthocyanins, inhibits monocyte adhesion and reduces atherosclerosis in apolipoprotein E-deficient mice. J Agric Food Chem. 2010; 58:12722-8. https://doi.org/10.1021/jf103427j PMid:21090717

11. Jawi IM, Suprapta DN, Dwi SU, Wiwiek I. Purple Sweet Potato reduced Mice' Blood and Liver MDA Levels after Maximum Physical Exertion. Jurnal Veteriner Jurnal Kedokteran Hewan Indonesia. 2008; 9(2):65-72.

12. Jawi IM, Wita IW, Suprapta DN. Aqueous Extract of Purple Sweet Potato Tuber Increases SOd-2 and decrease VCAM-1 Expression by increasing Nrf2 Expression in The Aortic Endothelia of Hypercholetrolemic rabbits. Journal of Biology, Agriculture and Healthcare. 2014; 4(10):76-84.

13. Woodruff TM, Thundyil J, Tang SC, Sobey CG, Taylor SM, Arumugam TV. Pathophysiology, treatment, and animal and cellular models of human ischemic stroke. Molecular neurodegeneration. 2011; 6(1):11. https://doi.org/10.1186/1750-1326-6-11 PMid:21266064 PMCid:PMC3037909

14. Jordan J, Moreno-Parrado L, Anton-Martinez D, A Jellinger K, F Galindo M. Modulation of apoptosis in acute ischemic stroke as treatment challenges. Current Immunology Reviews. 2012; 8(1):39-49. https://doi.org/10.2174/157339512798991209

15. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. Nature reviews Molecular cell biology. 2008; 9(1):47. https://doi.org/10.1038/nrm2308 PMid:18097445

16. Niizuma K, Yoshioka H, Chen H, Kim GS, Jung JE, Katsu M, Okami N, Chan PH. Mitochondrial and apoptotic neuronal death signaling pathways in cerebral ischemia. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2010; 1802(1):92-9. https://doi.org/10.1016/j.bbadis.2009.09.002 PMid:19751828 PMCid:PMC2790539

17. Vaux LD. Apoptogenic factor released from mitochondria. Biochim Biophys Acta. 2011; 1813: 546-550. https://doi.org/10.1016/j.bbamcr.2010.08.002 PMid:20713095

18. Adnyana IM, Sudewi AA, Samatra DP, Suprapta DN. A simple method to stimulate ischemic stroke in Wistar rat for animal testing. BALI MEDICAL JOURNAL. 2017; 6(1):156-60. https://doi.org/10.15562/bmj.v6i1.430

19. Jawi IM, Arijana IG, Subawa AA, Wirasuta IM. The Pharmacological Mechanisms of Anthocyanin in Aqueous Extract of Purple Sweet Potato as Antihyperglycemic Herbal Remedy. Global Journal of Medical Research. 2016.

20. Zhang H, Li Q, Li Z, Mei Y, Guo Y. The protection of Bcl-2 overexpression on rat cortical neuronal injury caused by analogous ischemia/reperfusion in vitro. Neuroscience research. 2008; 62(2):140-6. https://doi.org/10.1016/j.neures.2008.07.002 PMid:18723055

21. Suwarba IGN. Adjuvant Therapy of Purple Sweet Potato (Ipomoea batatas L.) Reduced 8-hydroxy-2 deoxyguanosine, Interleukin 6, Increased superoxide dismutase, Improved EEG, and reduced Refractory Focal Epileptic Seizures in Children. Dissertation. Doctoral Program Udayana University, Denpasar, 2016.

22. Han YT, Chen XH, Xie J, Zhan SM, Wang CB, Wang LX. Purple sweet potato pigments scavenge ROS, reduce p53 and modulate Bcl-2/Bax to inhibit irradiation-induced apoptosis in murine thymocytes. Cellular Physiology and Biochemistry. 2011; 28(5):865-72. https://doi.org/10.1159/000335801 PMid:22178939

23. Yao RQ, Qi DS, Yu HL, Liu J, Yang LH, Wu XX. Quercetin attenuates cell apoptosis in focal cerebral ischemia rat brain via activation of BDNF–TrkB–PI3K/Akt signaling pathway. Neurochemical research. 2012; 37(12):2777-86. https://doi.org/10.1007/s11064-012-0871-5 PMid:22936120

24. Hüttemann M, Pecina P, Rainbolt M, Sanderson TH, Kagan VE, Samavati L, Doan JW, Lee I. The multiple functions of cytochrome c and their regulation in life and death decisions of the mammalian cell: From respiration to apoptosis. Mitochondrion. 2011; 11(3):369-81. https://doi.org/10.1016/j.mito.2011.01.010 PMid:21296189 PMCid:PMC3075374

25. Ullah I, Park HY, Kim MO. Anthocyanins Protect against Kainic Acid-induced Excitotoxicity and Apoptosis via ROS-activated AMPK Pathway in Hippocampal Neurons. CNS neuroscience & therapeutics. 2014; 20(4):327-38. https://doi.org/10.1111/cns.12218 PMid:24393263

26. Li X, Wen W, Liu K, Zhu F, Malakhova M, Peng C, Li T, Kim HG, Ma W, Cho YY, Bode AM. Phosphorylation of caspase-7 by p21-activated protein kinase (PAK) 2 inhibits chemotherapeutic drugs-induced apoptosis of breast cancer cell lines. Journal of Biological Chemistry. 2011;jbc-M111.

27. ali Shah S, Ullah I, Lee HY, Kim MO. Anthocyanins protect against ethanol-induced neuronal apoptosis via GABAB1 receptors intracellular signaling in prenatal rat hippocampal neurons. Molecular neurobiology. 2013; 48(1):257-69. https://doi.org/10.1007/s12035-013-8458-y PMid:23645118

28. Badshah H, Kim TY, Kim MO. Protective effect of anthocyanin against amyloid beta-induced neurotoxicity in vivo and in vitro. Neurochemistry International. 2015; 80:51-59. https://doi.org/10.1016/j.neuint.2014.10.009 PMid:25451757

29. Tait SW, Green DR. Mitochondria and cell death: outer membrane permeabilization and beyond. Nature reviews Molecular cell biology. 2010; 11(9):621. https://doi.org/10.1038/nrm2952 PMid:20683470

30. Azad N, Iyer AKV. Reactive Oxygen Species and Apoptosis. In: Laher I ed. System Biology of Free Radical and Antioxidants. Berlin; Springer-Verlag Berlin Heidelberg. 2014; 113-127. https://doi.org/10.1007/978-3-642-30018-9_15

31. Shin WH, Park SJ, Kim EJ. Protective effect of anthocyanins in middle cerebral artery occlusion and reperfusion model of cerebral ischemia in rats. Life sciences. 2006; 79(2):130-7. https://doi.org/10.1016/j.lfs.2005.12.033 PMid:16442129

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Basic Science

Silencing HCV Replication in Its Reservoir

Samar Samir Youssef[1*], Moustafa Nouh Elemeery[1,2,3], Sameh Seif Eldein[4], Doaa Ahmed Ghareeb[5,6]

[1]Microbial Biotechnology Department, National Research Centre, Egypt; [2]Center for Systemic Biotechnology, Korea Institute of Science and Technology, Republic of Korea; [3]Division of Biomedical Science and Technology, Korea University of Science and Technology, Republic of Korea; [4]National Hepatology and Tropical Medicine Research Institute, Egypt; [5]Biochemistry Department, Faculty of Science, Alexandria University, Egypt; [6]Biomedical Technology, Faculty of Science, Beirut Arab University, Lebanon

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***Correspondence:** Samar Samir Youssef. Microbial Biotechnology Department, National Research Centre, Egypt. E-mail: samaryoussef67@gmail.com

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Abstract

BACKGROUND: HCV infection and its complications are among the leading public health challenges; the emergence of drug-resistant variants are expected to be a major problem. A novel combinatorial small interfering RNA (siRNA) could be a novel triple therapy that could be suitable for genotype 4. Although HCV is a hepatotropic virus, there is reliable evidence about its replication in peripheral blood mononuclear cells (PBMC) of chronically infected patients; these cells act as an extra-hepatic reservoir for viral recurrence and persistence. The patients with HCV-RNA in PBMC showed a significantly lower response to therapy that supports to be one of the factors influencing therapeutic response. Almost all regions of HCV show potential for siRNA target with relative efficiencies of individual siRNA sequences.

AIM: This study aims to test the efficacy of siRNA against HCV-4 replication in PBMC in vitro, to introduce an alternative therapeutic option for HCV-4 suitable to eradicate it from both hepatic and extra-hepatic reservoirs.

METHODS: Efficacy of synthesised siRNA molecule that targets 5′UTR of domain IIIC within IRES of HCV RNA to eradicate HCV intra-PBMC in vitro was tested and compared with IFN/RBV in vitro, by using both qRT-PCR and western blot. Sixty genotype-4 chronic HCV patients who are naïve for any HCV treatment were enrolled and tested for the presence of HCV intra-PBMC using qRT-PCR before and after siRNA treatment in vitro.

RESULTS: Real-time PCR analysis showed a significant reduction of HCV RNA levels after 24hr post-HCV-positive-PBMCs treatment by siRNA with cell vitality reached up to 98%. Besides a complete inhibition of NS5A viral protein expression, that is functionally essential for viral assembly, replication and egress.

CONCLUSION: So, Targeting HCV infection using RNA interference technology might be a reliable therapeutic option for chronic HCV patients with HCV minus strand within PBMCs.

Introduction

Hepatitis C virus (HCV) infection is a global blood-borne disease that affects almost 3% of the world population with morbidity and mortality that is second to HIV among the emerging infections [1]. Egypt has been reported as the highest in HCV prevalence worldwide, that ranged from 6% to exceed 40% among region and demographic groups with 11-14% of the population chronically infected with the virus [2]. The infection may get worse to cirrhosis with the subsequent development of complications including hepatocellular carcinoma [3], [4].

HCV is a positive-sense single-stranded RNA virus, classified as the sole member of the genus Hepacivirus in the family Flaviviridae. The HCV RNA genome is roughly 9.6 kb long with an open Reading Frame (ORF) that encode a vast viral polyprotein of around 3,01 thousand amino acids [4]. Viral translation is mediated through the internal ribosome entry site (IRES) found within the 5' untranslated region (5'UTR) which comprise of ~341 bp long that is profoundly conserved even between various HCV isolates. The 5'UTR does not encode for functional protein and contains the IRES that initiate viral polyprotein translation in a cap-independent manner [5]. The IRES is basic for translational as it independently binds to the 40S ribosomal subunit and guides the ribosome to the initiation codon of the HCV mRNA with a specific end goal to facilitate its translation in a cap-independent manner. It contains four profoundly organised stem-looped domains that

named I-IV which facilitate HCV RNA translation. Domain I is not essential for IRES activity but rather essential for HCV replication, IRES in domains II-IV play a role in viral genetic replication in a cap-independent manner while Domain III contains subdomains that are critical for the binding of 40S ribosomal subunit [6]. Translation of HCV mediated by a profoundly conserved internal ribosomal Entry site (IRES) within the 5'UTR making it a significant focus for new medication development [5].

Despite hepatotropic nature of HCV, many studies proved that PBMC represents another extrahepatic reservoir where HCV can replicate, not only that, but it may also be the real culprit in viral recurrence after liver transplantation [7]. The substantial goal of viral treatment is to attain a sustained virological response (SVR) that is defined as undetectable HCV-RNA in peripheral blood determined 24 weeks after the end of treatment (ETR). Nonresponders (NR) to therapy are sometimes obstinate to retreatment and do not necessarily benefit from escalating treatment dose [8].

Despite the great success of the current therapies, there are many reported concerns that may increase the demand for new therapy and diagnostic approach to cover it [4], for example; Human Biliverdin reductase (HBVr) reported to be upregulated in many studies upon Direct-acting antivirals (DAAs) therapy, HBVr usually elevated at mean of 52 days after first DAA dose and reported to cause ten DAA-therapy discontinuations [9]. These patients cannot make a benefit from DAAs, so alternative options are urgently necessary, out of this alternative is RNAi that represent an effective silencing approach for molecular therapeutics through a particular succession RNA degradation procedure to diminish infection or replication of its invaders [5].

siRNA is promising silencing mechanism, vector-based or synthetic siRNAs directed against 5' UTR, NS3, NS5B, NS4B and core represented significant specific ability in reducing both viral infection and replication. The 5′UTR of HCV-IRES has also been targeted by siRNA resulted in inhibition of HCV by more than 80% at a concentration of 2.5 nM. Moreover, consensus siRNAs against 5′UTR diminished viral replication level *in vitro* on infected Huh-7 cell-line [10]. While the dominant part of HCV-siRNAs is integral to the positive strand, a lessening in both strands replication has been reported [11]. It is conceivable that targeting the positive strand leads to a decline in negative strands synthesis [12].

Interferon (IFN) pathway can be initiated by dsRNAs, so it was important to address whether HCV-particular siRNAs could trigger IFN pathway. It was exhibited that hindrance of viral replication by HCV RNAi *in vitro* was not related to an up-regulation of IFN-stimulated genes. Targeting HCV by siRNAs were better at diminishing HCV-RNA levels than an oscillating dose of IFN-α [11].

All these possibilities motivate us to test the efficacy of specific synthetic-siRNA on clearance of HCV from PBMCs of chronic HCV-infected patients aiming to get a new therapeutic recommendation for inconvenient DAAs therapy patients, non-responders or relapse's patients. In addition to scavenging viral infection from PBMCs that act as a real culprit in HCV relapses after liver transplantation.

Patients and Methods

Patients and Samples collection

Total of sixty patients was enrolled in this study; all were naïve and newly diagnosed HCV patients, from National Hepatology and Tropical Medicine Research Institute (NHTMRI) Cairo, Egypt. All patients were pre-diagnosed HCV antibody positive by ELISA technique. HCV detected in serum and PBMC for all samples. Ethical committee approval was taken from National Hepatology and Tropical Medicine Research Institute in addition to a signed consent form from each patient. Patient's inclusion criteria were; first, they should be treatment naïve patients, then they should not have any other neurological disorders or undergo conservative treatment, also not to be co-infected by any hepatic viral infection than HCV that was detected by the presence of its antibodies (Ab) in serum. About 12 mL blood was withdrawn from each patient on heparin.

siRNA Choice

siRNA targeting domain IIIC within IRES was chosen that showed 100% alignment with HCV sequences in the gene bank (database), in addition to its ability to align specifically against domain IIIC within IRES. This siRNA was specially synthesised and ordered from thermo-scientific U.S.A (cat# K-005000-G1-01, E-007500 -01), to be HPLC purified and sterilised using ultra-filtration methods to assure removal of any materials that might interfere with transfection or might be lethal to cells.

Optimising siRNA Transfection Procedure

Since there is a great variation in the capacity of transfection according to each cell type, so the transfection procedure must be determined and adjusted for each cell line before transfection. Also, it is well known that PBMCs is hard to transfect cells. Therefore, the GAPDH siRNA (Thermo, USA) was used to optimise: a) optimal cell plating density; b) optimal amount of siRNA to be transfected; and c) either to transfect it in serum-free or serum-containing medium (FCS) and determine optimal serum concentration.

Self-delivery Accell™-siRNAs were synthesised by Dharmacon® that allows effective delivery in wide-ranging cell lines including primary cells. Ficoll-hypaque density gradient technique was used to isolate PBMC from the blood. PBMC was washed at least four times with PBS, re-suspended in Accell delivery media®, and counted; then 1 × 10^6 cells were used for detection of presence of HCV and 0.6 ×10^6 cells/well were plated in 48 well-plate for testing efficacy of different siRNA concentrations (50-100-200 μM) on silencing HCV replication and compare its effect with IFN/RBV in separate wells were concomitantly treated with 110 IU/ml IFN plus100 μM Ribavirin *in vitro* [13]. Total RNA was harvested at different times post-siRNA transfection using Viral RNA extraction kit (QIAamp-viral RNA mini kit) from QIAGEN according to the manufacturer's manual. Also, control for HCV-siRNA was used that was Human GAPDH siRNA in addition to positive and negative (non-targeting NTC) control for each.

Determination of HCV in Patient's Sera

Two hundred (μL) serum were used for RNA extraction by viral RNA extraction kit (QIAamp-viral RNA mini kit- QIAGEN-USA) consistent with the manufacturer's manual. 200 ng of RNA utilised to detect virus C using one-step RT-PCR kit (QIAGEN, USA) and used as stated by the manufacturer's protocol, and then the PCR amplicon was photographed on 2% agarose gel.

HCV Genotyping

Genotyping was done using the VERSANT HCV genotype 2.0 Assay (LiPA - Bayer).

Uncovering HCV Strands in Patient's PBMC

Qualitative analysis

Cellular RNA was extracted from counted uncultured PBMC, collected from HCV serum positive patients, using Viral RNA extraction kit (QIAamp-viral RNA mini kit- QIAGEN, USA) consistent with the manufacturer's manual. HCV strands were detected by RT-PCR; briefly, 200 ng of extracted RNA was reversely transcribed to cDNA in 50 μL mixture PCR beads (GE healthcare life science, USA) and 25 pmoles antisense primer 5'GGTGCACGGTCTACGAGACCTC3' for a positive strand or 5'AACTACTGTCTTCACGCAGAA3' for a negative one. 50 μL was the first round PCR volume, using 50 pmoles from each primer and 5'TGCTCATGGTGCACGGTCTA3'. Then the second round uses 20% of this volume for its amplification with the internal pair of primers 5CGCAGAAAGCGTCTAGCCAT3 in addition to 5ACTCGGCTAGCAGTCTCGCG3. The thermal cycler conditions were the same as the first round, then PCR amplicon was analysed on 2% agarose gel electrophoresis and documented.

Quantitative analysis

HCV quantitation was performed on a Piko-Real-Time PCR System (Thermo-Scientific) using sensifast SYBR kit (Bioline) according to the manufacturer procedure.

In vitro treatment of infected PBMC by siT and IFN/RBV

Patients proved to be infected with HCV in both serum and PBMCs were enrolled in this step. PBMCs were separated using ficoll, washed 4 times with PBS to avoid any attached viral particles on cell membrane, then resuspended in Accell delivery media® For each patient, cells were plated on a 48-well plate at a concentration of 0.6 × 10^6/mL, and cultured at 37˚C, 5% CO_2 for 72 hr in absence or presence different siRNA concentrations, siNTC, GAPDH siRNA and IFN/RBV separately (triplicate for each treatment). Cultured cells without any treatment were used as a control. Cells were harvested, washed 4 times with PBS, and subjected to total cellular RNA extraction. Total RNA extracted from cultured PBMCs was reverse transcribed and amplified using the same assay described previously for both qualitative and quantitative measurement [14].

Western blot analysis of HCV NS5A antigens in PBMC with and without siRNA

Cell lysates of PBMC either with or without siRNA treatment were subjected to SDS-PAGE. Then washed three times, after that membrane was incubated with diluted peroxidase-labelled anti-human IgG/IgM antibody mixture at 1:5000 in phosphate buffer saline (PBS) 3 g/L for previously treated strips with the anti-NS5A (Novocastra Laboratories, UK) for at least two hr at room temperature. Then Visualize of the immune complexes on the nitrocellulose membranes by promoting the strips with 0.01 mol/L PBS (pH 7.4) that contain 40 mg 3,3',5,5 tetramethylbenzidine and 100 μl of 30 ml/L hydrogen peroxide (Immunopure TMB substrate Kit, Rockford, USA).

Statistical Analysis

All experiments were performed in triplicates on three independent cultures. Data were shown in means ± SD. $P < 0.05$ was considered statistically significant.

Results

Clinical Aspects of HCV patients

Patients showed a significant difference (P<0.001) in liver function test ALT, AST, liver synthetic function assays; prothrombin and albumin (P<0.0001) and total leukocyte count (P=0.0002), on the other hand Age, BMI, gender, and total bilirubin showed no significant difference between normal and chronic HCV infected patients Table (1).

Table 1: Clinical data of chronic-HCV patients' vs healthy control

Variable	Chronic HCV patients (mean ± SD)	Healthy persons (mean ± SD)	P value
Age	49 ± 10	47±8	0.674 (NS)
BMI (Kg/m^2)	26.8 ± 5	26.2±5	0.7464 (NS)
Gender			
Male	22 (55%)	5 (50%)	0.5719 (NS)
Female	18 (45%)	5 (50%)	0.5687 (NS)
Platelet count (10^3/µL)	216 ± 68	265±120	0.1006 (NS)
ALT (IU/L)	68.1 ± 41.7	13±6	0.0003 (S)
AST (IU/L)	52.3 ± 32.6	12±8	0.0006 (S)
Bilirubin Total (mg/dl)	0.43 ± 0.16	0.39±0.19	0.1087 (NS)
Bilirubin Direct (mg/dl)	0.85 ± 0.37	0.29±0.16	0.086 (NS)
Prothrombin concentration %	69.9 ± 19.3	103±4.7	<0.0001 (S)
Albumin (g/dl)	3.28 ± 0.6	4.7±0.8	<0.0001 (S)
Total leukocyte count (10^3/µL)	6.08 ± 2.18	3.0±1.5	=0.0002 (S)
Serum HCV viral load			
<500,000 IU/ml	25 (62.5%)	0	
≥500,000 IU/ml	15 (37.5%)	0	
PBMC viral load			
PBMC + HCV	19 (47.5%)	0	
PBMC -HCV	21 (52.5%)	0	
Fibrosis level			
F (n: 0/1/2/3/4)	16/14/9/1	0/0/0/0	

NS= no significant difference; S = significant difference.

HCV RNA detectability in Patient's Serum and PBMC

All the 60 patients were HCV RNA positive in serum but only 19 out of them (31.7%) showed detectable HCV -RNA in PBMC using RT-PCR for HCV strands, from clinical data observations, 17(89.5%) out of these 19 PBMC positive HCV patient show non-responder state to PEG-IFN and 11(64.7%) showed non-response to sovaldi therapy at week 12.

Results of optimisation of siRNA concentration and delivery method in vitro

To assess the potentiality and efficacy of siRNA in Accell delivery media, optimisation of the various parameters carried out using GAPDH siRNA as a positive internal control. The results represented a significant silencing of GAPDH mRNA expression in transfected PBMCs when Accell delivery reagent was used ($p < 0.05$) (Figure 1). But there was no observed inhibition when lipofectamine nor electroporation was used compared to using Accell delivery with no siRNA as a negative control.

Two other transfection reagents were tested for transfection optimisation. Accell delivery media showed a significant knockdown of GAPDH compared to Lipofectamine and Electroporation; (A); $p < 0.05$. Values expressed as relative expression value (REV) with mean ± SD, n = 19.

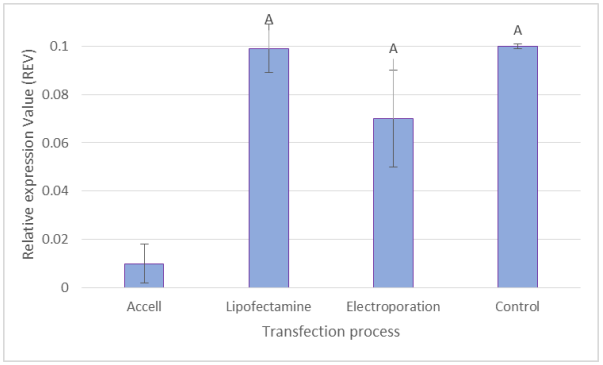

Figure 1: Effect of different delivery methods on GAPDH expression

Results of in vitro inhibition of HCV replication by siRNA in PBMC

Current study demonstrates that the introduction of siRNA targeting domain IIIC within 5'UTR IRES site into PBMC, that previously confirmed to be infected by HCV using quantitative PCR assay, caused a dramatic sharp decrease of HCV RNA by more than 94% compared to non-treated cells using qRT-PCR (Table 2), also NS5A protein expression levels detected by western blot assay was completely inhibited (Figure 3). was also noticed that the effect of siRNA on HCV replication and life-cycle occurs very early after 24 hours post siRNA transfection with variable treatment concentrations (Figure 2).

Table 2: The HCV RNA levels (%*) in HCV-infected PBMCs inhibited by siRNA for 72 h

	Doses of siRNA (nM)			
	10	50	100	200
Untreated	100 ± 11.74	100 ± 8.45	100 ± 12.47	100 ± 6.23
siGAPDH	96 ± 19.47	86 ± 9.24	80 ± 8.96	76 ± 10.04
siNTC	92 ± 8.25	92 ± 14.63	111 ± 16.28	108 ± 8.06
siT	29 ± 12.50[a]	18 ± 8.50[a]	6 ± 1.11[a,b]	8 ± 2.83[a,b]
IFN/RBV (110 IU/ml IFN plus100 µM Ribavirin)	57±11.8[a]			
RBV (100 µM Ribavirin)	8 ± 2.7[a]			

*data Presented as viral load (mean ± SD); [a] P<0.01, as compared with untreated control; [b]P<0.01, as compared with the 10nM group; [c]P<0.01, as compared with the 50nM group.

siRNA were applied to HCV-infected PBMCs cell-culture to assess the effects of siRNA on the HCV replication. Infected PBMCs that were untreated and transfected non-targeting (scrambled) siRNA (siNTC) served as negative controls while PBMCs that were untreated and transfected with GAPDH siRNA served as positive controls, Figure 3 and Table 2 illustrate viral RNA levels and protein expression for both transfected and control PBMCs, as quantitated by qPCR at 72 h after transfection. siRNA was significantly able to suppress HCV replication at 100 nM compared with untreated PBMCs (P < 0.01).

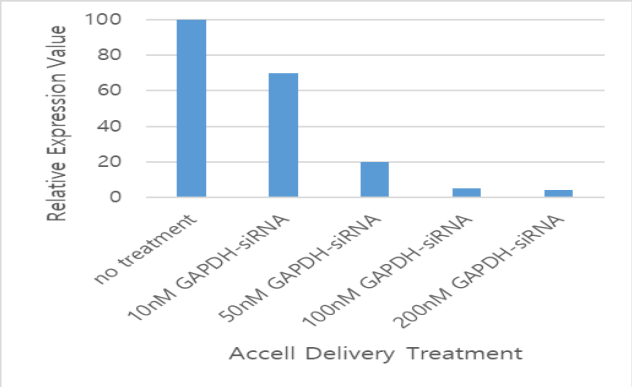

Figure 2: Concentration optimisation using GAPDH siRNA. All values were expressed as relative expression value (REV) with mean ± SD, n = 9

The result of the quantitative PCR of samples after these *in vitro* treatments shows significant viral reduction with siT compared to IFN/RBV (P = 0.001). As shown in Table 2, SiT decreased the viral load by mean ± SD 94.0 ± 1.11% while the combination therapy decreased it by mean 57 ± 11.8% and finally ribavirin alone decreases it by mean 8 ± 2.7% only that later complied with *in-vivo* response results for same patients of IFN/RBV and DAAs therapies.

NS5A levels were significantly decreased after 24 hr. of the transfection of 100 nM of SiT (Fig. 3). And maximum inhibition levels were observed on 72 hr. post transfection (mean silencing 94.0 ± 1.11%). Also, there are no significant inhibition levels- for HCV in cells transfected with either the NTC siRNA nor those with GAPDH siRNA- was detected.

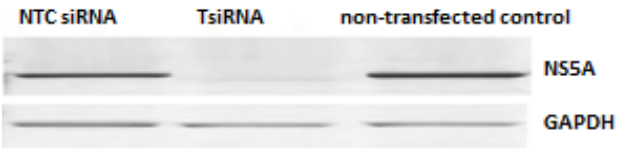

Figure 3: Composite picture for Western blot for HCV-NS5A compared to intracellular GAPDH

Upper row showed HCV-NS5A expression in different transfection for PBMC positive HCV cells after 24hr post-treatment. Lower row showed the GAPDH expression in same transfected cells.

Results of SiRNA cytotoxicity detection

To detect possible cytotoxic effects that may be induced by the siRNA, both trypan-blue exclusion method and MTT assay were performed. The cell viabilities reached up to 98% (Table 3) for both cells transfected by siRNA or for cells grown in Accell delivery media (transfection media). Thus, neither the transfection media (on another way transfection process) nor selected siRNA in the current study induced toxicity against PBMCs cells.

Table 3: Cell viability (%*) of PBMCs exposed to either siRNA for 72 h or IFN/RBV

	Doses of siRNA (nM)			
	10	50	100	200
Untreated	100 ± 4.47	100 ± 3.72	100 ± 2.46	100 ± 1.16
siGAPDH	96 ± 19.47	86 ± 9.24	80 ± 8.96	76 ± 10.04
siNTC	100 ± 9.86	100 ± 5.72	108 ± 4.28	105 ± 5.2
siT	102 ± 3.50	108 ± 6.50	116 ± 11.11	118 ±1 2.53
IFN/RBV (110 IU/ml IFN plus100 µM Ribavirin)	57±11.8[a]			
RBV (100 µM Ribavirin)	68±18.2[a]			

* data Presented as viral load (mean ± SD) siNTC= siRNA non targeting pool control.

Discussion

In the current study, 60 genotypes 4 chronic HCV infected patients were enrolled. They are naïve for HCV treatment, HCV RNA was detected in PBMCs of 19 patients out of 60 (about 31.7%), and the remainder show no HCV in its PBMCs, similarly this finding complies with our result that was published 2014 [14] that represent presence of HCV intra-PBMC in 10 out of 25 HCV infected patients that represent 40% of total study population.

Viral translation is mediated through IRES site found within the 5′UTR that consists of ~341 bp in length with highly conserved sequence even between different HCV isolates. IRES can initiate viral polyprotein translation in a cap-independent manner via independently binds to the 40S ribosomal subunit and directs the ribosome to the initiation codon of mRNA of HCV to expedite translation in a cap-independent manner and it is a vital target for the development of new antiviral compounds [15].

It is well known that viruses, particularly retroviruses and HCV, are notoriously prone to the high rate of errors during their replication process, and continuously undergoes mutation and mostly produce mutated viral proteins to escape immune-system defence mechanisms. These mutations may also escape attacking by various siRNAs [16]. The protein-coding sequence of the HCV genome that was targeted in the study by McCaffrey et al., 2002 varies considerably among different HCV genotypes, and even among strains of the same genotype [17]. Also, the high error rate of the non-proofreading HCV RNA-dependent RNA polymerase greatly showed that was called “siRNA escape mutants” which have silent mutations in the protein-coding sequence [18]. In contrast, the 5'UTR especially IRES IIIC, that was selected as a target in the current study, is almost identical among the known strains of HCV[7]. Moreover, structural fetters on the 5'UTR, regarding its ability to direct internal ribosome entry and translation of viral proteins, would not riddance escape mutations [19].

For this reason, the IRES domains within 5'UTR of the HCV genome appears to be an ideal target for siRNA in clinical applications. Not all 5'UTR-

directed siRNAs have the same efficacy; there are many siRNA were detected based on the consensus sequences of Egyptian HCV 5'UTR. In this study siT was chosen that showed 100% alignment with HCV sequences in the gene bank (database) and also can align in 5'UTR specifically against domain IIIC within IRES in silico then we validate this hypothesis in the wet lab.

In the current study, we were able to demonstrate that the introduction of SiT targeting domain IIIC within IRES in 5'UTR into PBMC that previously confirmed its infection by HCV using quantitative PCR assay, caused a dramatic sharp decrease of HCV RNA by more than 94% compared to non-treated cells, HCV RNA levels decreased 25-fold (P = 0.0005) in Huh-7 cells too after transfection with siRNA (data not shown), in addition to modulation of NS5A viral protein expression. It was noticed that the effect of siRNA on HCV replication and life-cycle occurs very early after 24 hours post siRNA transfection. Our data is in agreement with both McCaffrey et al., 2002 [17] who showed in his study that, HCV NS5B RNA polymerase gene fragment, which was transiently co-transfected with siRNA into mouse liver by hydrodynamic injection, was cleaved after treatment with specific siRNA [17]. Our data are also consistent with those of Randall et al., 2003 who demonstrated that siRNA could target and cleaves the HCV 5'UTR efficiently and specifically. More importantly, Randall and his associates showed that the cleavage of HCV-RNA not only suppressed viral protein synthesis but also blocked the replication of sub-genomic viral RNA [20] that was supported by our data from PBMCs too.

Up to our Knowledge, it's the first time for a comparative study between the effect of either ribavirin alone or with combination with IFN at recommended dose against 100 μM siRNA as optimal dose and we clearly observe the superior effect of siRNA over other treatment on PBMC *in vitro*, as the same pattern, Table 2 showed the superiority of siT that reached 94% viral clearance while the combination therapy decreased it by 57% and finally ribavirin decreased it by 8% only, the finding support siRNA efficiency on HCV silencing either in hepatic cell or extra-hepatic reservoir like PBMCs, Also it was the first time to test efficacy of siRNA to eradicate HCV replication on PBMCs that are hard to transfect cells acts as HCV reservoir.

As a safe and efficient way of delivery of siRNAs to cells that can suppress HCV replication in all infected cells in vivo have not been discovered yet, chemically specially modified synthetic siRNA might easily be made and delivered into cells on their own. In our study we compared three different ways of transfection and we conclude that Accell siRNA delivery method® is specially modified for use without a transfection reagent and works at a higher concentration than classical siRNA with minimal disruption of the expression profile and approved to transfect hard to transfect cells like PBMCs with no off-target effect (Figure 1).

Results showed that the siT that we selected, siT- (nt 59–79 from the 5'UTR beside nt 109–129 from the HCV core area), successfully inhibit HCV replication in PBMC culture without affecting housekeeping genes (Figure 3), it was clearly observed safety profile of siT on PBMCs in addition to cell vitality that exceeded 98% using trypan blue exclusion method and cytotoxicity MTT assay, thus confirming earlier reports on siRNA and suggesting the potential therapeutic value of siRNA in HCV type-4.

To confirm our result and data both Q-PCR of viral RNA, western blot for NS5A protein were performed to assess the effect of siT at 24 hr on such protein expression. NS5A may be an RNA binding protein because of its ability to bind to 3'UTR of the plus and minus HCV RNA strands with a key modulator role of host cell function and activity, especially IFN response of the host cell, despite siRNA showed superior efficacy in modulating NS5A gene expression in this study, results need more validation by comparing its efficacy to DAAs targeting the same NS5A like Declatasvir® and Elbasvir®.

Our preliminary data showed that siT efficiently suppresses HCV replication in PBMC in vitro. The result of this study overcomes our result of another study previously published in 2014; antisense SODN1 for inhibition of HCV in both PBMC and hepatoma cells because of failure of antisense to eradicate HCV within PBMCs compared to hepatoma cells [14]. Besides over-coming IFN+RBV too making it promising molecule for either non-responders or relapses' patients beside those non-suitable for current therapies.

Our data suggest that siRNAs targeting 5'UTR can induce an anti-HCV response in cell culture of PBMC harbouring the virus. Therefore, it represents a future therapy that could eradicate viral RNA from either hepatic cells or PBMCs, and consequently, it can potentially cure patients with HCV by eradicating it from its reservoir. The efficiency of siT in inhibiting HCV replication in PBMCs potentially suggests that this RNA-targeting approach might support and provide an effective therapeutic option for HCV infection especially for those hard to treated patients.

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References

1. Organization WHO. WHO fact sheet. 164-hepatitis C, 2014.

2. Lehman EM, Wilson ML. Epidemic hepatitis C virus infection in Egypt: estimates of past incidence and future morbidity and mortality. J Viral Hepat. 2009; 16(9): 650-8. https://doi.org/10.1111/j.1365-2893.2009.01115.x PMid:19413698

3. Itskowitz MS. Hepatitis C: epidemiology, diagnosis, and management. Compr Ther. 2007; 33(2): 87-93. https://doi.org/10.1007/s12019-007-8005-8 PMid:18004020

4. Elemeery MN, et al. Validation of a serum microRNA panel as biomarkers for early diagnosis of hepatocellular carcinoma post-hepatitis C infection in Egyptian patients. World J Gastroenterol. 2017; 23(21): 3864-3875. https://doi.org/10.3748/wjg.v23.i21.3864 PMid:28638226 PMCid:PMC5467072

5. Penin F, et al. Structural biology of hepatitis C virus. Hepatology. 2004; 39(1): 5-19. https://doi.org/10.1002/hep.20032 PMid:14752815

6. Khaliq S, et al. Down-regulation of IRES containing 5'UTR of HCV genotype 3a using siRNAs. Virology Journal. 2011; 8: 221-221. https://doi.org/10.1186/1743-422X-8-221 PMid:21569449 PMCid:PMC3116492

7. Shi ST, Lai MM. HCV 5′ and 3′ UTR: when translation meets replication. Hepatitis C Viruses: Genomes and Molecular Biology. 2006: 49-87.

8. Bare P. Hepatitis C virus and peripheral blood mononuclear cell reservoirs Patricia Bare. World J Hepatol. 2009; 1(1): 67-71. https://doi.org/10.4254/wjh.v1.i1.67 PMid:21160967 PMCid:PMC2999261

9. Pearlman BL, Traub N. Sustained virologic response to antiviral therapy for chronic hepatitis C virus infection: a cure and so much more. Clin Infect Dis. 2011; 52(7): 889-900. https://doi.org/10.1093/cid/cir076 PMid:21427396

10. Ansar M, et al. Inhibition of full length hepatitis C virus particles of 1a genotype through small interference RNA. Virol J. 2011; 8: 203. https://doi.org/10.1186/1743-422X-8-203 PMid:21535893 PMCid:PMC3094304

11. Ashfaq UA, et al. siRNAs: potential therapeutic agents against hepatitis C virus. Virol J. 2011; 8: 276. https://doi.org/10.1186/1743-422X-8-276 PMid:21645341 PMCid:PMC3118364

12. Berger KL, Randall G. Possibilities for RNA interference in developing hepatitis C virus therapeutics. Viruses. 2010; 2(8): 1647-65. https://doi.org/10.3390/v2081647 PMid:21994699 PMCid:PMC3185727

13. Buckwold VE, et al. Synergistic In Vitro Interactions between Alpha Interferon and Ribavirin against Bovine Viral Diarrhea Virus and Yellow Fever Virus as Surrogate Models of Hepatitis C Virus Replication. Antimicrobial Agents and Chemotherapy. 2003; 47(7): 2293-2298. https://doi.org/10.1128/AAC.47.7.2293-2298.2003 PMid:12821481 PMCid:PMC161860

14. Youssef SS, et al. In vitro inhibition of hepatitis C virus by antisense oligonucleotides in PBMC compared to hepatoma cells. Biomed Res Int. 2014; 2014: 196712. https://doi.org/10.1155/2014/196712 PMid:24991538 PMCid:PMC4058683

15. Khaliq S, et al. Down-regulation of IRES containing 5'UTR of HCV genotype 3a using siRNAs. Virol J. 2011; 8: 221. https://doi.org/10.1186/1743-422X-8-221 PMid:21569449 PMCid:PMC3116492

16. Carmichael GG. Medicine: silencing viruses with RNA. Nature. 2002; 418(6896): 379-80. https://doi.org/10.1038/418379a PMid:12140542

17. McCaffrey AP, et al. RNA interference in adult mice. Nature. 2002; 418(6893): 38-9. https://doi.org/10.1038/418038a PMid:12097900

18. Zekri ARN, et al. Consensus siRNA for inhibition of HCV genotype-4 replication. Virology Journal. 2009; 6: 13-13. https://doi.org/10.1186/1743-422X-6-13 PMid:19173711 PMCid:PMC2661880

19. Yokota T, et al. Inhibition of intracellular hepatitis C virus replication by synthetic and vector-derived small interfering RNAs. EMBO Rep. 2003; 4(6): 602-8. https://doi.org/10.1038/sj.embor.embor840 PMid:12740604 PMCid:PMC1319196

20. Randall G, Grakoui A, Rice CM. Clearance of replicating hepatitis C virus replicon RNAs in cell culture by small interfering RNAs. Proc Natl Acad Sci U S A. 2003; 100(1): 235-40. https://doi.org/10.1073/pnas.0235524100 PMid:12518066 PMCid:PMC140937

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Impact of Transphyseal Elastic Nailing On the Histostructure of the Tibia in Growing Animals (Non-Randomized Controlled Experimental Study)

Dmitrii A. Popkov, Tamara A. Silanteva*, Elena N. Gorbach, Eduard R. Mingazov, Natalia A. Kononovich, Arnold V. Popkov

Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics, 6, M. Ulyanova Street, Kurgan, Russia

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***Correspondence:** Tamara A. Silanteva. Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopaedics, 6, M. Ulyanova street , Kurgan, Russia. E-mail: tsyl@mail.ru

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Abstract

BACKGROUND: The use of intramedullary elastic nailing is a method of choice for prevention of complications in children with osteogenesis imperfecta. However, the morphology of the growing long bones in the conditions created was not investigated.

AIM: The purpose of our experiment was to study the impact of elastic intramedullary nailing on the histostructure of long bones in their physiological growth.

METHODS: Six mongrel dogs underwent intramedullary elastic transphyseal nailing of the intact tibia with two titanium wires. Six months after nailing, a light-optical microscopic and histomorphometric study of the operated and contralateral tibiae was performed.

RESULTS: It was found that asymmetric lesion of the distal physis induces a decrease in the height of the distal epimetaphysis. Adaptive changes in the hyaline cartilage of both articular ends were revealed corresponding to the initial stage of chondropathy. Intramedullary nailing promotes an increase in the thickness of the compact bone and the volume of the trabecular bone.

CONCLUSIONS: Elastic transphyseal nailing of the intact tibia has a shaping effect which is expressed by an increase in the volume of spongy and compact bone, adaptive changes in the hyaline cartilage. Asymmetric damage to growth zones should be avoided to prevent deformities.

Introduction

Osteogenesis imperfecta (OI) is a systemic bone disease that is accompanied by bone fragility that results in fractures and deformities [1] [2]. In 1987, J.P. Metaizeau proposed the use of counter directed intramedullary transphyseal elastic nailing to prevent deformities and treat fractures of the tubular bones. Currently, the use of transphyseal intramedullary nailing is a method of choice for prevention and treatment of complications in OI children [1] [2] [3] [4] [5] [6]. The advantage of elastic rods is the possibility to use them in a small bone canal, which is characteristic for OI children [3] [7]. Specific features of the method include possible elongation of the intramedullary fixator to stabilise bone fragments and to prevent bone deformities in the period of segment growth [3] [6] [7] [8] [9].

Earlier, we performed an X-ray diffraction study of the effect of the central retrograde and eccentric antegrade transphyseal placement of intramedullary elastic rods on the growth of the tibia without compromising its integrity. It was found that the segment lost the length and there was a change in the plane of distal articular surface inclination [10]. However, the morphology of the tibia in the conditions created by this previous study was not investigated.

The purpose of the present research was to study the shaping effect of bipolar transphyseal elastic intramedullary nailing on the histological structure of an intact long bone that continued physiological growth.

Material and Methods

This non-randomized controlled study was performed on six littermate healthy dogs of both sexes in the period of their active growth in 2014-2017 by the experimental department of the Russian Ilizarov Scientific Center for Restorative Traumatology and Orthopedics. The age of the animals at the time of surgery was six months and the weight range from 10 to 12 kg. The choice of the species was due to the similarity of the bone reparative process in dogs and humans in orthopaedic situations [11]. Approval was obtained from the institutional ethics committee before the experiment. All manipulations were carried out by "On the Approval of the Rules of Laboratory Practice", those of the "European Convention for the Protection of Animals used for Experimental and other Scientific Purposes" and approved by national ethics regulations.

Counter bipolar transphyseal elastic intramedullary nailing of the intact tibia on the right side was produced in dogs in the conditions of the operating room under intravenous anaesthesia with 20-25 mg of thiopental sodium per 1,000 g of body weight after premedication (0.1% atropine sulfate 0.1 ml, 1% dimedrol 1 ml, 2% xyiazinum 0.2 ml intramuscularly).

Two pre-curved titanium wires with a diameter of 1.8 mm and a length of 10 cm were used. The bending radius of the wires was 40°. One antegrade wire was inserted through the proximal paratendinous approach and the centre of the proximal tibial epiphysis. The second wire was introduced in the retrograde direction eccentrically at the level of the medial malleolus. The free ends of the wires were bent U-shaped and impacted into the epiphyses to prevent migration of the nails.

The experimental continued 180 days after the surgical intervention. At the time of its completion, the biological age of the animals corresponded to 12 months. Left tibia served as a control segment in all the animals.

The proximal and distal epiphyses, as well as the tibial diaphysis, were separated and fixed in 10% neutral formalin. Bone units were decalcified in a Richmann-Gelfand-Hill mixture, dehydrated in ethanol of increasing concentrations, and poured with celloidin. Histotopographic sections were cut with a sledge microtome (Reichard, Germany); sections 10-15 μm thick were stained with hematoxylin and eosin, Van Gieson and Masson methods.

Light-optical microscopic and histomorphometric study of epiphyseal preparations was carried out using AxioScope.A1 stereomicroscope and AxioCam ICc 5 digital camera supplied with Zen blue software (Carl Zeiss MicroImaging GmbH, Germany). The results of the microscopic investigation of the articular surfaces were objectified using the ICRS score system to assess the histological structure of the articular cartilage after mechanical or osteoarthritic damage [12].

Histomorphometric evaluation included the total thickness (h_{tot}, μm), height of non-calcified (h_{ncalc}, μm) and calcified (h_{calc}, μm) zones, cell density of chondrocytes in the articular cartilage (NA_{chc}, mm^{-2}); height of the epiphyseal parts of the tibia (h_{ep}, μm); thickness of the diaphysis compact plate (H_{cp}, μm); thickness of trabecular bone coupling around of the intramedullary wires (mm); trabecular bone area S_{tb} ($μm^2$) and its volume (S_{tb},%) in the bone marrow canal.

Statistical analysis was performed using StatSoft Statistica v6.0. The mean (M) and standard deviation of the mean (SD) were calculated. The Student's test was used to compare the differences between the two independent groups. A probability value of $P < 0.05$ was considered statistically significant.

Results

After 180 days of the experiment, the proximal epiphysis of the tibia of the experimental and control extremities had an anatomically correct orientation and a typical histological structure (Figure 1 a-f) as in dogs of the similar age [13].

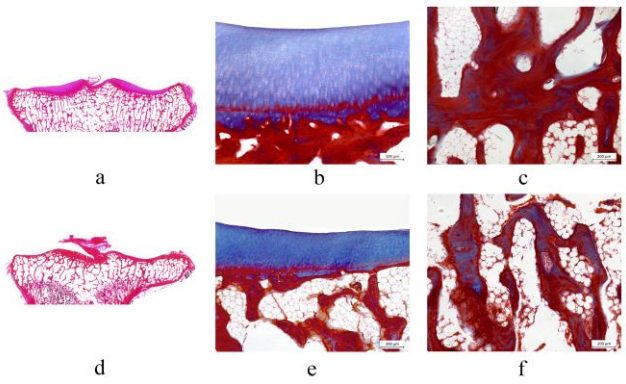

Figure 1: Histostructural organisation of the proximal epiphysis of the contralateral (a) and operated (d) canine tibiae of the dog after 6 months of transphyseal elastic intramedullary nailing. Changes in histoarchitectonics in the articular surface (e) and spongy bone substance (f) of the operated bone were noted in comparison with the corresponding sites (b, c) of the contralateral limb. Celloidine sections. Staining with hematoxylin and eosin (a, d), magnification × 6.5. Masson staining (b, c, e, f), magnification × 100

The articular surface of the tibial plateau in the control segment was covered with typical hyaline cartilage (Figure 1b). The ICRS score was I-VI: 3, which corresponded to the intact cartilage condition (Table 1).

Table 1: Semiquantitative histological evaluation of the articular surface of the tibial epiphyses of the control (C) and experimental (E) segments according to the ICRS scale [12]

Parameter and its histological evaluation (score)	Articular surface			
	Proximal epiphysis		Distal epiphysis	
	C	E	C	E
I. Surface Smooth/continuous (3) Discontinuities/irregularities (0)	3	0.3	3	0.3
II. Matrix Hyaline (3) Mixture: hyaline.fibrocartilage (2) Fibrocartilage (1) Fibrous tissue (0)	3	3	3	3
III. Cell distribution Columnar (3) Mixed.columnar-clusters (2) Clusters (1) Individual cells.disorganized (0)	3	2.3	3	1.3
IV. Cell population viability Predominantly viable (3) Partially viable (1) <10% viable (0)	3	3	3	3
V. Subchondral bone Normal (3) Increased remodeling (2) Bone necrosis.granulation tissue (1) Detached.fracture.callus at base(0)	3	3	3	2.3
VI. Cartilage mineralization (calcified cartilage) Normal (3) Abnormal.inappropriate location(0)	3	0.3	3	0.3

In the experimental segment, the total thickness of the hyaline cartilage of the articular surface was reduced, but the tidemark and the cement line retained a similar contour and continuity (Figure 1d). In the superficial zone, there was single surface undulation, but its smooth structure was retained. In the subchondral bone, there were alternating dense and rarefied areas; however, active restructuring or necrosis was not detected. The ICRS scores were I-VI: 3, very rarely-I:0.II: 3.III: 2.IV: 3.V:3.VI: 0 (Table 1).

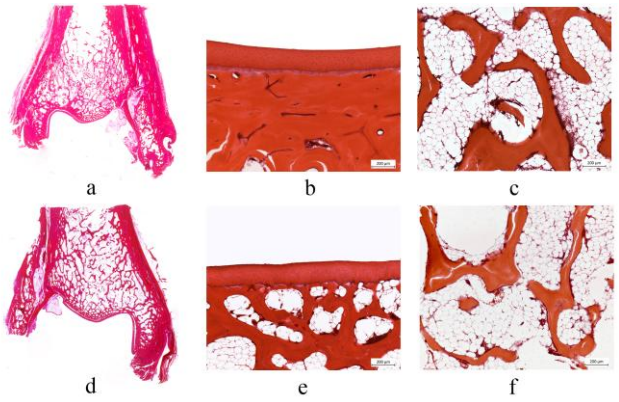

Figure 2: Histostructural organisation of the distal epiphysis of the control (a) and experimental (d) tibia of the dog after 6 months of transphyseal elastic intramedullary nailing. Changes in the histoarchitectonics of the articular surface (e) and spongy bone substance (f) of the operated bone were noted in comparison with the corresponding contralateral sites (b, c). Celloidin sections. Staining with hematoxylin and eosin, magnification × 6.5 (a, d); magnification × 100 (b, c, e, f)

The distal epiphysis of the control and experimental segments also had a typical histological structure (Figure 2 a-f). The control segment featured a smooth surface of the hyaline articular cartilage, its zonal cell organisation, homogeneity of the extracellular matrix, continuity of the basophilic line and the osteochondral junction. Numerous areas of the articular surface had signs of chondropathy in the experimental segment: strengthening of the fibrous matrix component in the upper third of the hyaline cartilage, the formation of rounded cell clusters in the zone of the columns, irregularity of the basophilic division lines and the osteochondral junction. On the ICRS scale, the score in the control segment was I-VI: 3, in the experimental one it was I-VI: 3 or II: 0. II: 3. III: 1. IV: 3. V: 2. VI: 0 (Table 1).

The tendency to decreased thickness of the zones and the overall height of the hyaline cartilage of the articular surface of the tibial epiphyses was statistically confirmed in its proximal part. The decrease in the thickness of the calcified zone was most pronounced, amounting to 78% and 25% for the proximal and distal epiphyses, respectively. The increase in the numeric density of chondrocytes in the articular cartilage of the proximal and distal epiphyses was 72% and 18%, respectively (Table 2).

Table 2: Histomorphometric evaluation of the parameters of the hyaline articular cartilage of the tibia of the control (C) and experimental (E) segments (M ± SD, μm)

Parameter	Proximal epiphysis		Distal epiphysis	
	C	E	C	E
h_{tot}	311±32.8	227±14.2[1]	223±39.1	216.8±42.1
h_{ncalc}	275±44.7	209±12.8[1]	198±32.6	185.9±31.9[1]
h_{calc}	87±40.6	19.3±9.6[1]	43.5±11.2	32.2±11.0[1]
NA_{xu}	2586±833.9	4439±709.2[1]	2739±422.8	3258±318.9[1]

[1]–statistical difference with control side, $p < 0.05$.

In the region of growth zone closure in the proximal tibial epimetaphysis of the control segment, a network of massive lamellar trabeculae with red or yellow bone marrow in the space between them was identified (Figure 1c). The height of the epiphyseal part at the level of the medial and lateral condyles of the articular surface did not differ significantly (Table 3).

Table 3: Histomorphometric evaluation of the height of the medial (M) and lateral (L) part of the tibial epiphyses, (M ± SD, μm)

Control segment				
Parameter	Proximal epiphysis		Distal epiphysis	
	M	L	M	L
h_{ep}	476 ± 8.1	473 ± 11.3	262 ± 8.92[1]	274 ± 10.7
Experimental segment				
Parameter	Proximal epiphysis		Distal epiphysis	
	M	L	M	L
h_{ep}	477 ± 14.0	460 ± 14.9[1,2]	190 ± 10.7[1,2]	271 ± 11.9

[1]–differences with the opposite part of the epiphysis are significant at $p < 0.05$; [2]–differences with the same parameter of the control segment are significant at $p < 0.05$.

In the experimental segment, a similar region was formed by a discontinuous network of lamellar and reticulofibrous bone trabeculae, including small cartilage foci (Figure 1f), while red or yellow bone marrow was slightly oedematous. The objective decrease in the height of the epiphysis at the level of the medial condyle of the articular surface was about 3% (Table 3). The growth plate of the distal epimetaphysis of the tibia in the control segment was replaced by a developed thick network of massive

lamellar trabeculae containing red or yellow bone marrow (Figure 2c). The height of the medial part of the epiphysis was reduced by 3% in comparison with its lateral part (Table 3). In the experimental segment, the spongy bone replacing the growth plate included atrophic trabeculae and red-to-yellow bone marrow and blood stagnation in the microcirculatory vessels (Figure 2f). The height of the distal epiphyseal part of the bone on the lateral side did not differ from the same value in the control segment but was reduced by 27% on the medial side (Table 3).

Significant differences in the structure of the experimental and control limbs were found on the anatomic and histological preparations of the tibial diaphysis. In the experimental limb, the tibial bone marrow canal was filled in with a trabecular bone substance forming a bone coupling around the wire which was 0.9-1.5 mm thick and closely adhered to the surface of the intramedullary implants. If the titanium implant contacted with the endosteal surface of the compact plate, its integration into the bone matrix was noted. The volume of the trabecular bone in the medullary canal of the experimental tibia was 6.7 ± 0.31 %, which significantly exceeded the value in the control limb equal to 1.9 ± 0.08 %. It meant a 2.8-fold increase in its absolute area. The thickness of the compact plate in the experimental diaphysis increased significantly, by 7.6 % as compared to the control tibia (Table 4).

Table 4: Morphometric parameters of the tibial diaphysis of the control (C) and experimental (E) segments after 180 days (M ± SD)

Thickness of compact plate. H_{cp} (μm)		The trabecular bone area in the bone marrow canal. S_{tb} (μm^2)	
C	E	C	E
2341 ± 8.42	2515 ± 9.11[1]	1669755 ± 8202	473512 ± 30.11[1]

[1]– the Significant difference with control segment, $p < 0.05$.

Discussion

Transphyseal insertion of implants implies some degree of damage to the articular cartilage and the bone growth zone. Previously, we reported that transphyseal intramedullary implants injure less than 6% of the physics area for 25 weeks. They did not lead to irreversible epiphysiodesis but caused a delay in bone growth with a loss of 15 to 18% of its length. We also found that the eccentric introduction of intramedullary nails through the medial malleolus resulted in ankle joint varus by the time of limb growth completion [10]. Radiographic study results correlated with histologically confirmed abnormalities of growth plates closure and a decreased rate of spongy bone formation in the distal tibial epiphysis on the side of the intervention. The findings obtained were confirmed by the studies of other authors who established the fact of angular deviation in the eccentric damage to the growth zone.

However, the central transphyseal introduction of elastic rods does not result in angulation [14] [15]. Seil R. et al. did not reveal any angular deviation during the subsequent growth of the segment in lambs after drilling the central canal [16]. Similar results were obtained in our study with the antegrade intramedullary insertion of the nails through the centre of the proximal tibial epiphysis. Our study also showed that elastic rods that passed outside the support areas of the articular surface and remained for 6 months in situ could cause initial signs of a dystrophic process in the hyaline cartilage of the articular surface. The decrease in the height of the articular cartilage without marginal osteophytes in the absence of fissuring and cracking of the cartilaginous matrix that was revealed using ultrasound [17] indicates the development of osteoarthritis grade I according to Outerbridge classification [18]. A similar to our data decrease in the height of the hyaline cartilage of the tibial plateau was observed in adolescents with arthropathies of non-inflammatory genesis. It is also one of the initial signs of osteoarthritis development in adults [19]. Reduction in the total height of the joint hyaline cartilage, and of the calcified zone thickness in the proximal femoral epimetaphysis in particular, was observed in the treatment of diaphyseal fractures in the conditions of locked intramedullary osteosynthesis [20]. Thinning of the calcified zone of the cartilage of the articular surfaces was also noted during its age involution [21].

The formation of both periosteal and endosteal bone callus during the healing of experimental fractures under the conditions of intramedullary elastic stress reinforcement was shown by us earlier [22]. Gradual traction of intramedullary elastic rods promotes the formation of the surrounding bone coupling, which was noted by limb bone elongation in experimental and clinical conditions [23].

In our experiment, traction of the implants was carried out due to spontaneous growth of the limb segment and was accompanied by thickening of the compact plate of the diaphysis and the formation of an endosteal formed spongy bone substance that was seen as a tight coupling around the intramedullary wires. Such an increase in bone mass along with augmentation in the mechanical strength of the bone due to its reinforcement with intramedullary wires could be useful for prevention of deformities and fractures and could be assisted with medication therapy in metabolic osteopathy [24].

Thus, bipolar transphyseal elastic intramedullary reinforcement has a form-building effect on the cost structure of the articular cartilage and subchondral bone of the epimetaphyses, as well as the on the diaphysis of the intact tibia in growing animals. Location of the rods (central or eccentric) affects the growth zone and the structural organisation of the articular cartilage by inducing initial changes of a dystrophic nature. These changes are less pronounced with the central positioning of the wires,

when the angular orientation of the epiphysis remains preserved, in comparison with their eccentric position inducing the formation of angular deformity.

The changes are adaptive, since quantitative rather than qualitative differences were observed in the histological structure of the control and experimental segments. Nevertheless, they might cause the development of osteoarthritis and deformity of the limb. Therefore, preventive measures are required. Transphyseal insertion of wires increases the opposition growth of the compact plate and stimulates the formation of additional trabecular bone in the medullary canal. The complex of intramedullary structures (wire + bone coupling round it) promotes an increase in the strength of the bone, which is important for the treatment of pediatric patients with systemic skeletal diseases.

We acknowledge the limitation of our study since the bone strength of the canine tibia was not affected as happens in osteogenesis imperfecta. Therefore, direct extrapolation of the results for osteogenesis imperfecta would need clinical trials.

References

1. Panzica M, Garapati R, Zelle B et al. Combination of femoral fracture treatment and corrective osteotomy in a child with osteogenesis imperfecta. Arch Orthop Trauma Surg. 2004; 124(5):341-5. https://doi.org/10.1007/s00402-004-0644-y PMid:15034724

2. Rosemberg DL, Goiano EO, Akkari M, Santili C. Effects of a telescopic intramedullary rod for treating patients with osteogenesis imperfecta of the femur. J Child Orthop. 2018; 12(1):97-103. https://doi.org/10.1302/1863-2548.12.170009 PMid:29456761 PMCid:PMC5813132

3. Boutaud B, Laville JM. L'embrochage centro-médullaire coulissant dans l'ostéogenèse imparfaite: Quatorze cas avec un recul moyen de 8 ans. Rev Chir Orthop. 2004; 90(4):304-311. https://doi.org/10.1016/S0035-1040(04)70125-7

4. Esposito P, Plotkin H. Surgical treatment of osteogenesis imperfecta: current concepts. Curr Opin Pediatr. 2008; 20(1):52-7. https://doi.org/10.1097/MOP.0b013e3282f35f03 PMid:18197039

5. Laron D, Pandya NK. Advances in the orthopedic management of osteogenesis imperfecta. Orthop Clin North Am. 2013; 44(4):565-73. https://doi.org/10.1016/j.ocl.2013.06.010 PMid:24095072

6. Ruck J, Dahan-Oliel N, Montpetit K, Rauch F. Fassier F. Fassier-Duval femoral rodding in children with osteogenesis imperfecta receiving bisphosphonates: functional outcomes at one year. J Child Orthop. 2011; 5(3):217-24. https://doi.org/10.1007/s11832-011-0341-7 PMid:22654983 PMCid:PMC3100465

7. Lascombes P. Flexible intramedullary nailing in Children. Springer-Verlag Berlin: Heidelberg, 2010. https://doi.org/10.1007/978-3-642-03031-4

8. El-Adl G, Khalil MA, Enan A, Mostafa MF, El-Lakkany MR. Telescoping versus non-telescoping rods in the treatment of osteogenesis imperfecta. Acta Orthop Belg. 2009; 75(2):200-8. PMid:19492559

9. Monti E., Mottes M, Fraschini P et al. Current and emerging treatments for the management of osteogenesis imperfecta. Ther Clin Risk Manag. 2010; 6:367-81.

10. Popkov DA, Kononovich NA, Shutov RB, Barbier D. Intramedullary Elastic Transphyseal Tibial Osteosynthesis and Its Effect on Segmental Growth. Vestn Ross Akad Med Nauk. 2015; 70 (4):441-49.

11. Pearce AI, Richards RG, Milz S, Schneider E, Pearce SG. Animal models for implant biomaterial research in bone: a review. Eur Cell Mater. 2007; 13:1-10. https://doi.org/10.22203/eCM.v013a01 PMid:17334975

12. Mainil-Varlet P, Aigner T, Brittberg M, Bullough P, Hollander A, Hunziker E, Kandel R, Nehrer S, Pritzker K, Roberts S, Stauffer E. Histological assessment of cartilage repair: a report by the Histology Endpoint Committee of the International Cartilage Repair Society (ICRS). JBJS. 2003; 85:45-57. https://doi.org/10.2106/00004623-200300002-00007

13. Von Pfeil DJ, DeCamp CE. The epiphyseal plate: physiology, anatomy, and trauma. Compend Contin Educ Vet. 2009; 31(8):E1-11.

14. Mäkelä EA, Vainionpää S, Vihtonen K, Mero M, Rokkanen P. The effect of trauma to the lower femoral epiphyseal plate. An experimental study in rabbits. J Bone Joint Surg Br. 1988; 70(2):187-91. https://doi.org/10.1302/0301-620X.70B2.3346285 PMid:3346285

15. Guzzanti V, Falciglia F., Gigante A., Fabbriciani C. The effect of intra-articular ACL reconstruction on the growth plates of rabbits. J Bone Joint Surg Br. 1994; 76(6):960-3. https://doi.org/10.1302/0301-620X.76B6.7983128 PMid:7983128

16. Seil R, Pape D, Kohn D. The risk of growth changes during transphyseal drilling in sheep with open physes. Arthroscopy. 2008; 24(7):824-33. https://doi.org/10.1016/j.arthro.2008.02.007 PMid:18589272

17. Kurzanceva OM, Murashkovskij AL, Trofimov AF, Fedorov VI. Differential diagnosis of deforming osteoarthritis and rheumatoid arthritis in knee joint injury using ultrasound. Sono Ace International. 2005; 13:78-81.

18. Lasmar NP, Lasmar RC, Vieira RB, de Oliveira JR, Scarpa AC. Assessment of the reproducibility of the Outerbridge and FSA classifications for chondral lesions of the knee. Rev Bras Ortop. 2015; 46(3):266-69. https://doi.org/10.1590/S0102-36162011000300006 PMid:27047818 PMCid:PMC4799221

19. Shevchenko NS. Ultrasonic parameters of the knee joint structure in adolescence. Orthopaedics, Traumatology and Prosthetics. 2012; 4:73-8. https://doi.org/10.15674/0030-59872012473-78

20. Silant'eva TA, Emanov AA. Histological findings of the proxyimal femoral epiphysis by repair of a diaphyseal fracture in the conditions of transosseous and locked intramedullary osteosynthesis. International Journal of Applied and Fundamental Research = Mezhdunarodnyj zhurnal prikladnyh i fundamental'nyh issledovanij. 2014; 11(4):675-9.

21. Lane LB, Bullough PG. Age-related changes in the thickness of the calcified zone and the number of tidemarks in adult human articular cartilage. J Bone Joint Surg Br. 1980; 62(3):372-5. https://doi.org/10.1302/0301-620X.62B3.7410471 PMid:7410471

22. Popkov AV, Kononovich NA, Gorbach EN, Tverdokhlebov SI, Irianov YM, Popkov DA. Bone healing by using Ilizarov external fixation combined with flexible intramedullary nailing versus Ilizarov external fixation alone in the repair of tibial shaft fractures: experimental study. The Scientific World Journal. 2014; 2014.

23. Jager T, Popkov D, Lascombes P, Popkov A, Journeau P. Elastic intramedullary nailing as a complement to Ilizarov's method for forearm lengthening: a comparative pediatric prospective study. Orthop Traumatol Surg Res. 2012; 98(4):376-82. https://doi.org/10.1016/j.otsr.2012.01.007 PMid:22560591

24. Kokavec M, Novorolský K, Pribilincová Z. Role of an interdisciplinary approach in the healing of long bone fractures in patients with osteogenesis imperfecta. Acta Chir Orthop Traumatol Cech. 2008; 75(3):185-9. PMid:18601816

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Evaluating *HER2* Gene Amplification Using Chromogenic In Situ Hybridization (CISH) Method In Comparison To Immunohistochemistry Method in Breast Carcinoma

Hadi Atabati[1], Amir Raoofi[2], Abdollah Amini[3*], Reza Masteri Farahani[3]

[1]Leishmaniasis Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran; [2]Student Research Committee, Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [3]Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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***Correspondence:** Abdollah Amini, Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Tel:00989126898452. E-mail: d.amini2008@yahoo.com

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Abstract

BACKGROUND: In patients with breast cancer, *HER2* gene expression is of a great importance in reacting to Herceptin treatment. To evaluate this event, immunohistochemistry (IHC) has been done routinely on the basis of scoring it and so the patients were divided into 4 groups. Lately, as there have been disagreements about how to treat score 2 patients, chromogenic in situ hybridization (CISH) and florescence in situ hybridization (FISH) are introduced. Since CISH method is more convenient than FISH for gene amplification study, FISH has been substituted by CISH.

AIM: The current study is conducted in order to investigate whether using CISH is a better method comparison to IHC method for determines HER2 expression in patients with breast cancer in.

METHODS: In this cross-sectional descriptive analytical study, information of 44 female patients with invasive ductal breast cancer were gathered from Imam Reza and Omid Hospital in Mashhad. IHC staining was done for all patients in order to determine the level of HER2 expression, and after scoring them into 4 groups of 0, +1, +2 and +3, CISH staining was carried out for all 4 groups. At the end, results from both methods were statistically evaluated using SPSS software V.22.0.

RESULTS: The average age of patients was 50.2 with the standard deviation of 10.96. Using IHC method was observed that 2.6% (1 patient), 26.3% (10 patients), 65.8% (25 patients) and 5.3% (2 patients) percentage of patients had scores of 0, +1, +2 and +3. On the other hand, CISH method showed 36 patients (90%) with no amplifications and 4 (10%) with sever amplifications. In a comparative study using Fisher's exact test (p = 0.000), we found a significant relation between IHC method and CISH method indicating that all patients showing severe amplifications in CISH method, owned scores of +2 and +3 in IHC method.

CONCLUSION: According to the present study and comparing the results with similar previous studies, it can be concluded that CISH method works highly effective in determining HER2 expression level in patients with breast cancer. This method is also able to determine the status of patients with score +2 in IHC for their treatment with herceptin.

Introduction

Breast cancer is the second death-causing cancer amongst women worldwide and is also the main death cause of women between 20 and 40 [1]. Breast cancer is the most prevalent malignancy in Middle Eastern women [2]. Previous review studies on breast cancer conducted in Iran, haves also shown an occurrence of about 17.2-22 cases in 100,000 individuals [3]. About 72% of patients in Iran had a tumor bigger than 2 centimeters at a time of diagnosis diagnosed [3].

In order to treat breast cancer patients who need to systemic or complementary treatment, after local treatment, there are three remedial processes including hormonal therapy, chemotherapy and human monoclonal antibody therapy like anti

Herceptin. Monoclonal antibody therapy which in this particular cases the human monoclonal antibody would be anti-herceptin. Choosing between different treatment processes depends on how the patient reacts to the hormonal therapy and their level of HER2 expression [4]. HER2 is an oncogene coding a 185-kDa transmembrane glycoprotein expressing intracellular tyrosine kinase activities. HER2 receptor belongs to epidermal growth factor receptors (EGFR) which effects on activation of cascades cellular mechanisms leading to growth and development [5, 6]. Increased gene expression of HER2 is observed in 18 to 20 percent of breast cancer cases [5] [7] [8]. Patients with high levels of HER2 expression respond better to medications like Herceptin. Also, the level of HER2 can expose patient's sensitivity or resistance to chemotropic drugs [9]. Furthermore, recent studies have shown that patients with HER2 expression are resistant to hormonal therapies [10].

Determining HER2 level is a necessity for choosing the best treatment for breast cancer patients and is done through three methods: IHC, FISH, CISH. In IHC method, patients are categorized into 4 scores, which due to mismatched results between different labs and therapy of choice disagreements, it is necessary to recheck HER2 level with either CISH or FISH. Since FISH method also has some drawbacks such as limitations on keeping results, high costs and the need of a florescence microscope, CISH is preferred as it offers a much higher accuracy and sensitivity level [7] [11] [12] [13] [14] [15].

In keeping with previous studies in this area, we decided to compare the evaluating competence of CISH and IHC methods in determining HER2 level in breast cancer patients.

Material and Methods

Female patients with invasive ductal breast cancer whose tissue samples and paraffin blocks were kept in Imam Reza (pbuh) and Omid hospital at Mashhad were included in this sectional descriptive analytical study. Cases with not enough tissue samples for IHC or CISH were excluded from the study, as well as those with in situ carcinoma or other form of breast cancer.

IHC staining was done for all the patients to determine HER2 expression level. New sections were prepared from each block to be stained. Blocks were deparaffinized at first and then was added Citrate buffer in 94°C for 30 min. After that, one overnight incubation with monoclonal antibody (HER2-pY 1248, Dako Denmark A/S) was done at 37°C. Diaminobenzidine was used as chromogen in a standard process of Avidin-Biotin- peroxidase staining in IHC method. After slides preparation, each of them was stained individually to determine the HER2 level.

After that, HER2 staining, results were reported in 4 different groups of 0, +1, +2 and +3. Score 0 (negative result is assigned to those cases showing no detectable stained cells or stained cells are observed in less than 10% of tumor cells). Score +1 is assigned to colorfulness with less than 10% weakly stained tumor cells membrane, score +2 includes cases with weakly to partial stained in more than 10% of tumor cells membrane, and +3 is for cases with more than 30% complete and strongly stained tumor cells membrane (Figure 1) [16].

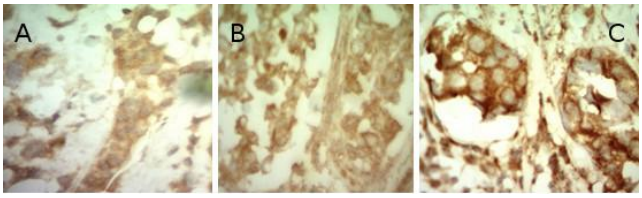

Figure 1: Microscopic pictures of immunohistochemistry samples in order to investigate HER2 gene in breast cancer; a) Score 0-1; b) Score +2; c) Score +3

After scoring the samples using IHC (according to the level of membrane staining intensity and the percentage of stained cells) samples were divided into 4 groups of 0, +1, +2 and +3 and then according to the provided instruction for ZytoDot Kit (ZytoDot 2C, Zytovision, Germany) staining was conducted for all samples. This technique was carried out on 4-5 µm sections of paraffinized tissue blocks.

In site hybridization technique (ISH) refers to an operation which a single-stranded DNA or RNA attach as a probe with a portion of the single-stranded DNA of the cell as a target. In-situ hybridization can be done in a liquid medium or in a solid medium. And the labeled material in probes can be chromogenic enzymes or fluorescent compounds. If hybridization is performed locally in the sample cells, hybridization is called in situ or Hybridization (ISH). So, the CISH method is a hybridization technique in the main site (ISH). In CISH, the generated signal is a kind of color that appears under the Bright field light microscope.

Generally, tissues 4–5 µm thick were mounted on Histogrip-treated microscope slides then dried at 37°C, and baked for 2–4 hours at 60°C. The slides were deparaffinized for 15 min three times in xylene at room temperature (22–27°C) and washed for 2 min three times in 100% ethanol.

These slides were microwaved in SPOT-Light Tissue Heat Pretreatment Buffer for 10 min at 92°C and cleaned for 3 min three times in phosphate-buffered saline (PBS). They were supported with 100 µl SPOT-Light Tissue Pretreatment Enzyme for 10 min at 37°C and washed for 3 min three times in PBS at room temperature. Then, the slides were dehydrated in 70%, 85%, 95%, and 100% ethanol for 2 min, after that air-dried. Probe (15 µl) had been

added to each sample and followed up with a 24 mm × 32 mm coverslip, after that the slides were denatured at 94°C for 3 min and located in a dark humidity box for 16–24 hours at 37°C.After removal of the rubber cement and coverslip, the slides were soaked in 0.5 × SCC buffer in a Coplin jar for 5 min at 75°C. Then they were washed for 2 min three times by PBS-Tween 20 buffer at RT. After Using ZytoDot 2C, Zytovision, Germany CISH kit, the slides were counterstained with 150 µl of Gill-2 hematoxylin and incubated for 3 min. They were then mounted with a coverslip (Figure 2).

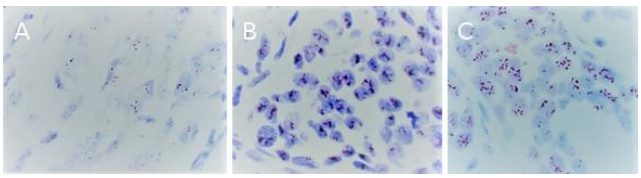

Figure 2: Chromogenic in situ hybridisation (CISH) of HER-2 oncogene in breast cancer; A) With normal HER2 copy number (1000 X) with no amplification; B) CISH on a breast carcinoma with low level HER2 gene amplification (1000 X); C) CISH on a breast carcinoma with high level HER2 gene amplification (1000 X)

Results were observed under the light microscope with 40x and 100x magnifications by 2 experienced pathologists, who were not aware of IHC results beforehand. Invasive tumor areas were recognized and dimensionally analyzed afterwards. HER2 signal, indicating a copy of its gene, was observed as a detectable green spot and signals of chromosome 17 centromere was observed as a red spot, where these two were simply distinguishable. In a normal diploid nucleus without amplifications, 2 green spots and 2 red spots with smooth rounded edges were seen in each nucleus. Then, 100 cells were counted and the ratio of hybridized probe HER2 genes to signals of chromosome 17 centromere was calculated. Necrotic zones, overlapped nuclei and nucleus with undetectable signals were omitted.

Since mitosis can cause extra signals in a few percent of non-neoplastic cells; in cases with heterogeneity, those areas in tumor which showed amplifications, were selected. Samples with the ratio of 2.5 or more were considered as having amplification and those wih the rate less than 2.5 amplifications. Results were categorized based on kit's instructions:

1. Less than 5 copies of HER2 gene in ratio with 2 signals of control (chromosome 17 centromere) in each cell nuclei of 100 counted cells mean there has been no amplifications.

2. Having 5 or more copies of HER2 gene in ratio with 2 signals of control (chromosome 17 centromere), which is sometimes seen as a gene cluster in tumor cell nucleus is considered as amplification.

In cases with no amplifications at diploid cells, 1 to 2 signals and in chromosomal aneuploidy situation, 2 to 4 signals were observed in each nucleus.

As an internal control, lymphocytes and stromal cells were examined and showed 2 signals with smooth rounded edges in each nucleus.

For quality control part, signals must be clear and easily detectable; also it's necessary to have internal control and morphologically healthy cells. Damaged nuclei show extra enzyme digestion which in turn causes dispersed signals or no signals at all.

At the end, statistical analysis was carried out between so called methods using software as below:

Data were analyzed using the statistical analysis software SPSS vs.22 in order to describe the data generally, statistical indexes like, mean, median standard deviation and ranges were used. For the main data analysis Chi-squared test was used to determine the relation between HER2 gene amplification and the score each carcinoma sample gained in IHC. $P < 0.05$ was regarded as statistically significant.

Results

A group of 40 patients with breast cancer were included in this study. The average age of the participant was 50.2 with the standard deviation of 10.96. The youngest patient was 26 and the oldest was 81 years old.

Among all patients, 92.6% were married and 7.4% were single. Tumor grade frequencies for scores I, II and III were 2.7%, 86.5% and 10.8% respectively (NA is assigned to those with no amplification and having amplification in the graph) (Figure 3). Furthermore, based on tumor (T) properties, 10%, 86.7% and 3.3% of patients were classified as T1, T2 and T3 (Figure 4).

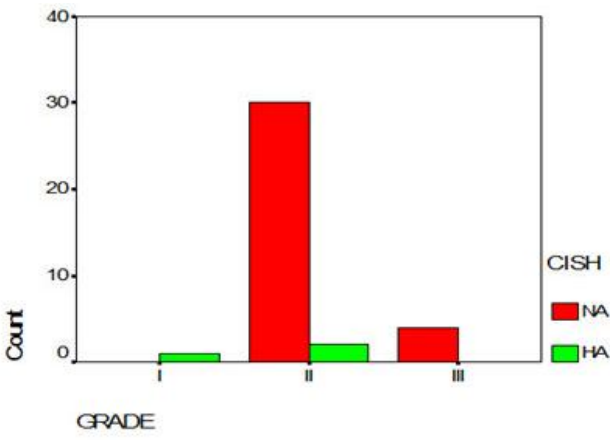

Figure 3: The frequency of grade tumor in samples examined by the method CISH

Evaluations using IHC method showed that respectively 2.6%, 26.3%, 65.8% and 5.3% of patients had scores of 0, +1, +2 and +3. In the other evaluation carried out by CISH method, 36 (90%) samples had not and 4 (10%) had amplification.

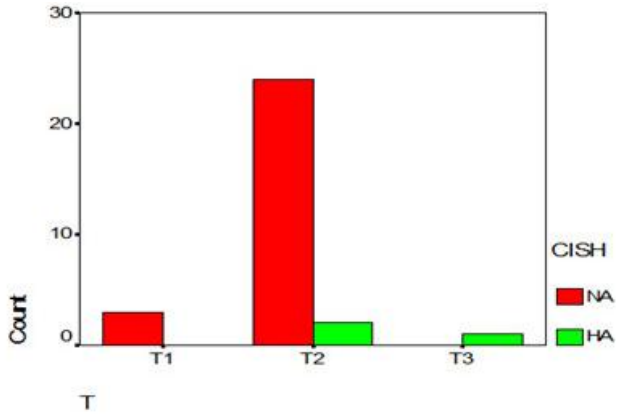

Figure 4: The frequency of tumor characteristics (T) in samples studied by CISH method

In comparative study between IHC and CISH methods, A significant relationship was found between the two methods using Fisher's exact test (p = 0.000) all the samples with no amplification in CISH method had the, scores of +2 and +3 in IHC. It was also recognized that among 25 samples with the score +2 in IHC, only 2 of them showed signs of amplifications in CISH while others were negative for amplification (Figure 5).

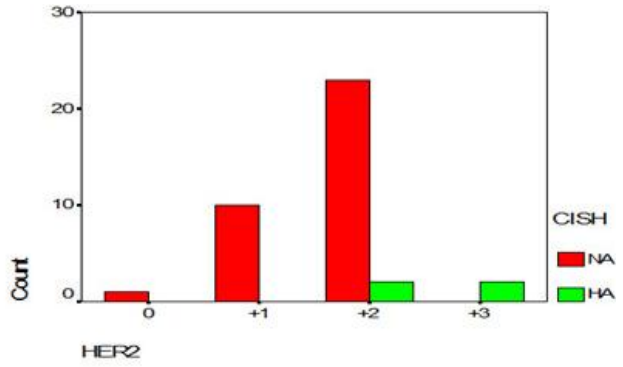

Figure 5: The frequency of results comparing IHC and CISH methods in diagnosis

Also Chi-square test showed a significant relation between CISH and tumor (T) properties (p = 0.009). On the other hand, the T-test didn't show any significant relation between CISH results and patient's age (p = 0.07).

In addition, when comparing between CISH and Tumor Grade, it came to our notice that most of the samples with no amplifications were of level II (p = 0.003). In the other comparison between IHC and tumor (T) properties, Kruskal-Walis test showed no significant relation between IHC and tumor (T) properties (p = 0.991). Also, in comparison between IHC and Tumor Grade, Kruskal-Walis test showed no significant relation between IHC and Tumor Grade (p = 0.993).

Discussion

Breast cancer is the second most prevalent cancer after skin cancer and is the second fatal cancer after lung cancer. It has been established through in various previous studies that HER2 gene amplification has appeared in 18-20% percent of breast cancer patients which in turn increases the mortality rate amongst these patients [17]. Since positive HER2+ patients demonstrate better responses to the treatment with monoclonal Ab Herceptin, it would be advantageous to locate HER2 gene. According to previous studies in determining the status of HER2 IHC method is used first. Soon after, with CISH having such unique properties, this method of staining was chosen to investigate HER2 gene amplification [6] [13] [14] [16] [18] [19] [20] [21].

Therefore, this study was conducted to investigate the quality of diagnosis of CISH and compare CISH with IHC in scoring efficacy of 40 patients with invasive ductal breast cancer. Our results demonstrated a relation between CISH and IHC in scoring patients. This means that all the patients examined in CISH method, also showed high amplifications, manifested high amplification for HER2 gene in IHC method as well (score +2 and score +3). So, for these patients, CISH can be used with high certainty.

In other studies conducted in France on 79 patients with invasive ductal breast cancer and in US involving 161 patient with the same problem confirmed that results obtained from CISH method for determining the level of HER2 gene amplification is more than 90% consistent with others method .and on the other hand, it can be used as a check test for patients with score +2 in IHC method [17] [22]. In a study carried out in Spain addressing the same issue, the compatibility of positive cases in CISH and IHC has been reported to be 72.5% and for negative cases, results of these 2 methods were 100% compatible. However, they could find no significant relationship between CISH, IHC with tumor grade which shows the specificity and novelty of this article. [23]. It has been clarified in other studies that HER2 gene expression level is crucial in how patients would respond to treatment with Herceptin. So in this study, IHC was done routinely and patients were classified into 4 scores and since there have been disagreements regarding treatment of choice for score +2 patients, CISH was used in this study confirmed the efficacy of CISH over IHC and showed a significant relation between CISH an IHC, meaning

that for any sample showing amplification in CISH, there would be a score +2 and +3 in IHC (p = 0.000).

Considering the result from the present study and comparing them with previous research on the issues it can be concluded that since CISH method is a more reliable method, with lower costs and which no need for Epi-fluorescent microscope, and since it also enables the researcher to simultaneously examine morphology and results of hybridization; it can be used as a highly effective method for determining HER2 expression level in patients with breast cancer. However, having some key limitations in this study like small sample size and unavailability of patient's detailed information such as the exact number of involved auxiliary lymph nodes and expanse of metastasis around the hand, it appears that conducting similar using a larger sample size can help in validating the results obtained from this study.

References

1. Darvishi M, Vasei N. Herpes zoster in breast cancer: a case report. J Biomed Res. 2017; 31(4): 370–372. PMid:28808209 PMCid:PMC5548998

2. Sadjadi A, Nouraie M, Ghorbani A, Alimohammadian M, Malekzadeh R. Epidemiology of breast cancer in the Islamic Republic of Iran: first results from a population-based cancer registry, 2009.

3. Mousavi SM, Montazeri A, Mohagheghi MA, Jarrahi AM, Harirchi I, Najafi M, Ebrahimi M. Breast cancer in Iran: an epidemiological review. The breast journal. 2007; 13(4):383-91. https://doi.org/10.1111/j.1524-4741.2007.00446.x PMid:17593043

4. King CR, Kraus MH, Aaronson SA. Amplification of a novel v-erbB-related gene in a human mammary carcinoma. Science. 1985; 229(4717):974-6. https://doi.org/10.1126/science.2992089 PMid:2992089

5. Owens MA, Horten BC, Da Silva MM. HER2 amplification ratios by fluorescence in situ hybridization and correlation with immunohistochemistry in a cohort of 6556 breast cancer tissues. Clinical breast cancer. 2004; 5(1):63-9. https://doi.org/10.3816/CBC.2004.n.011 PMid:15140287

6. Mrozkowiak, A., et al., HER2 status in breast cancer determined by IHC and FISH: comparison of the results. Pol J Pathol, 2004. 55(4): 165-71. PMid:15757204

7. Yaziji H, Goldstein LC, Barry TS, Werling R, Hwang H, Ellis GK, Gralow JR, Livingston RB, Gown AM. HER-2 testing in breast cancer using parallel tissue-based methods. Jama. 2004; 291(16):1972-7. https://doi.org/10.1001/jama.291.16.1972 PMid:15113815

8. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987; 235(4785):177-82. https://doi.org/10.1126/science.3798106 PMid:3798106

9. Hancock MC, Langton BC, Chan T, Toy P, Monahan JJ, Mischak RP, Shawver LK. A monoclonal antibody against the c-erbB-2 protein enhances the cytotoxicity of cis-diamminedichloroplatinum against human breast and ovarian tumor cell lines. Cancer research. 1991; 51(17):4575-80. PMid:1678683

10. Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, Somerfield MR, Hayes DF, Bast Jr RC. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. Journal of clinical oncology. 2007; 25(33):5287-312. https://doi.org/10.1200/JCO.2007.14.2364 PMid:17954709

11. Penault-Llorca F, Bilous M, Dowsett M, Hanna W, Osamura RY, Rüschoff J, van de Vijver M. Emerging technologies for assessing HER2 amplification. American journal of clinical pathology. 2009; 132(4):539-48. https://doi.org/10.1309/AJCPV2I0HGPMGBSQ PMid:19762531

12. Dalvandi M, Nazemi Rafie A, Kamali A, Jamshidifard A. Evaluation of the prognostic value of multimodal intraoperative monitoring in posterior fossa surgery patients with cerebellopontine angle tumors. Eur J Transl Myol. 2018; 28(1): 7260. https://doi.org/10.4081/ejtm.2018.7260 PMid:29686816 PMCid:PMC5895985

13. Powell WC, Roche PC, Tubbs RR. A new rabbit monoclonal antibody (4B5) for the immuno-histochemical (IHC) determination of the HER2 status in breast cancer: Comparison with CB11, fluorescence in situ hybridization (FISH), and interlaboratory reproducibility. Applied Immunohistochemistry & Molecular Morphology. 2008; 16(6):569. https://doi.org/10.1097/PAI.0b013e3181895d6c PMid:18931613

14. Gown AM, Goldstein LC, Barry TS, Kussick SJ, Kandalaft PL, Kim PM, Christopher CT. High concordance between immunohistochemistry and fluorescence in situ hybridization testing for HER2 status in breast cancer requires a normalized IHC scoring system. Modern Pathology. 2008; 21(10):1271. https://doi.org/10.1038/modpathol.2008.83 PMid:18487992

15. Li-Ning-T E, Ronchetti R, Torres-Cabala C, Merino MJ. Role of Chromogenic in Situ Hybridization (CISH™) in the Evaluation of HER2 Status in Breast Carcinoma: Comparison with Immunohistochemistry and Fish. International journal of surgical pathology. 2005; 13(4):343-51. https://doi.org/10.1177/106689690501300406 PMid:16273190

16. Wolff AC, HM, et al. ASCO–CAP HER2 Test Guideline Recommendations, 2013.

17. Arnould L, Denoux Y, MacGrogan G, Penault-Llorca F, Fiche M, Treilleux I, Mathieu MC, Vincent-Salomon A, Vilain MO, Couturier J. Agreement between chromogenic in situ hybridisation (CISH) and FISH in the determination of HER2 status in breast cancer. British journal of cancer. 2003; 88(10):1587. https://doi.org/10.1038/sj.bjc.6600943 PMid:12771927 PMCid:PMC2377115

18. Viale G, Slaets L, Bogaerts J, Rutgers E, Van't Veer L, Piccart-Gebhart MJ, de Snoo FA, Stork-Sloots L, Russo L, Dell'Orto P, Van Den Akker J. High concordance of protein (by IHC), gene (by FISH; HER2 only), and microarray readout (by TargetPrint) of ER, PgR, and HER2: results from the EORTC 10041/BIG 03-04 MINDACT trial. Annals of oncology. 2014; 25(4):816-23. https://doi.org/10.1093/annonc/mdu026 PMid:24667714 PMCid:PMC3969556

19. Naseh G, Mohammadifard M, Mohammadifard M. Upregulation of cyclin-dependent kinase 7 and matrix metalloproteinase-14 expression contribute to metastatic properties of gastric cancer. IUBMB Life. 2016; 68(10):799-805. https://doi.org/10.1002/iub.1543 PMid:27562173

20. Ghaffari SR, Sabokbar T, Dastan J, Rafati M, Moossavi S. Her2 amplification status in Iranian breast cancer patients: comparison of immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH). Asian Pac J Cancer Prev. 2011; 12(4):1031-4. PMid:21790246

21. Takahashi M, Inoue KI, Goto R, Tamura M, Taguchi K, Takahashi H, Suzuki H, Yamashiro K, Ogita M. Metastatic breast cancer of HER2 scored 2+ by IHC and HER2 gene amplification assayed by FISH has a good response to single agent therapy with trastuzumab: a case report. Breast Cancer. 2003; 10(2):170. https://doi.org/10.1007/BF02967645 PMid:12736573

22. Di Palma S, Collins N, Faulkes C, Ping B, Ferns G, Haagsma B, Kissin M, Layer G, Cook M. Chromogenic in-situ hybridisation (CISH) should be an accepted method in the routine diagnostic evaluation of HER2 status in breast cancer. Journal of clinical pathology. 2007. PMCid:PMC1972421

23. Madrid MA, Lo RW. Chromogenic in situ hybridization (CISH): a novel alternative in screening archival breast cancer tissue samples for HER-2/neu status. Breast Cancer Research. 2004; 6(5):R593. https://doi.org/10.1186/bcr915 PMid:15318940 PMCid:PMC549176

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Basic Science

Correlation of P38 Mitogen-Activated Protein Kinase Expression to Clinical Stage in Nasopharyngeal Carcinoma

Farhat Farhat[1*], Elvita Rahmi Daulay[2], Jessy Chrestella[3], Rizalina Arwinati Asnir[1], Ashri Yudhistira[1], Riko Radityatama Susilo[1]

[1]Universitas Sumatera Utara Fakultas Kedokteran, Otorhynolaryngology Head and Neck Surgery Jl. Dr. T. Mansyur No. 9, Medan, North Sumatera 20155, Indonesia; [2]Universitas Sumatera Utara Fakultas Kedokteran, Radiology Medan, North Sumatera, Indonesia; [3]Universitas Sumatera Utara Fakultas Kedokteran, Pathology Medan, Sumatera Utara, Indonesia

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Keywords: p38 MAPK; Nasopharyngeal carcinoma; Clinical stage; Correlation; Biomarker

***Correspondence:** Farhat Farhat. Universitas Sumatera Utara Fakultas Kedokteran, Otorhinolaryngology Head and Neck Surgery Jl. Dr T. Mansyur No.9, Medan, North Sumatera 20155, Indonesia. E-mail: farhat@usu.ac.id

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Abstract

BACKGROUND: Nasopharyngeal carcinoma (NPC) is uncommon and usually diagnosed at the advanced stage. A subfamily of mitogen-activated protein kinase which is called p38 mitogen-activated protein kinase (MAPK) involved in response to stress, and plays an important role in cell regulation. There is a suggestion that p38 mitogen-activated protein kinase could be a potential biomarker to determine the clinical stage of nasopharyngeal carcinoma.

AIM: The aim of this study is for observing and analysing the correlation of p38 mitogen-activated protein kinase expression in regards to nasopharyngeal carcinoma patient's clinical stage.

METHODS: This study involved 126 nasopharyngeal carcinoma patients admitted to Haji Adam Malik General Hospital.

RESULTS: The result of this study indicates that nasopharyngeal carcinoma mostly found in the age group 41-60 years, male, non-keratinizing squamous cell carcinoma, and stage IV group. In immunohistochemistry evaluation, most of p38 mitogen-activated protein kinase overexpressed in non-keratinizing squamous cell carcinoma, T3-T4, N2-N3 and clinical stage III-IV. Spearman's test for categorical correlation yield p-value of < 0.001.

CONCLUSION: In conclusion, there is a significant correlation between p38 mitogen-activated protein kinase expression and the clinical stage of nasopharyngeal carcinoma.

Introduction

Nasopharyngeal carcinoma (NPC) is not a rare entity, which has a distinct distribution especially in Asia [1] [2] [3] [4] [5]. There are more than 13.000 new cases of NPC in Indonesia and associated with a high mortality rate [1] [6]. The aetiology of NPC is considered to be related to environmental and genetic factors as well as EBV infection [7] [8]. Because of the location of NPC is in the silent and painless area; therefore the disease is usually diagnosed at the advanced stages; hence early detection of NPC is difficult [9]. Regulation of signaling molecules in intracellular signal transduction, which regulate cell proliferation, apoptosis, and adhesion, underlines the basis of NPC pathogenesis [10] [11] [12] [13] [14] [15] [16] [17].

Mitogen-activated protein kinase (MAPK) is an important signal molecule that affects a variety of cellular process such as proliferation, differentiation, migration, and apoptosis [18] [22]. Aside from its physiological functions, MAPK also plays a key role in many pathological conditions including cancer, cardiac hypertrophy, and diabetes [23] [24] [25] [26].

Two distinct classes of MAPKs have been identified so far: p42-p44 (ERK) MAPKs inducible by; and SAPKs (Stress-Activated Protein Kinases), which include p38 MAPKs, and p46-p54 JNKs inducible by cellular stress [24] [25]. Each MAPK class responds to distinct stimuli and induces a specific biological response, and they proofed to have a crucial role in cancer development [26].

The p38 MAPKs are a conserved subfamily of MAPKs involved in response to stress found in eukaryotic cells from yeast to mammals. p38 was found in 1994 as a MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells [27]. In the development of NPC, p38 MAPK activation plays an important role both in the ability to protect EBV-infected Raji cells from apoptosis and also for promoting EBV lytic gene expression [17].

The p38 MAPKs have been proposed as a novel biomarker for predicting the clinical stage of NPC [19] [20]. Considering the importance of a biomarker for NPC, the primary goal of the current study was to explore the prevalence and expression of p38 MAPK and their possible correlation to the clinical stage of NPC. This study might provide supportive evidence for the role of p38 MAPK in the clinical stage of NPC.

This study was analytic research, and we used paraffin samples of 126 NPC patients to analyse the correlation of p38 MAPK overexpression to the clinical stage of NPC.

Material and Methods

During July to October 2017, research with 126 samples of NPC patients was established in Adam Malik General Hospital, Medan. The samples were taken in 2015-2016 based on history taking, physical examination, and nasopharyngeal histopathological biopsy. The criteria include patients with NPC histopathologically examined and not yet received any radiotherapy, chemotherapy, or combination of both. To obtain an adequate result, non-probability consecutive sampling was used to receive a minimum of 68 patients in this study.

Paraffin-embedded pathological specimens of nasopharyngeal histopathological biopsy were obtained. All of these resection samples were treated with a standard fixation, dissection, and processing protocol. The blocks were then cut into 4 mm sections and processed for immunohistochemistry. After being washed with phosphate-buffered saline, the specimens were incubated with the primary antibody using GENETEX human p38 MAPK antibody.

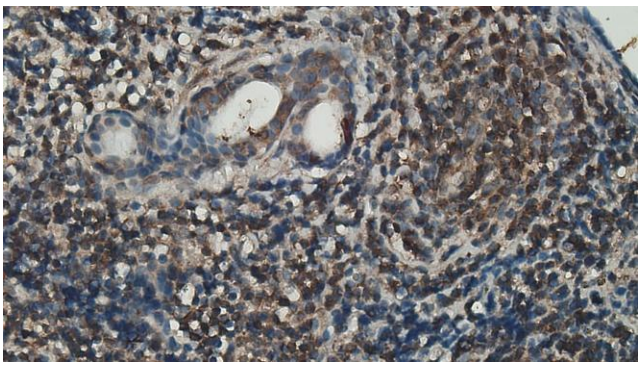

Figure 1: Cytoplasmic expression of p38 MAPK in non-keratinizing squamous cell nasopharyngeal carcinoma (x 400)

The tissues were examined using immunohistochemistry technique under fluorescent microscope evaluating the immunoreaction of p38 MAPK. Three pathologists who were blinded to the patients' clinicopathological data independently evaluated the p38 MAPK expressions. The results were evaluated using immunoreactivity score (score obtained by multiplying width score with intensity score) [21].

This study was approved by the Health Research Ethical Committee of the Medical Faculty of Universitas Sumatera Utara.

To define the correlation in this study, SPSS ver. 22 software was used to conduct all the statistical analysis. The association was identified by using Spearman's correlation, in which $p < 0.05$ was considered as statistically significant.

Results

There were 126 patients with NPC involved in this study that has fulfilled the study requirement. The presentation of demographic data was shown in table 1. Distributions of age-specific rates of NPC shows that NPC incidence increases simultaneously with age, but fells of after 60 years. Despite the same ratio of male: female over the year, the number of NPC based on gender are higher in men compared to women. After immunohistochemistry examination, the most frequent histological type was non-keratinizing squamous cell carcinoma. For the clinical stage, most of the samples in the study was stage IV. The main focus of this study showed most of p38 MAPK over-expressed was in non-keratinizing squamous cell carcinoma (63.5%), T3-T4 primary tumour size (53.1%), cervical nodes enlargement in the N2-N3 group (65.1%) and clinical stage in the III-IV group (71.8%).

The association uses Spearman's test to categorise the correlation which results in the p-value

of < 0.001 indicating that the correlation of clinical staging and the expression of p38 MAPK is statistically significant.

Table 1: Demographic Data and Correlation of p38 MAPK and Clinical Stage in NPC Patients

Characteristic	p38 MAPK Expression				*P*
	Overexpression	%	Negative	%	
Age (y)					
≤ 20	3	2.4	4	3.2	
21-40	21	16.7	7	5.6	
41-60	59	46.8	13	10.3	
> 60	17	13.5	2	1.6	
Sex					
Male	69	54.8	21	16,7	
Female	31	24.6	5	4.0	
Histopathology					
Keratinizing SCC	8	6.3	0	0.0	
Non-keratinizing SCC	80	63.5	20	15.9	
Undifferentiated carcinoma	12	9.5	6	4.8	
Primary Tumor (T)					
T1	12	9.5	13	10.3	
T2	21	16.7	11	8.7	
T3 T4	40	31.7	1	0.8	
	27	21.4	1	0.8	
Nodes (N)					
N0	8	7.8	13	12.6	
N1	11	10.7	4	3.9	
N2 N3	18	17.5	0	0.0	
	49	47.6	0	0.0	
Clinical Staging					
I	4	3.9	9	8.7	<0.001
II	8	7.8	5	4.9	
III	16	15.5	2	1.9	
IV	58	56.3	1	1.0	

*p < 0.05 = statistically significant.

Discussion

In this study, the largest age group of a patient diagnosed with NPC is between 41-60 years old (57.1%). Similar to Adham et al., (2012), 40-49 years old is the highest age of the NPC patients found in their study [1]. This occurred because the DNA repair mechanism function and immune system have been lessened as mutation develops at the age of more than 40 years [5] [6] [13].

In our study, a trend was conducted, with 71.4% male and 28.6% female resulting in a 2.5:1 ratio. The male: female ratio was relatively stable over the years. In Cao (2011) study, the predominance was found as well with the incidence rate of NPC was less in women than in men, with a ratio of 2-3:1 [3]. The exposure to environmental pollution from occupation and lifestyle caused males got a higher ratio than females diagnosed with NPC [2] [5].

In our study, non-keratinizing squamous cell carcinoma (79.4%) was the most common form of NPC, similar to the studies done in other high-risk countries. In an endemic area, over 90% of NPC is non-keratinizing squamous cell carcinoma [2] [4] [5].

The p38 pathway has also been playing a role in the activation of p53 and p53-mediated apoptosis [18] [28] [29]. Many cancers are associated with decreased p38 activity because in the majority of cancers studied, reduced p38 activity implicated in the continuous cell proliferation, with downstream target activation such as ATF-2 and Elk-1 [30] [33].

Many factors support the role of p38 MAPK as a tumour suppressor, and the negative regulation of cell cycle and apoptotic induction mediate this p38α function, although terminal differentiation induction also supports tumour suppressor [24] [34] [35]. However, p38 MAPK as well as an oncogenic function that is mediated by the involvement of cancer progression processes, such as invasion, inflammation, and angiogenesis [36] [40]. The conclusion of this study is a statistically significant correlation between clinical staging and the expression of p38 MAPK. Further research with larger and multiple centres is required for assessing the role of p38 MAPK in the progression of nasopharyngeal cancer.

References

1. Adham M, Kurniawan AN, Muhtadi AI, Roezin A, Hermani B, Gondhowiardjo S, et al. Nasopharyngeal carcinoma in Indonesia: epidemiology, incidence, signs, and symptoms at presentation. Chinese journal of cancer. 2012; 31(4):185. https://doi.org/10.5732/cjc.011.10328 PMid:22313595 PMCid:PMC3777476

2. Tang L-L, Chen W-Q, Xue W-Q, He Y-Q, Zheng R-S, Zeng Y-X, et al. Global trends in incidence and mortality of nasopharyngeal carcinoma. Cancer letters. 2016;374(1):22-30.Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA: a cancer journal for clinicians. 2015; 65(2):87-108.

3. Cao S-M, Simons MJ, Qian C-N. The prevalence and prevention of nasopharyngeal carcinoma in China. Chinese journal of cancer. 2011; 30(2):114. https://doi.org/10.5732/cjc.010.10377 PMCid:PMC4013340

4. Zhang L-F, Li Y-H, Xie S-H, Ling W, Chen S-H, Liu Q, et al. Incidence trend of nasopharyngeal carcinoma from 1987 to 2011 in Sihui County, Guangdong Province, South China: an age-period-cohort analysis. Chinese journal of cancer. 2015; 34(3):15. https://doi.org/10.1186/s40880-015-0018-6 PMid:26058679 PMCid:PMC4593377

5. Jayalie VF, Paramitha MS, Jessica J, Liu CA, Ramadianto AS, Trimartani T, et al. Profile of Nasopharyngeal Carcinoma in Dr. Cipto Mangunkusumo National Hospital, 2010. eJournal Kedokteran Indonesia. 2017:156-62.

6. Nawaz I, Moumad K, Martorelli D, Ennaji MM, Zhou X, Zhang Z, et al. Detection of nasopharyngeal carcinoma in Morocco (North Africa) using a multiplex methylation-specific PCR biomarker assay. Clinical epigenetics. 2015; 7(1):89. https://doi.org/10.1186/s13148-015-0119-8 PMid:26300994 PMCid:PMC4546349

7. Tulalamba W, Janvilisri T. Nasopharyngeal carcinoma signaling pathway: an update on molecular biomarkers. International journal of cell biology. 2012; 2012.

8. Fles R, Bos A, Rachmawati D, Waliyanti E, Tan I, Haryana S, et al. The role of Indonesian patients' health behaviors in delaying the diagnosis of nasopharyngeal carcinoma. BMC public health. 2017; 17(1):510. https://doi.org/10.1186/s12889-017-4429-y PMid:28545416 PMCid:PMC5445307

9. Tsang CM, Tsao SW. The role of Epstein-Barr virus infection in the pathogenesis of nasopharyngeal carcinoma. Virologica Sinica. 2015; 30(2):107-21. https://doi.org/10.1007/s12250-015-3592-5 PMid:25910483

10. Zeng MS, Zeng YX. Pathogenesis and etiology of nasopharyngeal carcinoma. InNasopharyngeal Cancer 2010 (pp. 9-25). Springer, Berlin, Heidelberg.

11. Liu T-H, Zheng F, Cai M-Y, Guo L, Lin H-X, Chen J-W, et al. The putative tumor activator ARHGEF3 promotes nasopharyngeal carcinoma cell pathogenesis by inhibiting cellular apoptosis. Oncotarget. 2016; 7(18):25836. https://doi.org/10.18632/oncotarget.8283 PMid:27028992 PMCid:PMC5041948

12. Petersson F, Editor. Nasopharyngeal carcinoma: a review. Seminars in diagnostic pathology. Elsevier, 2015.

13. Young LS, Dawson CW. Epstein-Barr virus and nasopharyngeal carcinoma. Chinese journal of cancer. 2014; 33(12):581. https://doi.org/10.5732/cjc.014.10197

14. Burgos JS. Involvement of the Epstein-Barr virus in the nasopharyngeal carcinoma pathogenesis. Medical Oncology. 2005; 22(2):113-21. https://doi.org/10.1385/MO:22:2:113 PMid:28184182

15. Chan AT, Teo PM, Huang DP. Pathogenesis and treatment of nasopharyngeal carcinoma. InSeminars in oncology 2004 Dec 1 (Vol. 31, No. 6, pp. 794-801). WB Saunders.

16. Raab-Traub N, editor. Epstein–Barr virus in the pathogenesis of NPC. Seminars in cancer biology. Elsevier, 2002.

17. Yong H-Y, Koh M-S, Moon A. The p38 MAPK inhibitors for the treatment of inflammatory diseases and cancer. Expert opinion on investigational drugs. 2009; 18(12):1893-905. https://doi.org/10.1517/13543780903321490 PMid:19852565

18. Martín-Blanco E. p38 MAPK signalling cascades: ancient roles and new functions. Bioessays. 2000; 22(7):637-45. https://doi.org/10.1002/1521-1878(200007)22:7<637::AID-BIES6>3.0.CO;2-E

19. Cargnello M, Roux PP. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. Microbiology and molecular biology reviews. 2011; 75(1):50-83. https://doi.org/10.1128/MMBR.00031-10 PMid:21372320 PMCid:PMC3063353

20. Fedchenko N, Reifenrath J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue–a review. Diagnostic pathology. 2014; 9(1):221. https://doi.org/10.1186/s13000-014-0221-9 PMid:25432701 PMCid:PMC4260254

21. Cuadrado A, Nebreda AR. Mechanisms and functions of p38 MAPK signalling. Biochemical Journal. 2010; 429(3):403-17. https://doi.org/10.1042/BJ20100323 PMid:20626350

22. Roux PP, Blenis J. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiology and molecular biology reviews. 2004; 68(2):320-44. https://doi.org/10.1128/MMBR.68.2.320-344.2004 PMid:15187187 PMCid:PMC419926

23. Wagner EF, Nebreda ÁR. Signal integration by JNK and p38 MAPK pathways in cancer development. Nature Reviews Cancer. 2009; 9(8):537. https://doi.org/10.1038/nrc2694 PMid:19629069

24. Obata T, Brown GE, Yaffe MB. MAP kinase pathways activated by stress: the p38 MAPK pathway. Critical care medicine. 2000; 28(4):N67-N77. https://doi.org/10.1097/00003246-200004001-00008 PMid:10807318

25. Coulthard LR, White DE, Jones DL, McDermott MF, Burchill SA. p38MAPK: stress responses from molecular mechanisms to therapeutics. Trends in molecular medicine. 2009; 15(8):369-79. https://doi.org/10.1016/j.molmed.2009.06.005 PMid:19665431 PMCid:PMC3016890

26. Bradham C, McClay DR. p38 MAPK in development and cancer. Cell cycle. 2006; 5(8):824-8. https://doi.org/10.4161/cc.5.8.2685 PMid:16627995

27. Wang B, Jiang H, Ma N, Wang Y. Phosphorylated-p38 mitogen-activated protein kinase expression is associated with clinical factors in invasive breast cancer. Springer Plus. 2016; 5(1):934. https://doi.org/10.1186/s40064-016-2636-0 PMid:27386378 PMCid:PMC4929108

28. Olson JM, Hallahan AR. p38 MAP kinase: a convergence point in cancer therapy. Trends in molecular medicine. 2004; 10(3):125-9. https://doi.org/10.1016/j.molmed.2004.01.007 PMid:15102355

29. Leelahavanichkul K, Amornphimoltham P, Molinolo AA, Basile JR, Koontongkaew S, Gutkind JS. A role for p38 MAPK in head and neck cancer cell growth and tumor-induced angiogenesis and lymphangiogenesis. Molecular oncology. 2014; 8(1):105-18. https://doi.org/10.1016/j.molonc.2013.10.003 PMid:24216180 PMCid:PMC3946852

30. Kennedy NJ, Cellurale C, Davis RJ. A radical role for p38 MAPK in tumor initiation. Cancer Cell. 2007; 11(2):101-3. https://doi.org/10.1016/j.ccr.2007.01.009 PMid:17292820

31. Riebe C, Pries R, Schroeder KN, Wollenberg B. Phosphorylation of STAT3 in head and neck cancer requires p38 MAPKinase, whereas phosphorylation of STAT1 occurs via a different signaling pathway. Anticancer research. 2011; 31(11):3819-25. PMid:22110204

32. Beisswenger C, Coyne CB, Shchepetov M, Weiser JN. Role of p38 MAP kinase and transforming growth factor-β signaling in transepithelial migration of invasive bacterial pathogens. Journal of Biological Chemistry. 2007; 282(39):28700-8. https://doi.org/10.1074/jbc.M703576200 PMid:17650505

33. Lara HHR, Monroy A. Prevalence of nasopharyngeal carcinoma among patients with nasopharyngeal mass in a Philippine tertiary training hospital. Philippine Journal of Otolaryngology Head and Neck Surgery. 2016; 31(1):35-8.

34. Han J, Sun P. The pathways to tumor suppression via route p38. Trends in biochemical sciences. 2007; 32(8):364-71. https://doi.org/10.1016/j.tibs.2007.06.007 PMid:17624785

35. Lin ML, Lu YC, Chung JG, Wang SG, Lin HT, Kang SE, et al. Down-regulation of MMP-2 through the p38 MAPK-NF-κB-dependent pathway by aloe-emodin leads to inhibition of nasopharyngeal carcinoma cell invasion. Molecular carcinogenesis. 2010; 49(9):783-97. https://doi.org/10.1002/mc.20652

36. Zhong Y, Naito Y, Cope L, Naranjo-Suarez S, Saunders T, Hong S-M, et al. Functional p38 MAPK identified by biomarker profiling of pancreatic cancer restrains growth through JNK inhibition and correlates with improved survival. Clinical cancer research. 2014; 20(23):6200-11. https://doi.org/10.1158/1078-0432.CCR-13-2823 PMid:24963048 PMCid:PMC4866510

37. Zhu G-H, Dai H-P, Shen Q, Ji O, Zhang Q, Zhai Y-L. Curcumin induces apoptosis and suppresses invasion through MAPK and MMP signaling in human monocytic leukemia SHI-1 cells. Pharmaceutical biology. 2016; 54(8):1303-11. PMid:26134921

38. Bistrović A, Grbčić P, Harej A, Sedić M, Kraljević-Pavelić S, Koštrun S, et al. Small molecule purine and pseudopurine derivatives: synthesis, cytostatic evaluations and investigation of growth inhibitory effect in non-small cell lung cancer A549. Journal of enzyme inhibition and medicinal chemistry. 2018; 33(1):271-85. https://doi.org/10.1080/14756366.2017.1414807 PMid:29271659 PMCid:PMC6009932

39. Wakeman D, Schneider JE, Liu J, Wandu WS, Erwin CR, Guo J, et al. Deletion of p38- alpha mitogen-activated protein kinase within the intestinal epithelium promotes colon tumorigenesis. Surgery. 2012; 152(2):286-93. https://doi.org/10.1016/j.surg.2012.05.009 PMid:22828150 PMCid:PMC3408636

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Basic Science

Protein 53 (P53) Expressions and Apoptotic Index of Amniotic Membrane Cells in the Premature Rupture of Membranes

Ketut Surya Negara[1*], Ni Luh Lany Christina Prajawati[1], Gede Putu Surya[1], Suhendro Suhendro[2], Komang Arijana[3], Ketut Tunas[4]

[1]Department of Obstetrics and Gynecology, Udayana University, Sanglah Hospital, Bali, Indonesia; [2]Department of Pathology, Sanglah Hospital, Bali, Indonesia; [3]Integrated Biomedical Laboratory, Medical Faculty of Udayana University, Bali, Indonesia; [4]Department of Public Health, Dhyana Pura University, Bali, Indonesia

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Keywords: Premature rupture of the membrane; p53 expression; Apoptotic index

***Correspondence:** Ketut Surya Negara. Department of Obstetrics and Gynecology, Udayana University, Sanglah Hospital, Bali. E-mail: tutsuryanegara@gmail.com

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Abstract

BACKGROUND: The premature rupture of membranes (PROM) represents an obstetric issue causing significant maternal and neonatal morbidity and mortality. Although protein 53 (p53), one of the proapoptotic proteins suspected of causing PROM at the molecular level is closely correlated with the occurrence of PROM, the exact mechanism remains still unclear.

AIM: This study aims to investigate the hypothesis that p53 expression and the apoptotic index play a role in the PROM mechanism.

METHODS: Placentas from 20 pregnancies (37–42 weeks gestation) and 20 pregnancies complicated by PROM were collected at delivery. The independent variable is represented by pregnant mothers with a single live fetus experiencing PROM (followed by labour and birth) while without PROM mothers represent the control. The research material was taken from the amnion tissue in the placenta. Also, p53 and apoptotic index (TUNEL) immunohistochemical examination were conducted at the Integrated Biomedical Laboratory, Medical Faculty of Udayana University, Bali. The correlation between the apoptotic index and p53 expression of the PROM group was tested using a McNemar Test.

RESULTS: No statistically significant differences were found between the two groups ($p > 0.05$). There was a significant difference in p53 expression in PROM cases compared to those without PROM (11.15 + 5.59% vs. 0.95 + 2.52%) with $\chi^2 = 19.538$ and $p = 0.001$. The apoptotic index in PROM cases was higher than in those without PROM (19.10 + 5.63% vs. 1.15 + 2.46%) with $\chi^2 = 32.40$ and $p = 0.001$. There was a strong correlation between p53 expression and PROM with PR = 3.449 (95% CI = 1.801-6.605; $p = 0.001$). There was a strong correlation between the apoptotic index and PROM with PR = 19 (95% CI = 2.81-128.69; $p = 0.001$).

CONCLUSION: p53 expression and the apoptotic index of amniotic membrane cells in cases of PROM was higher than in those without PROM, there was a strong correlation between p53 expression and apoptotic index with the occurrence of PROM.

Introduction

The premature rupture of membranes (PROM) is one factor causing increased maternal & neonatal morbidity and mortality rates due to the complications it causes [1], [2]. PROM involves the rupture of the fetal membrane before signs of labour occur [1]. This may occur at the end of the gestational period 37-42 weeks (term) and also far in advance of the normal gestational period, at less than 37 weeks (preterm) [1].

The rupture of the fetal membrane during labour is caused by weakening of the membrane due to uterine contraction and continuous stretching. In general, this membrane ruptures during uterine contraction; however, 10% of ruptures occur before uterine contractions in term pregnancies, and 40% occur during preterm pregnancy. This finding supports the case that contractions causing stretching of the uterus are not the only factor causing PROM [3].

Other factors suspected of causing PROM to include infection, hormonal changes, mechanical forces, and apoptosis [4]. The latest evidence is that PROM occurs before uterine contractions begin which is connected with amniotic cell apoptosis through caspase-dependent pathways mediated by p53 [5].

The proapoptotic effects of increasing p53 expression through intrinsic pathways induce amniotic cell's apoptosis. The increasing p53 expression via the molecular approach becomes a factor which plays a role in PROM pathogenesis, and this represents a very promising area for future research [6], [7]. Indeed, p53 is one of the key proapoptotic proteins implicated at the molecular level as it has a close correlation with the incidence of PROM, although the exact mechanism remains unclear [8].

The present study aims to show p53 expression, and the apoptotic index plays a role in the PROM mechanism and correlates p53 expression and the apoptotic index with the occurrence of PROM.

Material and Methods

Amniotic membranes were collected from participants suffering from PROM (n = 20) and those without PROM (i.e. normal labour) (n = 20) at term gestational age (37-42 weeks). Samples were only taken from patients who had been both well informed and signed a consent form. Amniotic membranes were taken from the edge of a rupture (2 cm in size) after labour using aseptic methods and samples were then placed into phosphate-buffered saline (PBS) solution as a transport-fixation solution.

Tissue Processing for Preparation of p53 Immunohistochemistry (IHC) and TUNEL

Amniotic membrane tissue was histopathologically processed into a paraffin block which was then cut at 3 to 5 µm, mounted on positively charged slides, and dried in an oven at 60°C for 30 minutes to ensure adherence to the slide. The sections were deparaffinized in four changes of xylene for 5 minutes each and then rehydrated through a series of graded alcohol with a final rinse in distilled water [9].

Endogenous peroxides quenched by soaking the section in two changes of 0.3% H_2O_2 in methanol. The immunostaining used the p53 kit from Labvision^R. The tissue section was treated with antigen retrieval and then incubated inside a steamer for 20 minutes. Initially, samples were heated to 99°C, then taken out of the steamer, and allowed to cool down for 20 minutes (still in the target retrieval solution). The slide was washed 3 times with distilled water and then put in a Tris-buffered saline solution for 5 minutes. The slide reacted with the p53 primary antibodies for 1 hour. The positive control was derived from human colonic tissue and PBS solution as a negative control. Streptavidin-biotin-based detection was used, then an antibody was added and incubated for 15 minutes, then diaminobenzidine (DAB) chromogen was added. Counterstaining with Harris-haematoxylin was performed for 1 minute. The slide was washed with 0.25% ammonia until it became blue and then washed with distilled water. The slide was dehydrated with graded alcohol and then cleared with xylol 4 times. Finally, it was mounted and covered with a coverslip [9], [10].

Protein 53 expressions were performed by counting the positive brown-stained nuclei on 200 epithelial and stromal nuclei of amniotic membrane cells at a hotspot area that expressed the highest number of apoptotic nuclei, and then the percentage was calculated [11]. Protein 53 expression was scored by the percentage and intensity of the staining: a score of 0 = negative; a score of 1+ = weak; a score 2+ = moderate; a score 3+ = strong; as presented in Table 1 [12], [13], [14].

Table 1: Interpretation of P53 Immunohistochemical Staining [14], [15]

Staining Pattern	Score
No nuclei staining or less than 10% nuclei staining	0
Staining more than 10% of nuclei, weak intensity, incomplete nuclei staining	1+
Staining more than 10% nuclei, weak to moderate intensity, complete nuclei staining	2+
Staining more than 10% nuclei, strong intensity, complete nuclei staining	3+

The normality of the data was tested using a Shapiro-Wilk Test, and homogeneity was tested by Levene's test. Protein 53 expression data were descriptively analysed using a Mann Whitney U-Test (due to abnormal distribution) to analyse the differences between the two groups. A Chi-Square Test was performed to analyse the correlation between p53 expression and apoptotic index with PROM. The correlation between the apoptotic index and p53 expression of the PROM group was tested using a McNemar Test. A significance level of $p < 0.05$ was considered significant.

After being deparaffinized and rehydrated, slides were then rinsed twice with PBS for two minutes each. The slides were incubated for 15-30 minutes at 21-37°C in a working solution Proteinase K (containing Proteinase K 2 µL in 98 µL PBS). The slides were then incubated in a blocking solution for ten minutes at 15-25°C (the blocking solution contained H_2O_2 3% in methanol). Slides were rinsed with PBS twice for two minutes each following the labelling protocol. Slides were rinsed with PBS twice for 2 minutes each, and then the area surrounding the samples was allowed to dry. The reagent of TUNEL was added to the samples, which were then covered and incubated for 60 minutes at 37°C in a dark humidity chamber. Slides were rinsed with PBS three times for two minutes each and then left to dry. Streptavidin-HRP solution (50 µL) was added to the

samples. Slides were incubated in a dark humidity chamber at 37°C for 30 minutes. Slides were rinsed with PBS three times for two minutes each. Then, 50-100 µL diaminobenzidine (DAB) substrate was added, and the slides were incubated for 10 minutes at 15-25°C (the DAB substrate contained 5 µL 10 mg/mL DAB buffer and 1 µL 30% H_2O_2 in µL PBS; freshly prepared). Slides were rinsed with PBS three times, covered with a coverslip, and analysed under a light microscope [9].

The apoptotic index of amniotic cells consists of the number of apoptotic amniotic cell nuclei in 100 cells observed under a microscope at 400 x magnification. The apoptotic index was observed by a pathologist at the hotspot area which contained the densest apoptotic nuclei on routine staining of haematoxylin-eosin (HE). The apoptotic index is determined as *strong* if > 10%, and weak if ≤ 10% [17].

$$\text{Apoptotic Index} = \frac{\text{The number of apoptotic nuclei}}{\text{The number of observed nuclei}} * 100\%$$

Results

The average age of mothers in the PROM group was 27.15 years: primigravida had the first rank with 10 cases (50%), the average gestation age (GA) of the PROM group was 38.7 weeks, and the average of body-mass index (BMI) was 24.60 kg/m^2. The average age of the without PROM mothers' group was 27.80 years, multigravida (G2) had the first rank with 10 cases (50%), primigravida accounted for 7 cases (35%), the average GA of the without PROM groups was 39.3 weeks, and their average BMI was 24.60 kg/m^2. The statistical test results illustrate there were no significant differences in mother's ages, gestation ages, *gravida*, and BMI between the PROM and the without PROM groups with a significance of 0.777, 0.153, 0.515, and 1.000, respectively, which all significances higher than 0.05 (p > 0.05). The statistics on mother's ages, gestation ages, and BMI are presented in Table 2.

Table 2: The Subject Characteristics of PROM and the Without PROM Group

Characteristic	PROM Group (n = 20)		Without PROM Group (n = 20)		p
	Mean	SD	Mean	SD	
Mother's Age (year)	27.15	± 6.85	27.80	± 7.52	0.777
Gestational Age (weeks)	38.70	± 1.5	39.30	± 0.98	0.153
BMI (kg/m^2)	24.60	± 4.48	24.60	± 4.48	1.000

Twenty samples from the PROM group expressed p53 on epithelial and stromal cells of the amniotic membrane with a strong intensity in the 4-30% in range. In the 20 samples from the without PROM group, most of them exhibited a negative expression of p53; several samples expressed p53 with a weak intensity in the 1-11% in range. The data on p53 expression in the PROM and without PROM groups are presented in Figure 1. The p53 expression in the PROM and without PROM groups data did not follow a normal distribution based on a normality test.

Table 3: Comparison of Amniotic Cells P53 Expression of PROM and Without PROM Group

Group	p53 Expression				χ^2	p
	Negative	Weak	Moderate	Strong		
PROM	7	0	2	11	19,538	0,001
PROM-free	19	1	0	0		

In the PROM group, 11 samples (55%) expressed strong p53; 2 samples (10%) expressed moderate p53, and 7 samples (35%) were absent of p53. In the without PROM groups, 1 sample (5%) expressed weak p53, and 19 samples (95%) were absent of p53. There were significant differences in p53 expression within the PROM group which was higher than in the without PROM groups (11.15 ± 5.59% vs 0.95 ± 2.52%) with p=0.001 using a Mann Whitney U-Test. The value of x^2 was 19.538 using a Chi-Square Test as presented in Table 3.

Table 4: Correlation between p53 Expression and PROM

Variable		PROM		PR	CI 95%	P
		Yes	No			
p53 Expression	Positive	13	1	3.449	1.801 – 6.605	0.001
	Negative	7	19			

There was a strong correlation between p53 expression and PROM with PR = 3.449 (CI95% = 1.801-6.605; p=0.001). This finding reveals that p53 was a proapoptotic protein that plays the role of a regulator of apoptosis through intrinsic pathways and had a strong correlation as a risk factor of PROM (PR > 1).

The PROM group had a strong apoptotic index based on 19 samples (95%) and a weak apoptotic index in 1 sample (5%). The without PROM groups had a strong apoptotic index based on 1 sample (5%) and a weak apoptotic index based on 19 samples (95%). The PROM group had an apoptotic index of 8-30%. Of the twenty samples from the without PROM groups, most of them had a weak amniotic-cell apoptotic index based on IHC TUNEL. One sample of the without PROM groups had a strong amniotic-cell apoptotic index, with a value of 11%. The amniotic cell's apoptotic index in the without PROM groups had a range of 0-11%. The data on the apoptotic index microscopy of the PROM and without PROM groups are presented in Figure 2.

There were significant differences between the apoptotic index of the PROM group which was higher than the without PROM groups (19.10 ± 5.63% vs 1.15 ± 2.46%) with x^2 = 32.40 and p = 0.001 using a Chi-Square and a Mann Whitney U-Test as presented in Table 6. There was a strong correlation between the apoptotic index and the occurrence of PROM with a value of PR = 19 (CI 95% = 2.81-128.69; p = 0.001) as presented in Table 7.

Table 5: The Distribution Value of Amniotic Cells Apoptotic Index of the PROM Group and the without PROM groups

Variable	PROM Group (n = 20)		Without PROM Group (n = 20)		p
	Mean	SD	Mean	SD	
Apoptotic Index	19.10	5.63	1.15	2.46	0.001

The results of the Chi-Square Test revealed that the value of χ^2 = 32.40 and p = 0.001. This finding shows there were significant differences in the amniotic-cell apoptotic index between the PROM group and the without PROM groups (p < 0.05). The apoptotic index of the PROM group was higher than the without PROM groups.

Table 6: The Differences between the Amniotic-Cell Apoptotic Index of the PROM and without PROM groups

Group	Amniotic Cells Apoptotic Index		χ^2	p
	Weak	Strong		
PROM	1	19	32.40	0.001
PROM-free	19	1		

The stronger p53 expression in the PROM group was also associated with a stronger apoptotic index compared to the PROM-free group.

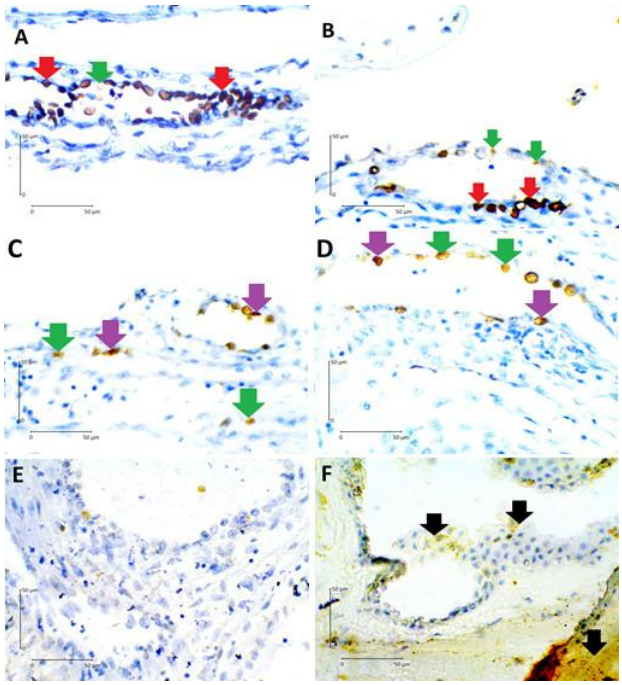

Figure 1: Protein 53 Expression in PROM and Without PROM Groups. A and B: Sample number 6 of the PROM group, p53 score 3+, strong expression (30%). Total 210 epithelial and stromal amniotic cells on A and B, 66 nuclei complete stained with strong intensity (red arrow), 4 nuclei incomplete stained with weak intensity (green arrow), and the others, 140 cells, negative. C and D: Sample number 15 of the without PROM groups, p53 score 3 1+, weak expression (11%). Total 200 epithelial and stromal amniotic cells on C and D, 8 nuclei incomplete stained with moderate intensity (purple arrow), 14 nuclei incomplete stained with weak intensity (green arrow), and the others, 178 nuclei, negative. E and F: Sample number 7 of the without PROM groups, p53 score 0, negative expression (0%). All of the 200 epithelial and stromal amniotic cells on E dan F were negative for p53 IHC. Figure F: artefact pool of residual IHC reagent brown on the part of the amniotic cell cytoplasm and the extracellular (black arrow), although there were no stained nuclei. 400 x magnification. Score 0: negative expression, score 1+: weak expression, score 2+: moderate expression, score 3+: strong expression

This result shows the stronger amniotic- cell p53 expression in the PROM group was caused by the higher apoptotic rate, which is mediated by p53 because the p53 expression has been confirmed by the amniotic-cell apoptotic index calculated using TUNEL-based methods.

Table 7: Correlation between Amniotic-Cell Apoptotic Index and PROM

Variable		PROM		PR	IC95%	p
		Yes	No			
Apoptotic Index	Strong	19	1	19.0	2.81 – 128.69	0.001
	Weak	1	19			

The result of a McNemar Test on the amniotic-cell apoptotic index and p53 expression in the PROM group shows a significance level 0.031 (p < 0,05), therefore, it can be concluded there was a correlation between a strong amniotic-cell apoptotic index and strong p53 expression in the PROM group at term pregnancy.

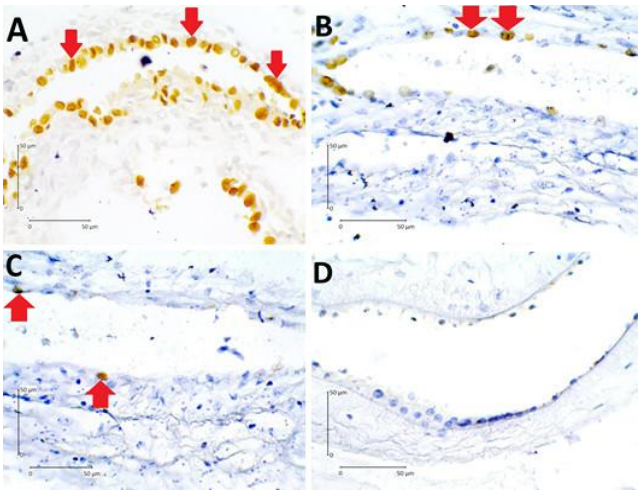

Figure 2: Apoptotic Index of PROM and without PROM Group. A) Sample number 6 of the PROM group. Strong apoptotic index, 30% (> 10%). From a total of 200 epithelial and stromal cells of amniotic membrane on one high power field (HPF), there were 60 nuclei with brown staining in TUNEL IHC (red arrow). B) Sample number 37 of the without PROM groups. Strong apoptotic index, 11% (> 10%). From a total of 100 epithelial and stromal cells of amniotic membrane on one HPF, there were 11 nuclei with brown staining in TUNEL IHC (red arrow). C) Sample number 31 of the without PROM groups. Weak apoptotic index, 2% (≤ 10%). From a total of 100 epithelial and stromal cells of amniotic membrane on 1 HPF, there were two nuclei with brown staining in TUNEL IHC (red arrow). D) Sample number 15 of the without PROM groups. Negative apoptotic index (0%). Total 50 epithelial and stromal cells of amniotic membrane on one HPF, there was no nuclei staining in TUNEL IHC. 400 x magnification. Strong apoptotic index: > 10% and weak apoptotic index: ≤ 10%

Discussion

The present study was conducted from January 2016-April 2018. Samples were taken by consecutive sampling from pregnant patients at Sanglah Hospital (and its educational facilities) in

Denpasar, Bali, in 2016. Inclusion criteria were: 37-42 weeks GA, single live fetus, no sign of maternal infection, and the mother's agreement to participate in this study. Exclusion criteria were: a history of preterm PROM in a previous pregnancy, polyhydramnios, Gemelli, macrosomia, and sexual intercourse in the previous 24 hours. A previous study on the epidemiology of PROM at Sanglah Hospital in 2015 showed the prevalence of labour with PROM was 179 cases (83.43%), while preterm PROM was 33 cases (15.57%) [19]. A comparative study from India reported that the prevalence of PROM at term pregnancy was 82.1% while the prevalence of preterm PROM was 17.6 % [20]. The results of the present study at Sanglah Hospital (and those of the Indian study as a comparison) were no different to the prevalence in the variation of PROM cases globally; with PROM affecting a total of 5-10% cases of all labours [21]. The premature rupture of membrane represents an as-yet unsolved problem in obstetrics: PROM accounts for more than a 24-hour increase in maternal mortality (68%), higher neonatal mortality (37.5%), and neonatal death (2%) [22].

The characteristics of the current study's samples show no differences in mother's age, gestational age, BMI, and gravida between the PROM and without PROM groups. These findings are consistent with a previous study on PROM carried out at Sanglah Hospital which revealed significance regarding the comparison of mother's age, GA, and BMI in the PROM and PROM-free groups that showed no significant statistical differences with all values $p > 0.05$ [23].

The cases of PROM were higher in the primigravida subjects with 10 cases of PROM from a total of 20 cases, which accounts for 50% of PROM cases in this study. Primigravida in the without PROM groups were 7 cases from a total of 20, which accounts for 35% of the without PROM cases. Primigravida in the PROM group was more frequent compared with primigravida in the without PROM groups (50% vs 35%), although a statistical analysis using a Mann Whitney U Test shows a significance value of 0.453 ($p > 0.05$), which represents no significant difference. The 2015 study at Sanglah Hospital found that PROM is more prevalent in primigravida; accounting for 69 cases (32.55%) in 179 births [19]. The finding of this study is that there is no difference between primigravida in PROM and without PROM labour which is consistent with Patil *et al.,* (2014), where the total cases of primigravida with PROM was 53 cases from 100 labours, compared with without PROM primigravida was 52 cases from 100 labours which are not a statistically significant difference [24]. The uteri of primigravida have a relatively low adaptation for gestation processes and so have a bigger risk of PROM.

Protein 53 expressions in the PROM group was higher than in the without PROM groups. The mechanism functions mainly through the intrinsic pathway, with the mitochondria as the centre of the process. The role of p53 begins after its activation by several agents; then p53 activates Bax and inhibits Bcl-2. The main mechanism of Bax on mitochondrial is decreased permeability of the mitochondrial membrane caused by cytochrome c leaking and affecting the Ca^{2+} level by the forming of a channel when Bax collaborates with several types of Bax. This channel becomes the entry point of Ca^{2+} ions: when the ions enter, the cytochrome c moves from the mitochondria to the cytoplasm. The cytochrome c on the cytoplasm will be bonded to the *apoptosis-activating factor* (apaf-1), a protein which is abutted or surrounded by Bcl-2 on the mitochondria's outer surface. The past-1 will then bond and form the CARD domain, and then form an apoptosome (i.e. a holoenzyme, combination of several proteins). This complex is a protease which cuts or degrades other proteins. Apoptosome will activate procaspase-9 to become caspase-9 (the first *caspase* activated by cytochrome c release), then caspase-9 will activate procaspase-3 to become caspase-3. This large amount of caspase-3 will cut the cytoskeleton, forming an apoptotic body, phagocytosis, and ends with the amniotic cells' apoptosis [25].

Numerous studies have revealed that PROM occurs before contractions begin due to a focal defect in an area near to the rupture site [5]. Malak and Bell in 1999 were the first to find an area of "high morphological change" on the amniotic membrane surrounding the cervix [26]. This area makes up 2-10% of the total amniotic membrane surface. A lot of studies support a different, zonal amniotic-membrane concept, especially surrounding the cervical which is significantly weaker compared with other zones due to changes in the biochemical and histological structure.

The amniotic membrane represents the most fragile part (compared with other areas) and is known as the paracervical weak zone where the highest prevalence of apoptosis is found [5]. Another study that supports this finding is Kataoka *et al.,* (2002) that reveals the apoptotic process on the amniotic membrane is highest at the area surrounding the cervix compared with the area at the fundus in PROM vs without PROM patients [22]. This paracervical weak zone had developed before PROM occurred and had a role as an initial breakpoint [26]. The amniotic membrane at this site shows an apoptotic process with an increase of apoptotic marker cleaved-caspase-3, cleaved-caspase-9, and a decrease of Bcl-2; these proteins represent the main proteins of the intrinsic pathway [5]. The other study that supports the current study's finding is by Menon, which reveals that the intrinsic pathway has a role in PROM due to increasing proapoptotic gene expression, p53, and decreasing Bcl-2 antiapoptotic gene expression [27]. Due to this apoptosis-based factor, the amniotic membrane becomes weaker and more susceptible to rupture in term pregnancies [5], [7].

The paracervical weak-zone's strength to

resist rupture is only 60% compared with others areas of the amniotic membrane. The paracervical weak-zone's position is at the centre above the cannabis cervical, and this zone is 10 cm in diameter [28] [29]. Another other study reveals epithelial amniotic cell apoptosis in PROM cases; this apoptosis not only caused weakness of the amniotic membrane but also became an activation factor for metalloprotein which will degrade the extracellular matrix inducing disintegration and this will increase the amniotic membrane's fragility, although the exact mechanism remains unclear [30].

A study by Suhaimi (2010), found a similar finding from an ELISA test: the p53 level was higher in PROM patients compared with those experiencing normal labour. The changes in the pro- and anti-apoptotic proteins at the paracervical area caused weakness in the integrity of the amniotic membrane structure and increased PROM risk. The increase of p53 expression-induced amniotic membrane cell apoptosis through the activation of Bax and then Bax-induced release of cytochrome c [7].

Menon *et al*., (2014) findings reveal that 79% of amnion cells and 89% of chorion cells in preterm PROM cases express p53. The average p53 expression in the preterm PROM group was 72% on amnion cells, and 80% on chorion cells which was higher than p53 expression in without PROM term labour, but the significance level between the two groups was 0.15 ($p > 0.05$) which is not significantly different [31].

Arofah *et al*., (2005) explain their finding that p53 expression in amniotic cells from the edge of a rupture taken from PROM patients was higher than p53 expression in without PROM patients in term pregnancy; the average p53 expression in the PROM group was 21.50 ± 1.27% compared with 5.40 ± 2.12% in the without PROM groups. A strong p53 expression from the edge of the rupture significantly correlated with PROM incidence according to statistical testing [32].

A study of amniotic membrane apoptosis in PROM cases using the IHC TUNEL method to highlight the apoptotic nuclei reveals that apoptotic activity on the amniotic membrane in PROM labour is higher than in without PROM labour [33]. A recent study explains that the proapoptotic protein signalling pathway (including p53) plays a role in the senescence process through amniotic membrane apoptosis which determines the time of labour [8].

Negara *et al*., (2017) reveal that apoptosis is increased in PROM patients through the increase of proapoptotic protein caspase-3, apoptosis-inducing factor (AIF), and Bcl-2. Apoptosis in both the caspase-dependent pathway and the caspase-independent pathway play a role in PROM occurrence [23].

Arofah et al., (2005) study suggests that the amniotic-membrane cell apoptotic index taken from the edge of a membrane rupture site of a PROM group was higher than the without PROM groups at term pregnancy, with the apoptotic-index average of the PROM group 37.30% ± 9,57% vs. 9.80% ± 3,33% in the without PROM groups. Amniotic-cell apoptotic index in this study was performed using a conventional method of routine haematoxylin-eosin staining [32].

A recent study showed that that the p53 signaling pathway played a role in the senescence process through amniotic membrane apoptosis, which would determine the time of labor [8].

In summary, p53 expression and the apoptotic index of amniotic membrane cells in cases of PROM were higher than those in without PROM cases; there was a strong correlation between p53 expression and the apoptotic index with the occurrence of PROM. The strong apoptotic index was correlated with strong p53 expression in PROM cases at term pregnancy.

References

1. Soewarto S. Ketuban Pecah Dini. Ilmu Kebidanan. Edisi Keempat. Jakarta: PT Bina Pustaka Sarwono Prawirohardjo, 2010: 677-82.

2. Getahun D, Strickland D, Ananth CV, Fassett MJ, Sacks DA, Kirby RS, Jacobsen SJ. Recurrence of preterm premature rupture of membranes in relation to interval between pregnancies. American journal of obstetrics and gynecology. 2010; 202(6):570-e1. https://doi.org/10.1016/j.ajog.2009.12.010 PMid:20132922

3. Parry S, Strauss JF. Premature Rupture of Membrane. The New England Journal of Medical. 1998; 338(10):663-70. https://doi.org/10.1056/NEJM199803053381006 PMid:9486996

4. Cunningham FG. 2010. Obstetrics Williams 23rd. Edition. United States of America: McGraw-Hill, 2010:257-9; 804-31.

5. Reti NG, Lappas M, Riley C, Wlodek ME, Permezel M, Walker S, Rice GE. Why do membranes rupture at term? Evidence of increased cellular apoptosis in the supracervical fetal membranes. American journal of obstetrics and gynecology. 2007; 196(5):484-e1. https://doi.org/10.1016/j.ajog.2007.01.021 PMid:17466714

6. Pedro MD, Fatima M. Apoptosis: Molecular Mechanisms. Encyclopedia of Life Science. John Willey and Sons Ltd., 2010.

7. Suhaimi D. Protein P53 Sebagai Faktor Risiko Terjadinya Ketuban Pecah Dini. Indonesian Journal of Applied Sciences. 2012; 2(2).

8. Deng W, Cha J, Yuan J, Haraguchi H, Bartos A, Leishman E, Viollet B, Bradshaw HB, Hirota Y, Dey SK. p53 coordinates decidual sestrin 2/AMPK/mTORC1 signaling to govern parturition timing. The Journal of clinical investigation. 2016; 126(8):2941-54. https://doi.org/10.1172/JCI87715 PMid:27454290 PMCid:PMC4966330

9. Dabbs DJ. Diagnostic immunohistochemistry: theranostic and genomic applications, Philadelphia: Saunders, 2017.

10. Labvision.com. 2018. Procedure of P53 Immunohistochemistry Staining. [Cited March 10]

11. Menezes HL, Jucá MJ, Gomes EG, Nunes BL, Costa HO, Matos D. Analysis of the immunohistochemical expressions of p53, bcl-2 and Ki-67 in colorectal adenocarcinoma and their correlations with the prognostic factors. Arquivos de gastroenterologia. 2010;

47(2):141-7. https://doi.org/10.1590/S0004-28032010000200005 PMid:20721457

12. Etebary M, Jahanzadeh I, Mohagheghi MA, Azizi E. Immunohistochemical analysis of P53 and its correlation to the other Prognostic factors in breast cancer. Acta Medica Iranica. 2002; 40(2):88-94.

13. Yemelyanova A, Vang R, Kshirsagar M, Lu D, Marks MA, Shih IM, Kurman RJ. Immunohistochemical staining patterns of p53 can serve as a surrogate marker for TP53 mutations in ovarian carcinoma: an immunohistochemical and nucleotide sequencing analysis. Modern pathology. 2011; 24(9):1248. https://doi.org/10.1038/modpathol.2011.85 PMid:21552211

14. Winata IG, Suwiyoga IK, Megadhana IW. An Expression of Protein 53 (P53) did not Correlate with Staging of Ovarian Cancer.

15. Rosai J. Rosai and Ackerman's surgical pathology e-book. Elsevier Health Sciences, 2011. PMCid:PMC3698689

16. enogene.com. Procedure of Terminal Deoxynucleotidyl Transferase-Mediated dUTP-Biotin Nick End Labeling TUNEL. [Cited 2018 March 10]

17. Loo DT. TUNEL assay. InIn Situ Detection of DNA Damage Humana Press, 2002:21-30. PMid:12073444

18. Santini D, Tonini G, Vecchio FM, Borzomati D, Vincenzi B, Valeri S, Antinori A, Castri F, Coppola R, Magistrelli P, Nuzzo G. Prognostic value of Bax, Bcl-2, p53, and TUNEL staining in patients with radically resected ampullary carcinoma. Journal of clinical pathology. 2005; 58(2):159-65. https://doi.org/10.1136/jcp.2004.018887 PMid:15677536 PMCid:PMC1770581

19. Budijaya M, Negara KS. Profil Persalinan dengan Ketuban Pecah Dini di RSUP Sanglah Denpasar Periode 1 Januari – 31 Desember 2015. Denpasar: Specialism Study Program, Obstetric and Gynecologic Department of Medical Science Faculty Udayana University/Sanglah Hospital, 2016.

20. Kumar S. Impact of premature rupture of membranes on maternal & neonatal health in Central India. Journal of Evidence Based Medicine and Healthcare. 2015; 2(48):8505-8.

21. Adeniji AO, Atanda OO. Interventions and neonatal outcomes in patients with premature rupture of fetal membranes at and beyond 34 weeks gestational age at a tertiary health facility in Nigeria. British Journal of Medicine and Medical Research. 2013; 3(4):1388. https://doi.org/10.9734/BJMMR/2013/3428

22. Kataoka S, Furuta I, Yamada H, Kato EH, Ebina Y, Kishida T, Kobayashi N, Fujimoto S. Increased apoptosis of human fetal membranes in rupture of the membranes and chorioamnionitis. Placenta. 2002; 23(2):224-31. https://doi.org/10.1053/plac.2001.0776 PMid:11945090

23. Negara KS, Suwiyoga K, Arijana K, Tunas K. Role of Caspase-3 as Risk Factors of Premature Rupture of Membranes. Biomedical and Pharmacology Journal. 2017; 10(4):2091-8. https://doi.org/10.13005/bpj/1332

24. Patil S, Patil V. Maternal and Foetal Outcome in Premature Rupture of Membranes. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2014; 13(12): 56-81. https://doi.org/10.9790/0853-131275683

25. Hongmei Z. Extrinsic and intrinsic apoptosis signal pathway review. In Apoptosis and Medicine. Intech, 2012. https://doi.org/10.5772/50129 PMCid:PMC3705112

26. McLaren J, Malak TM, Bell SC. Structural characteristics of term human fetal membranes prior to labour: identification of an area of altered morphology overlying the cervix. Human reproduction. 1999; 14(1):237-41. https://doi.org/10.1093/humrep/14.1.237 PMid:10374127

27. Menon R, Fortunato SJ. The role of matrix degrading enzymes and apoptosis in repture of membranes. Journal of the Society for Gynecologic Investigation. 2004; 11(7):427-37. https://doi.org/10.1016/j.jsgi.2004.04.001 PMid:15458739

28. Khwad ME, Pandey V, Stetzer B, Mercer BM, Kumar D, Moore RM, Fox J, Redline RW, Mansour JM, Moore JJ. Fetal membranes from term vaginal deliveries have a zone of weakness exhibiting characteristics of apoptosis and remodeling. Journal of the Society for Gynecologic Investigation. 2006; 13(3):191-5. https://doi.org/10.1016/j.jsgi.2005.12.010 PMid:16638590

29. Moore RM, Mansour J, Redline R, Mercer B, Moore JJ. The physiology of fetal membrane rupture: insight gained from the determination of physical properties. Placenta. 2006; 27(11-12):1037-51. https://doi.org/10.1016/j.placenta.2006.01.002 PMid:16516962

30. Wang W, Liu C, Sun K. Induction of amnion epithelial apoptosis by cortisol via tPA/plasmin system. Endocrinology. 2016; 157(11):4487-98. https://doi.org/10.1210/en.2016-1464 PMid:27690691

31. Menon R, Boldogh I, Hawkins HK, Woodson M, Polettini J, Syed TA, Fortunato SJ, Saade GR, Papaconstantinou J, Taylor RN. Histological evidence of oxidative stress and premature senescence in preterm premature rupture of the human fetal membranes recapitulated in vitro. The American journal of pathology. 2014; 184(6):1740-51. https://doi.org/10.1016/j.ajpath.2014.02.011 PMid:24832021

32. Arofah D, Hariadi HR, Watadianto. Perbandingan Indeks Apoptotik dan Derajat Ekspresi p53 Selaput Ketuban pada Ibu Hamil Cukup Bulan yang Disertai Ketuban Pecah Dini dengan yang Tanpa Ketuban Pecah Dini. Surabaya: Spesialism Study Program, Obstetric and Gynecologic Department of Medical Science Faculty Airlangga University/dr. Soetomo Hospital, 2005.

33. George RB, Kalich J, Yonish B, Murtha AP. Apoptosis in the chorion of fetal membranes in preterm premature rupture of membranes. American journal of perinatology. 2008; 25(01):029-32. https://doi.org/10.1055/s-2007-1004828 PMid:18075963

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MicroRNA-150 down Regulation in Acute Myeloid Leukaemia Patients and Its Prognostic Implication

Dalia A. Abdelhalim[1*], Basma M. Elgamal[2], Mona R. ElKafoury[1], Naglaa M. Hassan[2], Marwa M. Hussein[3], Mahmoud M. Elhefnawi[4], Asmaa M. Elfiky[5], Mohammad N. Hamdy[1]

[1]Department of Clinical and Chemical Pathology, Medical Division, National Research Centre, Cairo, Egypt; [2]Department of Clinical Pathology, National Cancer Institute, Cairo University, Cairo, Egypt; [3]Department of Medical Oncology, National Cancer Institute, Cairo University, Cairo, Egypt; [4]Informatics and System Department, Engineering Research Division, National Research Centre, Cairo, Egypt; [5]Environmental and Occupational Medicine Department, Environmental Research Division, National Research Centre, Cairo, Egypt

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***Correspondence:** Dalia A. Abdelhalim. Department of Clinical and Chemical Pathology, Medical Division, National Research Centre, Cairo, Egypt. E-mail: oodaliaadel@yahoo.com

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Abstract

BACKGROUND: MicroRNAs (miRNAs) are small, non-coding RNAs that are important for post-transcriptional gene regulation in both healthy and morbid conditions. Numerous miRNAs promote tumorigenesis, while others have a tumour suppressive effects. Acute myeloid leukaemia (AML) is a heterogeneous group of genetically diverse hematopoietic malignancies with variable response to treatment.

AIM: Our study aimed to investigate the possible role of miR-150 in de novo adult AML and the impact of its level on survival, and we used *in the silicon* analysis to predict the main target genes involved in miR-150 mediated cancer pathway.

MATERIAL AND METHODS: We evaluated miR-150 expression profiling assay using TaqMan primer probes Real time-PCR in the plasma of 50 adult AML patients, before the start of treatment and at day 28 of treatment, along with 20 normal adult control samples. miR-16 was used as an endogenous reference for standardisation. Follow-up of patients during treatment at day 28 of induction chemotherapy and after one year was done.

RESULTS: In this study, we found a significantly lower level of miR-150 in AML patients when compared to controls ($p = 0.005$) with 0.62 fold change than in healthy controls. Patients were divided into two groups: the low miR-150 group (miR-150 $\leq$ 1) and the high miR-150 group (miR-150 > 1). A statistically significant difference was found between the two groups regarding initial total leukocytic count and initial PB blast count while for the TLC, HB and PLT count at follow up. No difference in the overall survival between the low and the high miR-150 groups could be demonstrated.

CONCLUSION: Our results suggest that miR-150 functions as a tumour suppressor and gatekeeper in inhibiting cell transformation and that its downregulation is required for leukemogenesis.

Introduction

Acute myeloid leukaemia (AML) is a clonal disorder of hematopoietic progenitor cells which is characterised by diverse heterogeneity regarding genotypic, phenotypic and clinical features [1], [2]. AML is the most common acute leukaemia in adult patients and can arise “de novo” or as a secondary event [3]. Among the genetic aberrations that control disease development, there are microRNAs.

MicroRNAs (miRNAs) are short non-coding RNAs (~20-24 nucleotides) that are involved in post-transcriptional regulation of gene expression in multicellular organisms. This is achieved by affecting both the stability and translation of mRNAs [4]. miRNAs play an important role in many biological processes in the body such as cell growth, proliferation, differentiation, and apoptosis. They also act as both oncogenes and tumour suppressors contributing to a malignant transformation in solid and haematological tumours, including AML [5].

MiR-150 is a family of microRNA precursors found in mammals, including humans. The mature miRNA sequence is a 22 nucleotide, which is excised from the precursor hairpin by the enzyme Dicer [6]. This sequence then associates with RISC (RNA-induced silencing complex), that directly bind to the potential target site in the 3' untranslated region (3'UTRs) of specific target mRNA, leading to the repression of mRNA translation or the degradation of target mRNAs [7]. In normal hematopoiesis, miR-150 regulates genes whose downstream products encourage the differentiation of stem cells towards becoming megakaryocytes rather than erythrocytes [8]. It is also thought that together with miR-155, control B and T cell differentiation [9].

MiR-150 has been linked to the development of some cancers [10]. Mraz et al. reported that the expression of miR-150 was shown to regulate levels of GAB1 and FOXP1 proteins in malignant and normal B cells, which influences their BCR signalling [11].

Aberrant miRNA expression is a feature of different cancers including haematological malignancies. In a study by Morris et al., they identified that the expression of miR-150 is low or absent in blastic crisis (BC) of chronic myeloid leukaemia (CML) and acute myeloid leukaemia (AML) patients' samples and also in cell lines [12]. They found that the expression of miR-150 in AML cell lines, CD34+ progenitor cells from healthy individuals and primary BC CML and AML patients' samples at levels similar to miR-150 expression in normal bone marrow promotes myeloid differentiation of these cells.

They also reported that in AML cell lines, differentiation of miR-150 expressing cells occurs independently of retinoic acid receptor α (RARA) signalling and that the high throughput gene expression profiling (GEP) studies of the AML cell lines HL60, PL21 and THP-1 suggest that activation of CEPBA, CEBPE and cytokines associated with myeloid differentiation in miR-150 expressing cells, as compared to control cells, contributes to myeloid differentiation. They concluded from these data that miR-150 promotes myeloid differentiation, a previously uncharacterized role for this miRNA and that absent or low miR-150 expression contributes to down-regulated myeloid differentiation in acute leukaemia cells [12].

From two genomes wide large-scale miRNA expression profiling assays of various subtypes of primary AML samples and normal controls, Jiang et al., [13] identified miR-150 as one of the most significantly and consistently down-regulated miRNAs in most of the AML cases they have studied. They furthermore confirmed this significant down-regulation by a subsequent study [14].

Material and Methods

This is a matched case-control study included 50 adults de novo AML cases that were recruited from the Medical Oncology Department of the National Cancer Institute, Cairo University, Egypt, from January 2015 to March 2016. As well as 20 healthy volunteers were included as a control group. Approval from the Ethical Committee was obtained (Medical Ethical Research Committee—National Research Centre, Number P100510) to carry over this study and informed consents were signed by the patients by the Helsinki declaration. The demographic characteristics were documented at a presentation in addition to morphological, cytochemical, immunophenotypic and genetic analyses which were done at initial diagnosis. The follow-up data including the response to treatment and survival were documented for all patients.

Sampling and Extraction of micro-RNA

Two ml of peripheral blood were collected on EDTA from the newly diagnosed adult AML cases (n = 50) before starting therapy, follow up samples were collected at day 28 of treatment for 31 of them. Samples were also collected from the twenty healthy adults.

miRNA was isolated from plasma samples using miRNeasy Mini Kit (Cat number# 217004, Qiagen, USA) as recommended by the manufacturer's instructions, the purity and the concentration of the purified miRNA was detected using spectrophotometer Nano-drop (Maestrogen, Taiwan, MN-913) and stored at -80 ˚C till further assessments.

Detection of miRNA expression using Real-Time PCR

Gene-specific complementary DNA (cDNA) was prepared from miRNA by using reversing TaqMan microRNA RT-Kit, Cat number # 4366596, (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. miRNA expression for enrolled samples was quantified using TaqMan 2x universal master mix II Cat number # 4440043, (Applied Biosystems, Foster City, CA, USA) and TaqMan microRNA Assay Mix containing PCR primers and TaqMan probes for miR-150. MiR-16 was used as endogenous control for normalisation. Fluorescence was acquired and detected by ABI step one- Applied Biosystems. To determine miRNA relative expression, it was reported as fold change (ΔC_t and $\Delta\Delta C_t$ calculations).

The overall survival (OS) was calculated from the date of diagnosis to the date of death or last follow up visit. Disease-free survival was calculated from the date of complete remission to the date of relapse,

death or last follow up visit.

Statistical analysis

The data were presented as mean ± SD or median. The results were calculated using the Student's *t*-test, χ^2, paired sample T-test, Mann-Whitney *U* test or Kruskal-Wallis test where appropriate. Categorical variables were described with count and percentage. Kaplan-Meier method was used to calculate the survival rates and the log-rank test was used to test the significance in the difference in the patients' survival. Statistical analyses were performed using SPSS software (version 16.0 for Windows; SPSS INC., Chicago, IL, USA) where P values were two-tailed and considered statistically significant when less than 0.05.

Bioinformatics analysis

In a silico analysis for the miR-150 was performed to find target genes regulated by miR-150 and identifying the possible cellular pathways in which these target genes are involved. The miRNA target genes were predicted with the miRBase (www.mirbase.org). MicroRNA target gene analysis was performed using miRWalk 2.0 server [http://mirwalk.uni-hd.de/] which is a database that gives both predicted and experimentally validated miRNA-targets [15], [16]. The predicted target genes were obtained with cut off p-value 0.05, in addition to the validated ones. Both predicted and validated target genes were combined to furtherly undergo functional enrichment analysis.

Functional enrichment analysis for the miRNA target genes was done using the DAVID server [Database for Annotation, Visualization and Integrated Discovery], (https://david.ncifcrf.gov) [17], [18]. Pathway enrichment analyses of the predicted miRNA target genes were performed with KEGG pathway (www.genome.jp/kegg) [19].

Results

We included in our study 50 de novo adult AML patients (before starting treatment and at D28 of treatment). Thirty two of them were males (64%) and 18 were females (36%); mean age was (37.48 ± 12.38), as well as 20 age and sex-matched controls. We estimated the expression level of miR-150 in both the patient group initially and at D28 and the control group using TaqMan primer-probe assay Real Time PCR. We also evaluated parameters of clinical importance in the AML group such as; total leukocytic count (TLC), haemoglobin concentration (HB), platelet count (PLTs), bone marrow (BM) cellularity and blast % in both peripheral blood (PB) and bone marrow, initially and at D28. Immunophenotypic markers, cytogenetics and FLT-3 mutational status, were investigated too.

The expression level of miR-150 in both the patients group initially and the control group was estimated using Real-Time PCR. A significant difference between the initial level of miR-150 in patients and controls (p = 0.005) was found.

Plasma initial miR-150 was down-regulated in adult AML with 0.62 fold change than in healthy controls as demonstrated in Figure 1. A statistically significant lower values of relative expression of miR-150 in patients initially and at D28 compared to controls (p = 0.004) (Fig. 2).

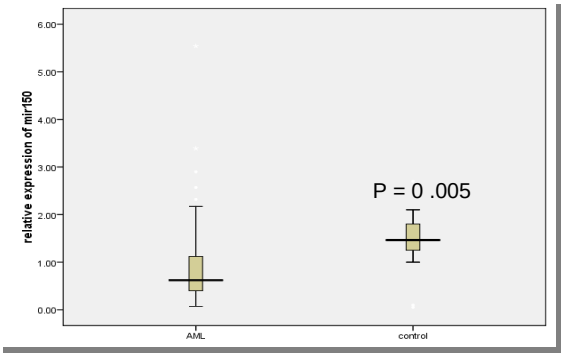

Figure 1: Relative initial plasma miR-150 levels in adult AML patient and control population. Expression levels of miR-150 were normalised to miR-16. Data were represented as the median value, and the Mann-Whitney U test was used to define statistical significance

We followed up our patients for their miR-150 at D28 of treatment. We found a significant decrease in the TLC and increase PLT count when measured initially in patients compared to its measurement at D28. No significant difference was found regarding HB concentration and miR-150 level (Table 1).

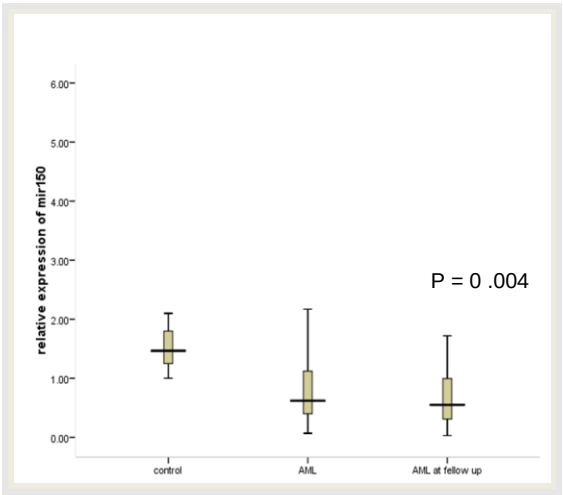

Figure 2: Relative plasma miR-150 levels in AML patients initially, at D28 of treatment and control population. Expression levels of miR-150 were normalised to miR-16. Data were represented as the median value, and Kruskal Wallis Test was used to define statistical significance

Table 1: Demographic and clinical characteristics of patients before treatment and at D28 of treatment

Parameters	Initial value	D28 value	P value
miR-150 n=31	1.1 ± 1.18	0.85 ± 0.92	0.3
TLC x10^9/L	34.5 ± 49.05	3.8 ± 4.1	0.001**
HB gm/dL	7.9 ± 1.5	7.9 ± 1.2	0.9
PLTs x10^9/L	57.96 ± 68.3	1.85x10^2 ± 172.	<0.001**

TLC: total leucocytic count, HB: haemoglobin, PLTs: platelet count, **highly significant statistical difference.

AML patients who achieved complete remission (CR) after induction chemotherapy at D28 were 32 cases (64%), while 7 cases were relapsed (21%) later on. No statistically significant difference was found in the relative expression of miR-150 before and after treatment in patients undergo CR (p = 0.59).

Table 2: Various clinical parameters in low and high groups when miR-150 was estimated initially

Parameters		Mir-150 initial ≤ 1 N = 35	Mir-150 initial > 1 N = 15	P value
Age (years)	Mean ± S.D	39.4 ± 10.9	32.8 ± 14.5	> 0.05
Sex n (%)	Male Female	22 (62.8) 13 (37.2)	10 (66.7) 5 (33.3)	> 0.05
TLC initial x10^9/L	Median Range	9.5 0.7- 42.3	36 1.68-112.58	0.023*
Plts initial x10^9/L	Median Range	40 (2.0-211)	33 (2.0-290)	0.649
HB initial gm/dl	Median Range	7.9 5.6-12.1	7.3 4.8-11.5	0.094
PB initial Blasts%	(mean ± S.D)	40.06 ± 27.85	58.2 ± 25.87	0.036*
BM initial cellularity n(%)	Normocellular Hypocellular Hypercellular Extrahypercellular	9 (25.7) 3 (8.6) 14 (40) 9 (25.7)	3 (20.0) 2 (13.3) 9 (60.0) 1 (6.7)	0.362
BM initial blast%	(mean ± S.D.)	62.6 ± 20.07	67.93 ± 20.0	0.393
FAB classification n(%)	M0 M1 M2 M3 M4 M5 M7 Others (phenotypic)	1 (2.9) 7 (20) 13 (37.1) 1 (2.9) 8 (22.9) 2 (5.7) 2 (5.7) 1 (2.9)	0 1 (6.7) 5 (33.3) 4 (26.7) 2 (13.3) 0 2 (13.3) 1 (6.7)	0.185
IPT n(%)	Myeloid Mono Myeloma Myeloid with aberrant Myeloid-B Others	20(57.1) 1(2.9) 6(17.1) 5(14.3) 1(2.9) 2(5.7)	9 (60) 0 0 2 (13.3) 1 (6.7) 3 (20)	0.353
FLT3 n(%)	Wild Mutant NA	26(74.3) 5(14.3) 4(11.8)	8 (53.3) 4 (26.7) 3 (20)	0.346
Cytogenetics n(%)	T (9, 22) T (8, 21) T (15, 17) Normal karyotype Others	1(4.8) 2(9.5) 1(4.8) 7(33.3) 10(47.6)	0 0 3 (27.3) 6 (54.2) 2 (18.2)	0.138
BM Cellularity N(%) D28	Normocellular Hypocellular Hypercellular NA	13(52) 9(36) 1(4) 2(8)	6 (50) 2 (16.7) 4 (33.3) 0	0.067
Blast n(%) D28	Less than or equal 5 More than 5	18(75.0) 6(25)	9 (75) 3 (25)	0.05
TLC x10^9/L D28	Median Range	2.0 (0.1-16.3)	4.27 (0.56-16)	0.023*
HB gm/dl D28	Median Range	7.7 (3.3-10.3)	8.7 (7-10)	0.013*
Plts x10^9/L D28	Median Range	84.5 (2.00-579)	333 (5-558)	0.014*
PB blasts n(%) D28	Equal zero More than 1	16(76.2) 5(23.8)	7 (87.5) 1 (12.5)	0.50
Response to treatment n(%)	CR Refractory NA	10(62.5) 3(18.8) 3(18.8)	22 (64.7) 6 (17.6) 6 (17.6)	0.98
Relapse	Relapse No relapse NA	2(16.7) 9(75) 1(8.3)	5 (19.2) 17 (65.4) 4 (15.4)	0.79
Early death	Before D28 After D28	3(50) 3(50)	7 (53.8) 6 (46.2)	0.87

TLC: total leucocytic count, HB: haemoglobin, PLT: platelet count, PB: peripheral blood, BM: bone marrow, FAB: French American British classification, IPT: immunophenotyping, D28: day 28 of treatment, NA: non-available, CR: complete remission, OS: overall survival.

Table 2 shows a comparison between different clinical parameters when miR-150 was measured before the start of treatment. Patients were divided into two groups; the low expressers for the miR-150 group (miRNA-150 ≤ 1) and the high expressers for the miR-150 group (miR-150 > 1). A statistically significant correlation was found between high miR-150 and higher initial TLC and PB blast %, while this significant correlation was observed with higher TLC, HB concentration and PLT count at D28 (p = 0.023, 0.036, 0.023, 0.013, 0.014 respectively). A relation also was found between initial low miR-150 and normocellular marrow at D28 samples but not reach a statistical significance (p = 0.067**).**

Table 3 shows a comparison between different clinical and laboratory parameters in the low miR-150 group and the high miR-150 group when miR-150 was measured at D28. A statistically significant relation was found between low miR-150 and myeloid phenotype (p = 0.030) and also with no relapse status for the patients (p = 0.035).

Table 3: Various clinical parameters in low and high groups when miR-150 was estimated at D28

Parameter		miR-150 at D28≤ 1 n = 23	miR-150 at D28> 1 n = 8	P value
Age (years)	Mean ± S.D	33.9 ± 10.5	38.85 ± 10.27	0.262
Sex n (%)	Male Female	10 (45.5) 13 (54.5)	5 (62.5) 3 (37.5)	0.409
TLC x10^9/L initial	Median Range	10 (1.2-42.3)	15.95 (1.68-65.35)	0.963
PLT x10^9/L	Median Range	33 (2.0-208)	18 (9-290)	0.37
HB gm/dL	Median Range	8.1 (5.9-12.1)	7.9 (4.8-9.1)	0.1
PB Blasts%	Median Range	40 (4-94)	18.5 (0-65)	0.139
BM cellularity n(%)	Normocellular Hypocellular Hypercellular Extrahypercellular	5 (21.7) 2 (8.7) 10 (43.5) 6 (26.1)	1 (12.5) 3 (37.5) 4 (50) 0 (0)	0.089
FAB n (%)	M0 M1 M2 M3 M4 M7 Other	1 (4.3) 4 (17.4) 10 (43.5) 2 (8.7) 5 (21.7) 0 (0) 1 (4.3)	0 (0) 1 (12.5) 1 (12.5) 1 (12.5) 2 (25.0) 3 (37.5) 0 (0)	0.084
IPT n (%)	Myeloid Myelomonocytic Myeloid with aberrant Myeloid B Other	15 (65.2) 2 (8.7) 4 (17.4) 1 (4.3) 1 (4.4)	4 (50) 0 (0) 0 (0) 0 (0) 4 (50)	0.030*
FLT-3 n (%)	Wild Mutant NA	19 (82.6) 4 (17.4) 0 (0)	7 (87.5) 0 (0) 1 (12.5)	0.117
Cytogenetics n (%)	t(8,21) t(15,17) Normal karyotype Others	2 (13.3) 2 (13.3) 6 (40) 5 (33.3)	0 (0) 0 (0) 4 (66.7) 2 (33.3)	0.524
BM D28 cellularity n (%)	Normocellular Hypocellular Hypercellular	9 (45) 8 (40) 3 (15)	3 (37.5) 3 (37.5) 2 (25)	0.817
BM Blasts% D28	Median Range Less than or equal 5 More than or equal 6	25 (0-67) 15 (75) 5 (25)	2.5 (0-30) 6 (75) 2 (25)	0.69
TLC x10^9/L at D28	Median Range	2.6 (0.1-16.3)	2.94 (0.38-16)	0.663
HB gm/dL at D28	Median Range	7.9 (3.3-10)	8 (6.9-10.3)	0.788
PLT x10^9/L at D28	Median Range	111.5 (2-558)	110 (5-579)	0.826
PB blasts % at D28	Equal zero More than or equal 1	15 (71.4) 6 (28.6)	7 (100) 0	0.1
Response to treatment	Refractory CR	4 (18.2) 18 (81.8)	2 (25) 6 (71.475)	0.98
Relapse status	No relapse Relapse	16 (84.2) 3 (15.8)	3 (42.9) 4 (57.1)	0.035*
Early death	Before D28 After D28	2 (22.2) 6 (75)	1 (100) 0 (100)	0.13

TLC: total leucocytic count, HB: hemoglobin, PLT: platelet count, PB: peripheral blood, BM: bone marrow, FAB: French American British classification, IPT: immunophenotyping, D28: day 28 of treatment, NA: non-available, CR: complete remission, OS: overall survival, RFS: Relapse-free survival.

Survival analysis

Kaplan-Meier survival curves were used to estimate overall survival (OS) and relapse-free survival (RFS) in the AML patients which are shown in figures (3A and 3B) and are summarised in Table 4.

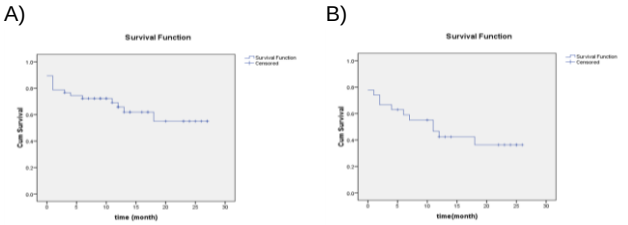

Figure 3: A) Overall survival of AML patients; B) relapse-free survival of AML patients

Moreover, there was no difference in the overall survival between the low and the high miR-150 groups when measured initially before the start of treatment (Figure 4A). Also, there was no difference in the overall survival between the low and the high miR-150 groups when measured at D28 as illustrated in (Figure 4B).

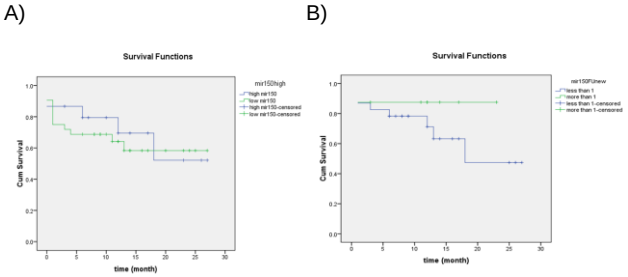

Figure 4: A) Overall survival compared between high and low miR-150 when measured initially; B) Overall survival compared between high and low miR-150 when measured at follow up

Table 4: Overall survival and disease/relapse-free survival

Parameter	AML patients	Mean	No of dead/relapse n (%)	No of alive/ relapse-free n (%)
Overall survival (time /month) (95%CI)	N = 47	17.73 (14.2-21.21)	17 (36.17)	30 (63.8)
Disease/relapse free survival (95%CI)	N = 27	12.82 (8.56-17.07)	16 (59.3)	11 (40.7)

We used in the silico analysis to predict the target genes, as shown in Table 5. We identified the most promising potential target genes and highly significant enrichment pathways for miR-150. The main target genes are *ACOX1, BDNF, RAPGEF3, FGF7, FGF12, PIK3R3, TNF, E2F3, SMAD4, STAT5B* which are involved in *cAMP, MAPK, ErbB, mTOR*, chronic myeloid leukaemia signalling pathway and pathway in cancer.

Most of the gene ontology (GO) annotations were associated with cell process, regulation of biological process, regulation of cell communication, intracellular signalling cascade, localisation and others Figure 5.

Table 5: Prediction of the target genes miR-150 and its pathways

	Target genes	Pathways
miR-150	*ACOX1, PPARA, ADCY1, ADCY2, ADCY5, ADCY6, GABBR1, CNGB1, ADORA1, ATP2B1, BDNF, ATP2B3, ATP2B4, GRIN2B, TIAM1, PDE4A, PAK1, RAPGEF3,*	cAMP signaling pathway
	FGF7, ZAK, PDGFB, FGF9, GNA12, FGF12, MAX, BDNF, MAPT, PAK1, FGF1, IL1A, AKT3, AKT2, PRKCA, BRAF, CACNG8, TP53, CACNG4, CACNG2, PRKCB,	MAPK signaling pathway
	GRB2, CAMK2G, STAT5B, ELK1, PAK3, CAMK2B, SHC1, PAK1, SHC3, PIK3R3, AKT3, PIK3R1, PIK3R2, SHC4, AKT2	ErbB signaling pathway
	PRKCA, TNF, BRAF, STK11, PIK3CB, IGF1, RICTOR, PRKCB, EIF4B, RPS6KA6, RPS6KA3, AKT1S1, TSC1, ULK1, ULK2, ULK3, PRKAA2, PIK3R3, AKT3, PIK3R1, AKT2, PIK3R2	mTOR signaling pathway
	E2F3, BRAF, GRB2, PIK3CB, TGFBR1, CBL, STAT5B, TP53, SMAD4, CDK4, CBLB, CCND1, CDKN1B, ARAF, MDM2, SHC1, PIK3R3, SHC3, CRK, PIK3R1, AKT3, AKT2, PIK3R2, SHC4	Chronic myeloid leukemia signaling pathway
	ADCY1, PPARD, E2F3, ADCY2, FGF7, PDGFB, FGF9, ADCY5, ADCY6, STAT5B, GNA12, SPI1, FGF12, CTNNB1, MAX, PAX8, SLC2A1, FGF1	Pathway in cancer

Discussion

The miRNA network is highly redundant, as a single miRNA could have multiple target mRNAs, and on the other hand, a single mRNA may be targeted by many miRNAs [20]. Many miRNAs have been shown to be involved in a myriad of cellular processes which include differentiation, apoptosis, metabolism and development [21]. Physiologically, as well as pathologically, miRNAs have been reported to play roles in cancers, inflammatory responses, diabetes and autoimmune diseases [22].

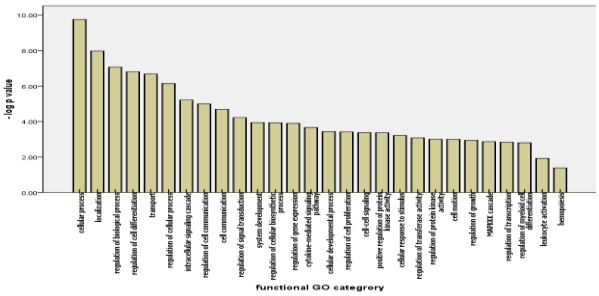

Figure 5: Functional gene ontology terms for miR-150 target genes

In this work, we investigated the role of miR-150 level in de novo adult AML; where we found a significantly lower level in patients when compared to controls (P = 0.012). These results are supported by the clarification of a previously uncharacterized role for miR-150 in promoting myeloid differentiation, where Morris et al. [25], demonstrated that low miR-150 expression contributes to the leukemic phenotype in various AML subtypes and BC of CML. Also, Fayyad-Kazan et al. [14] and Wang et al. [23], reported similar results to ours, where they found lower miR-150 expression in AML patients as

compared to controls (P = 0.0026, P = 0.006 respectively). Also, Xu DD et al. reported that miR-150 was downregulated in leukaemia stem cells (LSCs; CD34+CD38- cells) and clinical samples. Ectopic expression of miR-150 suppressed the LSCs biological behaviours, both *in vitro* and *in vivo* by its effect on Nanog signalling pathway [24].

Similarly, the expression levels of miR-150 were decreased in AML cases as in a study by Morris et al. [12], and overexpression of miR-150 promoted myeloid differentiation of cancer cells and suppressed their proliferation [25]. Also, Fayyad-Kazan et al. [14] reported that in a recent study, plasma levels of tumour necrosis factor alpha, interleukin-10, and interleukin-18, which all have a sequence, complementary to miR-150, were negatively correlated with the plasma levels of miR-150. This could impact on the immune system response to leukaemias. These data revealed that miR-150 could be a promising biomarker for AML at diagnosis and suggest that microRNA expression signature in plasma can serve as a valuable diagnostic and potential prognostic marker for human AML.

MiR-150 was measured twice, first; before the start of treatment, then; at D28 after induction chemotherapy for the 31 cases that they're follow up samples could be reached, the comparison in between did not reveal any statistical significance. Also, the comparison between levels of MiR-150 before treatment and at D28 for cases that undergo complete remission did not reveal any statistical significance. So, our results showed no difference between miRNA levels initially and at D28 with and without discrimination of the response to treatment. This was in contrast to Wang et al. [23] and Fayyad-Kazan et al. [14], who reported that there was up-regulation of miR-150 level in AML patients who achieved CR reaching the level of controls (P < 0.01) compared to its down-regulation before treatment. In our study, this could be explained by the fact that there was no difference in miRNA level between the cytogenetically good and bad prognostic groups of patients in our study, in contrast to Wang et al. [23] and Morris et al. [25], who reported a significant decrease in miRNA level in the poor and intermediate risk cytogenetics groups as compared to the favorable group.

Our patients were divided into two groups; the low expressers for the miR-150 group (miR-150 $\leq$ 1) and the high expressers for the miR-150 group (miR-150 > 1). The correlation between plasma miR-150 levels and clinicopathological data were verified. A statistically significant correlation was found between high miR-150 and both a higher initial TLC and PB blast % and with a higher TLC, HB and PLT count at D28 (p = 0.023, 0.036, 0.023, 0.013, 0.014 respectively). A relation was also found between low miR-150 and normocellular marrow at D28 samples but could not reach a statistical significance (p = 0.067). However, no significance could be recorded with others. Wang et al., [23] reported a significant correlation with the percentage of initial BM blast% (P = 0.020). However no significant difference was found with initial TLC, PLT number (P > 0.05). He also found a significant relation with FAB classification (P = 0.013) and cytogenetics (P = 0.012). However, no significant difference was found between the level of miR-150 and gender, age and extramedullary disease (P > 0.05)

Wang et al. [23], reported that AML patients with M5 subtype had a lower serum miR-150 level than those with other subtypes including M0, M1, M2 and M4 (P < 0.01). Our study included only 2 patients with M5, so, monocytic leukaemias (M4 and M5) in our study were summed up together and showed lower miR-150 level than other FAB subtypes, although with no statistically significant difference. We accordingly expect that with increasing the number of patients, comparable results could be obtained.

We found no statistically significant relation regarding Flt-3 mutational status between the high and low miR-150 groups; either initially or at D28. Our results are supported by Jiang et al. [13], who reported that forced expression of miR-150, reduced the levels of Flt-3 to 40-65%. Their results indicated that Flt3 functions as a direct target of miR-150 in regulating leukemic cell self-renewal and at least in part, responsible for the inhibitory effects of forced expression of miR-150 on leukemogenesis [30].

As regarding the prognostic value of initial plasma miR-150 in AML adult patients, our follow up data did not reveal any relation between different levels of our target miR-150 and either; the spectrum of response to treatment involving CR and refractory cases; those undergo relapse or not, or those undergo early death before D28 or not. But revealed a statistically significant relationship between lower levels of miR-150 at D28 and cases that did not show relapse (P = 0.035). Also, our survival analysis data revealed no significant relation between levels of miR-150 and overall or relapse-free survival, which was inconsistent to Wang et al. [23], who showed that AML patients in his low miR-150 group had significantly shorter five-year overall survival (P = 0.009) and event-free survival (P = 0.004) than patients in his high miR-150 group.

As regard target genes pathway analysis, it showed that the predicted target genes might play their roles through cellular pathways. Those target genes are implicated in proliferation, adhesion, and apoptosis. The main target gene of miR-150 included *AKT2, CBL*, and *PRKCA* [29].

Mir-150 targets and downregulates *AKT2* gene that is involved in Erb, MAPK, CML and mTOR signalling pathway, resulting in reduced the phosphorylated levels of AKtser473/4. Subsequently, this increases the levels of tumour suppressor genes such as *Bim* and *P53*, which leads to telomerase activation and immortalisation of cancer cells [26].

Also, CBL gene is implicated in AML signalling pathway and downregulated by miR-150 [27], [28].

The α isoform of Protein kinase C (PKCα), has been recognised as a tumour growth regulator in different types of cancers. Targeting PKCα-mediated signal transduction induces cell death in AML cells inhibiting BCL-2 phosphorylation and blocking ERK activation [29]. Overexpression of miR-150 significantly suppressed the endogenous expression of PKCα, and luciferase reporter/mutagenesis assays confirmed that PRKCA is a transcriptional target of miR-150 [31].

In conclusion, miRNAs have emerged as a class of gene expression important regulators contributing to AML pathogenesis and as potential biomarkers [32].

We conclude that miR-150 functions as a pivotal gatekeeper in inhibiting cell transformation and functions as a tumour suppressor and its repression are required for leukemogenesis. Moreover, miRNAs regulate different mRNA targets, and their modulation can represent a potential therapeutic target in combination with current chemotherapy in the eradication of leukemic progenitors.

References

1. Dohner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015; 373:1136–1152. https://doi.org/10.1056/NEJMra1406184 PMid:26376137

2. Papaemmanuil E, Dohner H, Campbell PJ. Genomic classification in acute myeloid leukemia. N Engl J Med. 2016; 375:900–901. https://doi.org/10.1056/NEJMc1608739

3. De Kouchkovsky I, Abdul-Hay M. Acute myeloid leukemia: A comprehensive review and 2016 update. Blood Cancer J. 2016; 6: e441. https://doi.org/10.1038/bcj.2016.50 PMid:27367478 PMCid:PMC5030376

4. Vitsios DM, Davis MP, van Dongen S, Enright AJ. Large-scale analysis of microRNA expression, epi-transcriptomic features and biogenesis. Nucleic Acids Res. 2017; 45:1079–1090. https://doi.org/10.1093/nar/gkw1031 PMid:28180281 PMCid:PMC5388392

5. Wallace JA, O'Connell RM. MicroRNAs and acute myeloid leukemia: Therapeutic implications and emerging concepts. Blood. 2017; 130:1290–1301. https://doi.org/10.1182/blood-2016-10-697698 PMid:28751524 PMCid:PMC5600138

6. Ambros V. microRNAs: tiny regulators with great potential. Cell. 2001; 107 (7): 823–6. https://doi.org/10.1016/S0092-8674(01)00616-X

7. Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell. 2005; 123 (4): 631–40. https://doi.org/10.1016/j.cell.2005.10.022 PMid:16271387

8. Edelstein LC, Bray PF. MicroRNAs in platelet production and activation. Blood. 2011; 117(20): 5289–96. https://doi.org/10.1182/blood-2011-01-292011 PMid:21364189 PMCid:PMC3109704

9. Vasilatou D, Papageorgiou S, Pappa V, Papageorgiou E, Dervenoulas J. The role of microRNAs in normal and malignant hematopoiesis. European Journal of Haematology. 2010; 84 (1): 1–16. https://doi.org/10.1111/j.1600-0609.2009.01348.x PMid:19744129

10. Lulla RR, Costa FF, Bischof JM, Chou PM, Bonaldo MF, Vanin EF, Soares MB. Identification of Differentially Expressed MicroRNAs in Osteosarcoma. Sarcoma. 2011; 732690. https://doi.org/10.1155/2011/732690

11. Mraz M, Chen L, Rassenti LZ, Ghia EM, Li H, Jepsen K, Smith EN, Messer K, Frazer KA, Kipps TJ. miR-150 influences B-cell receptor signaling in chronic lymphocytic leukemia by regulating expression of GAB1 and FOXP1. Blood. 2014; 124(1): 84–95. https://doi.org/10.1182/blood-2013-09-527234 PMid:24787006 PMCid:PMC4125356

12. Morris V, Zhang A, Yang T, Derek L, Stirewalt, Ramamurthy R, Meshinchi S, Vivian G. Oehler. MicroRNA-150 expression induces myeloid differentiation of human acute leukemia cells and normal hematopoietic precursors. PLoS One. 2013; 8(9): e75815. https://doi.org/10.1371/journal.pone.0075815 PMid:24086639 PMCid:PMC3782459

13. Jiang X, Huang H, Li Z, Li Y, Wang X, Gurbuxani S et al. Blockade of miR-150 maturation by MLL-fusion/MYC/LIN-28 is required for MLL-associated leukemia. Cancer Cell. 2012; 22(4):524–535. https://doi.org/10.1016/j.ccr.2012.08.028 PMid:23079661 PMCid:PMC3480215

14. Fayyad-Kazan H, Bitar N, Najar M, Lewalle P, Fayyad-Kazan M, Badran R et al. Circulating miR-150 and miR-342 in plasma are novel potential biomarkers for acute myeloid leukemia. J Transl Med. 2013; 11: 31. https://doi.org/10.1186/1479-5876-11-31 PMid:23391324 PMCid:PMC3579719

15. Dweep H, Sticht C, Pandey P, Gretz N. miRWalk-database: prediction of possible miRNA binding sites by "walking" the genes of three genomes. JBI. 2011; 44 (5): 839–47. https://doi.org/10.1016/j.jbi.2011.05.002

16. Dweep, H. and N. Gretz, miRWalk2.0. A comprehensive atlas of microRNA-target interactions. Nat Methods. 2015; 12(8): 697. https://doi.org/10.1038/nmeth.3485 PMid:26226356

17. Dennis Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki, RA. "DAVID: Database for Annotation, Visualization, and Integrated Discovery". Genome Biology. 2003;4 (5): P3. https://doi.org/10.1186/gb-2003-4-5-p3

18. Huang DW, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature Protocols. 2009; 4(1):44–57. https://doi.org/10.1038/nprot.2008.211 PMid:19131956

19. Kanehisa M, Goto S. "KEGG: Kyoto Encyclopedia of Genes and Genomes". Nucleic Acids Res. 2000; 28 (1): 27–30. https://doi.org/10.1093/nar/28.1.27 PMid:10592173 PMCid:PMC102409

20. Wang XS, Gong JN, Yu J, Wang F, Zhang XH et al. MicroRNA-29a and microRNA-142-3p are regulators of myeloid differentiation and acute myeloid leukemia. Blood 2012; 119:4992-5004. https://doi.org/10.1182/blood-2011-10-385716 PMid:22493297

21. Vitsios DM, Davis MP, van Dongen S, Enright AJ. Large-scale analysis of microRNA expression, epi-transcriptomic features and biogenesis. Nucleic (2013) Acids Res. 2017; 45:1079–1090. https://doi.org/10.1093/nar/gkw1031 PMid:28180281 PMCid:PMC5388392

22. Li Y, Gao L, Luo X, Wang L, Gao X et al. Epigenetic silencing of microRNA-193a contributes to leukemogenesis in t (8;21) acute myeloid leukemia by activating the PTEN/PI3K signal pathway. Blood 121: 499-509. https://doi.org/10.1182/blood-2012-07-444729 PMid:23223432

23. Wang Y, Wang J, Yin Z, Zhang W, Hu X, Wang Y. Serum miR-150 as a novel prognostic biomarker for acute myeloid leukemia. Int J Clin Exp Pathol. 2017; 10(6):6906-6911.

24. Xu DD, Zhou PJ, Wang Y, Zhang Y, Zhang R, Zhang L, Chen SH, Fu WY, Ruan BB, Xu HP, et al. miR-150 suppresses the proliferation and tumorigenicity of leukemia stem cells by targeting the Nanog signaling pathway. Front Pharmacol. 2016; 7:439. https://doi.org/10.3389/fphar.2016.00439 PMid:27917123

PMCid:PMC5114241

25. Morris VA, Cummings CL, Korb B, Boaglio S, Oehler VG. Deregulated KLF4 expression in myeloid leukemias alters cell proliferation and differentiation through microRNA and gene targets. Mol Cell Biol. 2015; 36: 559-573. https://doi.org/10.1128/MCB.00712-15 PMid:26644403 PMCid:PMC4751692

26. Watanabe A, Tagawa H, Yamashita J, Teshima K, Nara M, Iwamoto K, et al. The role of microRNA-150 as a tumor suppressor in malignant lymphoma. Leukemia. 2011; 25:1324–1334. https://doi.org/10.1038/leu.2011.81 PMid:21502955

27. Bousquet, Marina, et al. miR-150 blocks MLL-AF9–associated leukemia through oncogene repression. Molecular Cancer Research. 2013; 11(8): 912-922. https://doi.org/10.1158/1541-7786.MCR-13-0002-T PMid:23604034

28. Fleischmann K, Pagel P, von Frowein J, Magg T, Roscher AA, Schmid I. The leukemogenic fusion gene MLL-AF9 alters microRNA expression pattern and inhibits monoblastic differentiation via miR511 repression. Journal of Experimental & Clinical Cancer Research 2016; 35:9. https://doi.org/10.1186/s13046-016-0283-5 PMid:26762252 PMCid:PMC4712549

29. Fang ZH, Wang SL, Zhao JT, Lin Z J, et al. miR-150 exerts antileukemia activity in vitro and in vivo through regulating genes in multiple pathways. Cell death & disease. 2016; 7(9):e2371. https://doi.org/10.1038/cddis.2016.256 PMid:27899822 PMCid:PMC5059860

30. Fernandez N, Cordiner RA, Young RS, Hug N, Macias S, Caceres JF. Genetic variation and RNA structure regulate microRNA biogenesis. Nat Commun. 2017; 8:15114. https://doi.org/10.1038/ncomms15114 PMid:28466845 PMCid:PMC5418625

31. Wallace JA, O'Connell RM. MicroRNAs and acute myeloid leukemia: Therapeutic implications and emerging concepts. Blood. 2017; (130):1290–1301. https://doi.org/10.1182/blood-2016-10-697698 PMid:28751524 PMCid:PMC5600138

32. Trino S, Lamorte D, Caivano A, Laurenzana I, Tagliaferri D, Falco G, et al. MicroRNAs as New Biomarkers for Diagnosis and Prognosis, and as Potential Therapeutic Targets in Acute Myeloid Leukemia. Int J Mol Sci. 2018; 19:460. https://doi.org/10.3390/ijms19020460 PMid:29401684 PMCid:PMC5855682

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Basic Science

Cyclooxygenase-2 Expression and Its Correlation with Primary Tumor Size and Lymph Node Involvement in Nasopharyngeal Carcinoma

Jessy Chrestella[1], Farhat Farhat[2*], Elvita Rahmi Daulay[3], Rizalina Arwinati Asnir[2], Ashri Yudhistira[2], Indah Afriani Nasution[2]

[1]Universitas Sumatera Utara, Fakultas Kedokteran, Pathology, Sumatera Utara, Indonesia; [2]Universitas Sumatera Utara Fakultas Kedokteran, Otorhinolaryngology Head and Neck Surgery Jl. Dr T. Mansyur No. 9, Medan, North Sumatera 20155, Indonesia; [3]Universitas Sumatera Utara Fakultas Kedokteran, Radiology, Medan, Sumatera Utara, Indonesia

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***Correspondence:** Farhat Farhat. Universitas Sumatera Utara Fakultas Kedokteran, Otorhinolaryngology Head and Neck Surgery Jl. Dr T. Mansyur No.9, Medan, North Sumatera 20155, Indonesia. E-mail: farhat@usu.ac.id

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Abstract

AIM: This study aimed to observe the cyclooxygenase-2 expression and its correlation with tumour size and lymph node involvement in nasopharyngeal carcinoma.

METHODS: This study was cross-sectional, that enrolled 126 samples diagnosed with nasopharyngeal carcinoma in Haji Adam Malik General Hospital, Medan, Indonesia which fulfilled the inclusion criteria.

RESULTS: Based on this study, we found that the age peak incidence of nasopharyngeal carcinoma patients about a 41-60-year-old group (57.1%), dominated by men (71.4%). Through histopathological examination, non-keratinizing squamous cell carcinoma is the most predominant type (79.4%). We also found T3 is the most prevalent primary tumour size (32.5%) with prominent lymph node involvement N3 (45.2%), and stage IV (54.8%). Cyclooxygenase-2 overexpression is prevalent among nonkeratinizing squamous cell carcinoma (81.1%), T3 primary tumour size (41.1%), N3 node involvement (60.0%), and IV clinical stage (71.6%). In addition, we found a significant relationship between cyclooxygenase-2 expressions towards tumor size ($p < 0.001$) and lymph node involvement ($p < 0.001$) in nasopharyngeal carcinoma.

CONCLUSION: It is proved that the overexpression of cyclooxygenase-2 will increase the susceptibility of nasopharyngeal carcinoma patients having advanced primary tumour size and lymph node involvement.

Introduction

Nasopharyngeal carcinoma (NPC) is a malignancy originating from nasopharyngeal epithelial cells [1]. NPC incidence is dominated by Asia population, especially South China and South- East Asia [2]. In Indonesia, there are approximately 6.2 of 100,000 people diagnosed with NPC. Based on the data in 2008, NPC becomes the fifth leading cause of cancer mortality with annual incidence 13,000 [3].

Cyclopentane-fatty acid derivatives, largely known as prostaglandin, are produced in the human cell. It is well-known as a chemical mediator of inflammation. Meanwhile, COX or prostaglandin endoperoxidase synthase is an enzyme involved in the formation of prostaglandin [4], [5]. COX has two isoforms: COX-1 and COX-2. For maintaining homeostasis, COX-1 is expressed by most cells. In contrast, COX-2 expression related to some pathological conditions, particularly in malignancy. COX-2 can induce angiogenesis and inhibit apoptosis. Also, COX-2 has a certain role in resistance to cancer immunotherapy [6], [7], [8], [9]. COX-2 converts arachidonic acid into five major prostanoids: PGE_2, PGD_2, PGI_2 (prostacyclin), $PGF_{2\alpha}$, and thromboxane A_2 (TXA_2) [10], [11].

A number of studies have shown that COX-2 will induce angiogenesis by several mechanisms, such as (1) VEGF, it has been explained in the previous section; (2) formation of eicosanoid product (TXA2, PGI2, PGE2), it will directly stimulate endothelial formation, growth, and migration; (3) endothelial cell will be less susceptible to apoptotic by elevation anti-apoptotic Bcl-2 protein expression and activation of the PI3K-Akt pathway; (4) matrix metalloproteinase (MMP) will increase its expression, related to vascular invasion; (5) function of angiogenic protein will increase $\alpha_V\beta_3$ integrin; (6) IL-12 expression as angiogenesis inhibitor will decrease [2], [12], [13], [14], [15], [16], [17]. In another side the effect of COX-2 on angiogenesis, the effect of COX-2 on lymph nodes involvement still poorly understood but there is an opinion that said macrophages had been suggested as a major source of lymphangiogenic growth factor appeared chronic inflammatory lesions [18].

Our study will show that PGE_2 expression, the only one prostanoid, increase as a response to the COX-2 overexpression. COX-2/PGE_2 pathway proved its importance in stimulating myeloid-derived suppressor cells production. Myeloid cells can support tumour growth by suppressing immune function and induce angiogenesis [19], [20], [21], [22]. COX or prostaglandin endoperoxidase synthase is an enzyme involved in prostaglandin formation, and its existence is related to inflammation and tumour growth.

We looked at the COX-2 overexpression and compared them COX has two isoforms: COX-1 and COX-2. COX-2 is associated with prostaglandin synthesis in inflammatory tissues and neoplastic processes. In the previous study, COX-2 overexpression is well-documented involved in oncogenesis for certain malignancy, especially NPC [6], [23], [24], [25].

Material and Methods

In this study researchers aimed to observe the cyclooxygenase-2 expression and its correlation with tumour size and lymph node involvement in nasopharyngeal carcinoma. This cross-sectional study enrolled 126 samples diagnosed with nasopharyngeal carcinoma in Haji Adam Malik General Hospital, Medan, Indonesia which fulfilled the inclusion criteria. This study was conducted to analyse the correlation between cyclooxygenase-2 overexpression with tumour size and lymph node involvement in nasopharyngeal carcinoma. It was also expected that it could be used as one of the factors that affect the prognosis in patients with nasopharyngeal carcinoma.

This cross-sectional study conducted in Haji Adam Malik General Hospital and pathology department, Medical Faculty of Universitas Sumatera Utara (USU), Medan, Indonesia. This study enrolled 126 patients diagnosed with NPC by doing anamnesis, physical examination, imaging studies, and histopathological examination.

Then, the samples had to fulfil the inclusion criteria, such as NPC patient diagnosed by histopathological examination and also had never undergone radiotherapy, chemotherapy, or in combination. If the paraffin blocks were not in good condition and the patients were positive with other malignancy, the subject would be excluded from the study. We used non-probability consecutive sampling to avoid tendency and bias and provided the demographic data including gender, age, and some variables related to the samples, condition, including histopathologic type (based on World Health Organization/WHO).

While primary tumour size (T), lymph node involvement (N), and clinical staging were listed based on the American Joint Committee on Cancer (AJCC) 2010 classification. Tissue sections from paraffin block embedded NPC biopsies were stained with Genetex Human COX-2 Antibody. Slides were incubated with the universal peroxidase-labelled polymer (PolyVuePlus) HRP/DAB Detection System and counterstained with hematoxylin. Then, the result classified into four categories by using broad and intensity score, such as 0 = negative, 1 = < 10% of the cell stained or weak-stained, 2 = 10-50% of cells stained or moderate-stained, 3 = > 50% of cells stained or strong-stained. COX-2 immunostaining expression on tissue sections of paraffin block NPC biopsies can be seen in Figure 1.

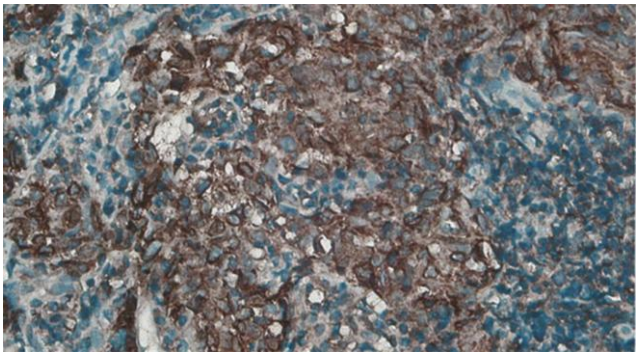

Figure 1: Strong cytoplasmic expression of COX-2 in non-keratinizing squamous cell nasopharyngeal carcinoma (x 400)

Eventually, the final score was obtained by multiplying broad scores with intensity scores. The score was called as immunoreactive scores, 4 or more defined as positive or overexpression. Histopathological examination and the process related to immunohistochemical staining were done by three professional pathologists.

The statistical analysis was done by using SPSS 16.0 software (SPSS, Chicago, IL). Demographic data were listed in a univariate variable. Then, bivariate analysis was performed using chi-square (χ^2) test to determine the relationship between COX-2 overexpression with primary tumour size and lymph node involvement in NPC. This study also had been approved by the Health Research Ethical Committee of Medical Faculty of Universitas Sumatera Utara, Medan, Indonesia.

Results

This study was conducted on 126 NPC patients who fulfilled the inclusion criteria. The highest prevalence of NPC was found in the age group of IV to VI decades (57.1%), dominated by males (71.4%) with non-keratinizing squamous cell carcinoma (79.4%), primary tumor size T3 (32.5%), lymph node involvement N3 (45.2%), and clinical stadium IV (54.8%). Based on immunohistochemical examination overexpression of COX-2 were also found in nonkeratinizing squamous cell carcinoma, primary tumour size T3, lymph node involvement N3, and clinical stadium IV. Based on the results of this study, we found a significant relationship between COX-2 expression with tumour size and enlarged lymph nodes with a value of $p < 0.001$.

Table 1: COX-2 overexpression frequency based on histopathology type, lymph node involvement (N) frequency, and clinical staging

Type	COX-2 Expression				
Over-expression		**%**	**Negative**	**%**	***P*****
Histopathology					0.253
*Keratinizing SCC**	7	(7.4)	1	(12.5)	
*Non-keratinizing SCC**	77	(81.1)	23	(23)	
Undifferentiated carcinoma	11	(11.6)	7	(38.9)	
Primary Tumor Size (T)					0.000
T1	5	(5.3)	20	(64.5)	
T2	24	(25.3)	8	(25.8)	
T3	39	(41.1)	2	(6.5)	
T4	27	(28.4)	1	(3.2)	
Lymph node involvement (N)					0.000
N0	9	(9.5)	18	(58.1)	
N1	14	(14.7)	5	(16.1)	
N2	15	(15.8)	8	(25.8)	
N3	57	(60.0)	0	(0.0)	
Clinical staging					0.000
I	2	(2.1)	15	(48.4)	
II	7	(7.4)	9	(29.0)	
III	18	(18.9)	6	(19.4)	
IV	68	(71.6)	1	(3.2)	

*SCC: Squamous Cell Carcinoma
**p: P-value

Discussion

In our study, a positive correlation between COX-2 overexpression with primary tumour size and lymph node involvement was proved in NPC. It explains COX-2 overexpression is more common in advanced primary tumour size and lymph node involvement. Bin Yang et al., also discovered the same result related to COX-2 overexpression and malignant process, including metastasis [26]. A study conducted by Gui Yang et al., also found that COX-2 and advanced clinical stage of NPC are positively correlated (OR 5.39; 95% CI: 3.79-7.66) [27]. Also, One study conducted by Li et al. found by using nasopharyngeal carcinoma cell lines treated with nonsteroid anti-inflammatory drugs (NSAIDs), particularly celecoxib, invasion, and migration will decrease through suppression of MMP-2 and -9 activity [28]. Fendri et al., also discovered the similar result related to COX-2 overexpression that There was a significant association between COX-2 expression with lymph node involvement (N+) in NPC patients with $p < 0.0001$ [29].

Besides VEGF, Epidermal growth factors (EGF) also play an important role in tumour proliferation and invasion. Ross found that COX-2 overexpression occurred in 79% of NPC patients, and it related to EGFR status but not with latent membrane protein (LMP) -1 or inducible NOS. Meanwhile, Tan K-B prevailed that COX-2 might be involved in the multistep process of NPC carcinogenesis since the COX-2 expression is more common in a dysplastic nasopharyngeal cell [30], [31]. It is also stated by Kwong et al. which enrolled 53 NPC patients, all patients who had intense staining for COX-2 were dysplastic [32]. Many clinical studies have shown that COX-2 induces angiogenesis, but there is no evidence to prove the relationship between COX-2 and lymph node involvement although many previous studies suggested that expression of COX-2 correlated with lymph node metastasis [18].

Also, a prognostic study conducted by Pan et al. uncovered that survival rate, including overall survival, disease-free survival, locoregional control, and distant metastasis-free survival are also related to COX-2 overexpression. Chen et al. conducted a study in T4 NPC patients treated with radiation therapy, 5-year survival rates for patients who have COX-2 overexpression was 27% compared with 60% in low COX-2 expression group. Furthermore, Xinhua et al., also stated that COX-2 might be used to predict prognosis, particularly local recurrence and distant metastasis [33], [34], [35]. Prognostic study related to COX-2 overexpression was evident in many cancer one of them is glottis cancer, COX-2 overexpression related to more aggressive tumour and low survival rate [36], [37], [38]. Otherwise, Long et al., and Y. J. Kim et al. found a contradictive result in our study. By using smaller sample sizes, both studies concluded that COX-2 expression and tumour size did not correlate [39], [40].

Our study provided evidence that the correlation between COX-2 overexpression with primary tumour size and lymph node involvement

are significant ($p < 0.001$). The COX-2 expression will increase as the tumor size increases. COX-2 overexpression is one of the tumor lymphangigenesis factors. Also, COX-2 contributes in the process of carcinogenesis, and it allows COX-2 to be used as a therapeutic target in the future for nasopharyngeal carcinoma.

References

1. Chua ML, Wee JT, Hui EP, Chan AT. Nasopharyngeal carcinoma. The Lancet. 2016; 387(10022):1012-24. https://doi.org/10.1016/S0140-6736(15)00055-0

2. Wang Z, Dabrosin C, Yin X, Fuster MM, Arreola A, Rathmell WK, Generali D, Nagaraju GP, El-Rayes B, Ribatti D, Chen YC. Broad targeting of angiogenesis for cancer prevention and therapy. In Seminars in cancer biology. 2015; 35:S224-S243. https://doi.org/10.1016/j.semcancer.2015.01.001 PMid:25600295 PMCid:PMC4737670

3. Adham M, Kurniawan AN, Muhtadi AI, Roezin A, Hermani B, Gondhowiardjo S, Tan IB, Middeldorp JM. Nasopharyngeal carcinoma in Indonesia: epidemiology, incidence, signs, and symptoms at presentation. Chinese journal of cancer. 2012; 31(4):185. https://doi.org/10.5732/cjc.011.10328 PMid:22313595 PMCid:PMC3777476

4. Divvela AK, Challa SR, Tagaram IK. Pathogenic role of cyclooxygenase-2 in cancer. Journal of Health Science. 2010; 56(5):502-16. https://doi.org/10.1248/jhs.56.502

5. Smith WL, Song I. The enzymology of prostaglandin endoperoxide H synthases-1 and-2. Prostaglandins & other lipid mediators. 2002; 68:115-28. https://doi.org/10.1016/S0090-6980(02)00025-4

6. Chen W, Hu GH. Biomarkers for enhancing the radiosensitivity of nasopharyngeal carcinoma. Cancer biology & medicine. 2015; 12(1):23. PMid:25859408 PMCid:PMC4383846

7. Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. International journal of cell biology. 2010; 2010.

8. Liu B, Qu L, Yan S. Cyclooxygenase-2 promotes tumor growth and suppresses tumor immunity. Cancer cell international. 2015; 15(1):106. https://doi.org/10.1186/s12935-015-0260-7 PMid:26549987 PMCid:PMC4635545

9. Dixon DA. Regulation of COX-2 expression in human cancers. COX-2. 2003; 37:52-71).

10. Rouzer CA, Marnett LJ. Cyclooxygenases: structural and functional insights. Journal of lipid research. 2009; 50(Suppl):S29-34. https://doi.org/10.1194/jlr.R800042-JLR200 PMid:18952571 PMCid:PMC2674713

11. Clària J. Cyclooxygenase-2 biology. Current pharmaceutical design. 2003; 9(27):2177-90. https://doi.org/10.2174/1381612033454054 PMid:14529398

12. Gately S, Li WW. Multiple roles of COX-2 in tumor angiogenesis: a target for antiangiogenic therapy. Seminars in Oncology. 2004; 31:2-11. https://doi.org/10.1053/j.seminoncol.2004.03.040 PMid:15179620

13. Imig JD. Epoxyeicosatrienoic acids and 20-hydroxyeicosatetraenoic acid on endothelial and vascular function. Advances in Pharmacology. 2016; 77:105-141. https://doi.org/10.1016/bs.apha.2016.04.003 PMid:27451096 PMCid:PMC5510644

14. Liu CH, Chang SH, Narko K, Trifan OC, Wu MT, Smith E, Haudenschild C, Lane TF, Hla T. Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. Journal of Biological Chemistry. 2001; 276(21):18563-9. https://doi.org/10.1074/jbc.M010787200 PMid:11278747

15. Huang T, Chen MH, Wu MY, Wu XY. Correlation between expression of extracellular matrix metalloproteinase inducer and matrix metalloproteinase-2 and cervical lymph node metastasis of nasopharyngeal carcinoma. Annals of Otology, Rhinology & Laryngology. 2013; 122(3):210-5. https://doi.org/10.1177/000348941312200311 PMid:23577575

16. Lasek W, Zagożdżon R, Jakobisiak M. Interleukin 12: still a promising candidate for tumor immunotherapy? Cancer Immunology, Immunotherapy. 2014; 63(5):419-435. https://doi.org/10.1007/s00262-014-1523-1 PMid:24514955 PMCid:PMC3994286

17. Gately S. The contributions of cyclooxygenase-2 to tumor angiogenesis. Cancer and metastasis reviews. 2000; 19(1-2):19-27. https://doi.org/10.1023/A:1026575610124 PMid:11191059

18. Iwata C, Kano MR, Komuro A, Oka M, Kiyono K, Johansson E, Morishita Y, Yashiro M, Hirakawa K, Kaminishi M, Miyazono K. Inhibition of cyclooxygenase-2 suppresses lymph node metastasis via reduction of lymphangiogenesis. Cancer Research. 2007; 67(21):10181-9. https://doi.org/10.1158/0008-5472.CAN-07-2366 PMid:17974958

19. Lu X, Qian CN, Mu YG, Li NW, Li S, Zhang HB, Li SW, Wang FL, Guo X, Xiang YQ. Serum CCL2 and serum TNF-α–Two new biomarkers predict bone invasion, post-treatment distant metastasis and poor overall survival in nasopharyngeal carcinoma. European journal of cancer. 2011; 47(3):339-46. https://doi.org/10.1016/j.ejca.2010.09.025 PMid:20951575

20. Elliott LA, Doherty GA, Sheahan K, Ryan EJ. Human tumor-infiltrating myeloid cells: phenotypic and functional diversity. Frontiers in immunology. 2017; 8:86. https://doi.org/10.3389/fimmu.2017.00086 PMid:28220123 PMCid:PMC5292650

21. Schmid MC, Varner JA. Myeloid cells in the tumor microenvironment: modulation of tumor angiogenesis and tumor inflammation. Journal of oncology. 2010; 2010.

22. Cotechini T, Medler TR, Coussens LM. Myeloid cells as targets for therapy in solid tumors. Cancer journal (Sudbury, Mass.). 2015; 21(4):343. https://doi.org/10.1097/PPO.0000000000000132 PMid:26222088 PMCid:PMC4948591

23. Iacovelli N, et al. Emerging prognostic factors in Nasopharyngeal carcinoma. Journal of Naso Pharyngeal Carcinoma. 2014; 1(8).

24. Kim TJ, Lee YS, Kang JH, Kim YS, Kang CS. Prognostic significance of expression of vegf and cox-2 in nasopharyngeal carcinoma and its association with expression of C-erbB2 and EGFR. Journal of surgical oncology. 2011; 103(1):46-52. https://doi.org/10.1002/jso.21767 PMid:21031415

25. Chou J, Lin YC, Kim J, You L, Xu Z, He B, Jablons DM. Nasopharyngeal carcinoma—review of the molecular mechanisms of tumorigenesis. Head & Neck: Journal for the Sciences and Specialties of the Head and Neck. 2008; 30(7):946-63. https://doi.org/10.1002/hed.20833 PMid:18446839 PMCid:PMC3046044

26. Wu YD, Zhou BP. TNF-α/NF-κB/Snail pathway in cancer cell migration and invasion. British journal of cancer. 2010; 102(4):639. https://doi.org/10.1038/sj.bjc.6605530 PMid:20087353 PMCid:PMC2837572

27. Yang G, Deng Q, Fan W, Zhang Z, Xu P, Tang S, Wang P, Yu M. Cyclooxygenase-2 expression is positively associated with lymph node metastasis in nasopharyngeal carcinoma. PloS one. 2017; 12(3):e0173641. https://doi.org/10.1371/journal.pone.0173641 PMid:28301518 PMCid:PMC5354404

28. Soo R, Putti T, Tao Q, Goh BC, Lee KH, Kwok-Seng L, Tan L, Hsieh WS. Overexpression of cyclooxygenase-2 in nasopharyngeal carcinoma and association with epidermal growth factor receptor expression. Archives of Otolaryngology–Head &

Neck Surgery. 2005; 131(2):147-52. https://doi.org/10.1001/archotol.131.2.147 PMid:15723947

29. Fendri A, Khabir A, Hadhri-Guiga B, Sellami-Boudawara T, Ghorbel A, Daoud J, Frikha M, Jlidi R, Gargouri A, Mokdad-Gargouri R. Overexpression of COX-2 and LMP1 are correlated with lymph node in Tunisian NPC patients. Oral oncology. 2008; 44(7):710-5. https://doi.org/10.1016/j.oraloncology.2007.09.006 PMid:18061524

30. Li WW, Long GX, Liu DB, Mei Q, Wang JF, Hu GY, Jiang JZ, Sun W, Gan L, Hu GQ. Cyclooxygenase-2 inhibitor celecoxib suppresses invasion and migration of nasopharyngeal carcinoma cell lines through a decrease in matrix metalloproteinase-2 and-9 activity. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2014; 69(2):132-7.

31. Tan KB, Putti TC. Cyclooxygenase 2 expression in nasopharyngeal carcinoma: immunohistochemical findings and potential implications. Journal of clinical pathology. 2005; 58(5):535-8. https://doi.org/10.1136/jcp.2004.021923 PMid:15858127 PMCid:PMC1770665

32. Kwong DL, Nicholls J, Sham J. Cyclooxygenase-2 expression in nasopharyngeal carcinoma. International Journal of Radiation. 2007.

33. Pan J, Tang T, Xu L, Lu JJ, Lin S, Qiu S, Chen G, Tham IW. Prognostic significance of expression of cyclooxygenase-2, vascular endothelial growth factor, and epidermal growth factor receptor in nasopharyngeal carcinoma. Head & neck. 2013; 35(9):1238-47. https://doi.org/10.1002/hed.23116 PMid:22972415

34. Chen WC, McBride WH, Chen SM, Lee KF, Hwang TZ, Jung SM, Shau H, Liao SK, Hong JH, Chen MF. Prediction of poor survival by cyclooxygenase-2 in patients with T4 nasopharyngeal cancer treated by radiation therapy: Clinical and in vitro studies. Head & Neck: Journal for the Sciences and Specialties of the Head and Neck. 2005; 27(6):503-12. https://doi.org/10.1002/hed.20178 PMid:15772955

35. Xinhua XU, Guoqing HU, Song LI, Feng XU, Daojun LI, Delan DA, Yan CH. Expression of cyclooxygenase-2 in nasopharyngeal carcinoma and its relation to angiogenesis and prognosis. The Chinese-German Journal of Clinical Oncology. 2006; 5(2):104-7. https://doi.org/10.1007/s10330-005-0400-y

36. Chang BW, Kim DH, Kowalski DP, Burleson JA, Son YH, Wilson LD, Haffty BG. Prognostic significance of cyclooxygenase-2 in oropharyngeal squamous cell carcinoma. Clinical cancer research. 2004; 10(5):1678-84. https://doi.org/10.1158/1078-0432.CCR-03-0354 PMid:15014019

37. Gallo O, Masini E, Bianchi B, Bruschini L, Paglierani M, Franchi A. Prognostic significance of cyclooxygenase-2 pathway and angiogenesis in head and neck squamous cell carcinoma. Human pathology. 2002; 33(7):708-14. https://doi.org/10.1053/hupa.2002.125376 PMid:12196922

38. Loong SL, Hwang JS, Li HH, Wee JT, Yap SP, Chua ML, Fong KW, Tan TW. Weak expression of cyclooxygenase-2 is associated with poorer outcome in endemic nasopharyngeal carcinoma: analysis of data from randomized trial between radiation alone versus concurrent chemo-radiation (SQNP-01). Radiation Oncology. 2009; 4(1):23. https://doi.org/10.1186/1748-717X-4-23 PMid:19591688 PMCid:PMC2715417

39. Kim YJ, Lee SH, Wu HG, Go H, Jeon YK. Immunohistochemical study to evaluate the prognostic significance of four biomolecular markers in radiotherapy of nasopharyngeal carcinoma. J Korean Soc Ther Radiol Oncol. 2010; 28(2):57-63. https://doi.org/10.3857/jkstro.2010.28.2.57

40. Sackett MK, Bairati I, Meyer F, Jobin É, Lussier S, Fortin A, Gélinas M, Nabid A, Brochet F, Têtu B. Prognostic significance of cyclooxygenase-2 overexpression in glottic cancer. Clinical Cancer Research. 2008; 14(1):67-73. https://doi.org/10.1158/1078-0432.CCR-07-2028 PMid:18172254

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Serum Calcium and Magnesium Levels in Normal Ghanaian Pregnant Women: A Comparative Cross-Sectional Study

Robert Djagbletey[1], Ebenezer Owusu Darkwa[1*], Papa Kobina deGraft-Johnson[2], Daniel Akwanfo Yaw Sottie[2], Raymond Essuman[1], George Aryee[1], Ernest Aniteye[1]

[1]Department of Anaesthesia, School of Medicine and Dentistry, College of Health Sciences, University of Ghana, P. O. Box 4236, Accra, Ghana; [2]Department of Anaesthesia, Korle-Bu Teaching Hospital, Accra, Ghana

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***Correspondence:** Ebenezer Owusu Darkwa. Department of Anaesthesia, School of Medicine and Dentistry, College of Health Sciences, University of Ghana, P. O. Box 4236, Accra, Ghana. E-mail: eoddarquah@yahoo.co.uk

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Abstract

BACKGROUND: Pregnancy is described as a normal physiologic state associated with various biochemical changes. Magnesium and calcium are essential macronutrients required for foetal growth. Complications associated with their deficiency during normal pregnancy include; low neonatal birth weight, pre-eclampsia, eclampsia and preterm labour. Changes in serum levels of magnesium and calcium in normal pregnancy have not been extensively studied among Ghanaian women.

AIM: To determine the variation in serum magnesium and calcium levels with gestational age in normal pregnancy in Ghanaian women.

METHODS: A hospital-based comparative cross-sectional study was conducted among 32 normal non-pregnant women (Group A) and 100 normal pregnant women (Group B) attending the clinic at the Korle-Bu Teaching hospital. The group B pregnant women were further divided into Group B1 (n = 33), Group B2 (n = 37) and Group B3 (n = 30) based on their pregnancy gestation as first, second and third trimester respectively. Blood samples were obtained from the antecubital vein of subjects and total serum calcium, magnesium, protein and albumin were estimated. Data obtained were analysed using SPSS for windows version 20. Analysis of variance (ANOVA) was employed to determine the statistical differences between the groups. A p-value of ≤ 0.05 was considered significant.

RESULTS: Mean serum total calcium and magnesium in first, second and third trimester normal pregnant women were 2.14 ± 0.16, 2.13 ± 0.44, 2.13 ± 0.35 mmol/L and 0.77 ± 0.11, 0.77 ± 0.16 and 0.76 ± 0.14 mmol/L respectively. Mean serum total calcium and magnesium levels in non-pregnant women were 2.20 ± 0.16 and 0.80 ± 0.10 mmol/L respectively. There was a statistically non-significant difference in serum total calcium and magnesium between non-pregnant and normal pregnant women, with p-values of 0.779 and 0.566 respectively. Mean total serum protein and albumin in first, second and third-trimester normal pregnant women were 68.42 ± 10.37, 70.46 ± 6.84, 66.70 ± 7.83 g/L and 39.92 ± 3.22, 40.75 ± 8.06, 38.26 ± 3.02 g/L respectively. Mean total serum protein and albumin in non-pregnant women were 73.13 ± 7.02 and 42.94 ± 3.03 g/L respectively. Mean total serum protein and albumin levels were lower in pregnant women as compared to non-pregnant women with the difference being significant in the third trimester (p-values of 0.012 and 0.002).

CONCLUSION: Total serum calcium and magnesium levels in normal pregnancy were non-significantly lower compared to non-pregnant women in Ghana. There was a reduction in total serum protein, and albumin levels during pregnancy with a significant reduction noticed during the third trimester compared to the non-pregnant state.

Introduction

Magnesium and calcium are essential for foetal growth. Various physiological changes occur during pregnancy for maternal adaptation as well as to meet the nutritional demands of the developing foetus [1]. Maternal nutritional status during pregnancy has been reported to influence the development of the foetus [2], [3]. Imbalances in maternal nutritional status and foetal demands can produce catastrophic complications [4], [5] especially in the third trimester where there is a rapid development of the foetus [6]. Hormonal alterations during pregnancy may alter serum nutritional status including macronutrient levels [7].

Various biological processes in the body require calcium as it is the most abundant mineral in

the body. Total blood calcium consists of ionic/free (50-65%), protein-bound mainly albumin (30-45%) and calcium complexed to anions (5-10%) mainly bicarbonate, citrate and lactate [8]. These three forms exist in equilibrium with each other maintaining a constant serum level in health. The readily available form of calcium to cells is the ionised form which therefore reflects accurate physiological calcium status. About 50% of maternal serum calcium is bound to proteins in healthy non-pregnant states. Maternal serum calcium levels have been reported to vary with the gestational age of pregnancy [9], [10]. Most studies have reported a significantly reduced total serum calcium levels in normal pregnancy especially the third trimester of pregnancy [11], [12], [13]. This has been explained on account of dietary deficiency, increasing demand of the growing foetus [14], [15] as well as haemodilution from fluid retention during the latter stages of pregnancy [16]. This is compounded by increasing urinary loss of calcium [17] and a fall in serum albumin levels [18], [19]. A normal growing foetus accumulates about 21g of calcium (range 13-33g) [20]. However, 80% of this accumulation occurs rapidly in the third trimester due to skeletal mineralisation [18], [21]. However, other studies have reported no variation in total serum calcium levels with increasing gestational age [22] while others have reported an increase in maternal serum calcium levels compared to the non-pregnant state [23].

Magnesium is the fourth commonest mineral ion as well as the second commonest intracellular cation in the body. About 50% of total body magnesium is located in bones while the other 50% is mainly located intracellularly. Approximately 1% is found intravascularly, and its level is tightly controlled [24] Magnesium acts as a co-factor for various enzymatic processes. It also has a role in peripheral vascular relaxation [25] Serum levels of magnesium has been reported to be between 1.5-2.1 mg/dl [26]. The decrease in serum magnesium levels with increasing gestational age has been reported in some studies and initiation of labour has been attributed by some to myometrial magnesium deficiency [27], [28]. Maternal to foetal transfer (with almost 80% of the transfer occurring during the third trimester) and haemodilution during pregnancy has been proposed as mechanisms for the observed decrease in serum magnesium during pregnancy [16].

Magnesium is an important mineral for foetal growth. Deficiency of magnesium during pregnancy has been associated with pre-eclampsia, eclampsia, preterm labour and delivery of babies with low birth weight [29].

A wide range of serum magnesium levels has been reported among non-pregnant women and pregnant women at all trimesters [30]. Other studies have reported no difference in serum magnesium levels between pregnant and non-pregnant women [31].

Therefore in the serum levels of total calcium and magnesium during normal pregnancy seem inconclusive. The paucity of literature on the subject in our setting, therefore, necessitated this study.

Material and Methods

This is a comparative cross-sectional study conducted between June and August 2016 at the Korle-Bu Teaching Hospital

The site of this study was the Korle-Bu Teaching Hospital which is the largest tertiary referral hospital in Ghana. The obstetrics and gynaecology department has approximately 400 beds and conducts over 11,000 deliveries annually.

All healthy pregnant and non-pregnant women between the ages of 18 and 35 years inclusive who visited the antenatal and gynaecology clinic of the hospital were included in the study.

Pregnant and non-pregnant women with diabetes and hypertension, renal disease, chronic and acute infections, history of thyroid; parathyroid; pancreatic; hepatic disease, and women who were on calcium and magnesium supplements/medications containing these minerals were excluded from the study.

On fulfilling the inclusion criteria and obtaining informed consent, consecutive recruitment of one hundred and thirty-two (132) women was done, consisting of 32 normal non-pregnant women as controls (Group A) and 100 normal pregnant women (Group B).

Normal pregnant women in Group B were further divided into three groups (B1, B2 and B3) based on the gestational ages of their pregnancies. Group B1 (gestational age 1-13 weeks, n = 33), Group B2 (gestational age 14-28 weeks, n = 37) and Group B3 (gestational age 29-40+ weeks, n = 30).

The age, weight, and height of the recruited women were recorded. Their blood pressures were also measured in the sitting position using the right arm on two occasions separated by 15 minutes interval and averaged using mercury sphygmomanometer and a stethoscope.

Without the application of a tourniquet, four (4) millilitres of venous blood was obtained from the cubital vein of the left arm with the patient in the sitting position using a 19G hypodermic needle and a syringe and the blood sample was immediately transferred into a coded plain test tube. Within two (2) hours of collection, samples were sent to the laboratory and centrifuged at 4,000 rpm for 10 minutes to obtain the serum which was subsequently stored at -20°C before analysis within 24 hours of

sample collection. Serum magnesium and total serum calcium were determined using an atomic absorption spectrometer in acetylene-air flame (Variant 240FS; Varian Australia Pty Ltd, VIC, Australia) with reference ranges of 0.74–1.03 mmol/L and 2.12–2.62 mmol/L, respectively.

Data collected was handled confidentially with anonymous code identifiers for each subject and stored in Microsoft access data base 2010. Analysis of the data was done using the SPSS version 20. Comparison of means of total serum calcium and magnesium between the groups were done using analysis of variance (ANOVA). Statistical significance was considered at a *p*-value < 0.05.

Approval for this study was obtained from the ethical and protocol review committee of the University of Ghana School of Medicine and Dentistry.

Results

There was no significant difference in age, and systolic blood pressure in the groups of women studied. There was, however, a significant difference in BMI, and diastolic blood pressure of the groups of women studied.

Table 1: General characteristics of subjects

Characteristic	Non-pregnant	First trimester	Second trimester	Third trimester	
	n = 32	n = 33	n = 37	n = 30	
	Mean (SD)				*p*-value
Age (years)	31 (5.11)	29 (3.76)	30 (3.97)	30 (2.60)	0.291
BMI (kg/m^2)	29 (6.23)	25 (6.25)	28 (5.52)	30 (5.50)	0.001*
Systolic BP (mmHg)	118 (16.08)	111 (14.70)	112 (12.11)	116 (13.38)	0.118
Diastolic BP (mmHg)	77 (9.35)	71 (10.46)	66 (7.68)	68 (8.54)	< 0.001*

*significant at p ≤ 0.05, kg = kilogram, m = metres, mmHg = millimetres of mercury, g = gram, dl = decilitre SD = standard deviation.

The median (ranges) of parity of subjects were; non-pregnant 1(0-5), first trimester 1(1-2), second trimester 1(0-3) and third trimester 1(0-7). There was no significant difference (*p*-value = 0.965) in parity.

Table 2: Total serum calcium and magnesium levels of subjects

Characteristic	Non-pregnant	First trimester	Second trimester	Third trimester	
	n = 32	n = 33	n = 37	n = 30	
	Mean (SD)				*p*-value
Total serum calcium (mmol/l)	2.20 (0.16)	2.14 (0.16)	2.13 (0.44)	2.13 (0.35)	0.779
Serum magnesium (mmol/l)	0.80 (0.10)	0.77 (0.11)	0.77 (0.16)	0.76 (0.14)	0.566
Serum albumin (g/l)	42.94 (3.03)	39.92 (3.22)	40.75 (8.06)	38.26 (3.02)	0.004*
Total serum protein (g/l)	73.13 (7.02)	68.42 (10.37)	70.46 (6.84)	66.70 (7.83)	0.014*

*significant at p ≤ 0.05, mmol/l = millimole per litre, g/l = gram per litre, SD = standard deviation.

There was no significant change in total serum calcium and magnesium among the groups of women studied. There was, however, a significant difference in serum albumin and total serum protein between the groups of women studied.

Table 3: Serum electrolytes range of values in normal pregnancy and non-pregnant women

Characteristics	Non-pregnant	First trimester	Second trimester	Third trimester
	n = 32	n = 33	n = 37	n = 30
	Range			
Total serum calcium (mmol/l)	1.95-2.66	1.58-2.52	1.21-3.70	1.33-2.83
Serum magnesium (mmol/l)	0.62-1.07	0.45-1.04	0.46-1.09	0.49-1.02

There was a wide range of total serum calcium in normal pregnancy (1.21-3.70 mmol/l) and non-pregnant women (1.95-2.66 mmol/l).

There was a wide range of serum magnesium in normal pregnancy (0.45-1.09 mmol/l) and non-pregnant women (0.62-1.07mmol/l).

Table 4: Pairwise comparison analysis of differences in diastolic blood pressure

Comparison	*p*-value
First trimester *vrs* Second trimester	0.137
First trimester *vrs* Third trimester	0.445
Second trimester *vrs* Third trimester	0.937
First-trimester *vrs* Non-pregnant	0.068
Second trimester *vrs* Non-pregnant	< 0.001*
Third-trimester vrs Non-pregnant	0.001*

*significant at p ≤ 0.05, *vrs* = versus.

There was no significant change in diastolic blood pressure with gestational age among the normal pregnant women. The diastolic blood pressures were however significantly lower among women in the second and third trimester of pregnancy compared to the non-pregnant state.

Table 5: Pairwise comparison analysis of differences in total protein

Comparison	*p*-value
First trimester *vrs* Second trimester	0.719
First trimester *vrs* Third trimester	0.836
Second trimester *vrs* Third trimester	0.239
First-trimester *vrs* Non-pregnant	0.094
Second trimester *vrs* Non-pregnant	0.526
Third-trimester vrs Non-pregnant	0.012*

*significant at p ≤ 0.05, *vrs* = versus.

There was a reduction in total serum protein levels during pregnancy with a significant reduction noticed during the third trimester compared to the non-pregnant state. There was no significant inter-trimester change in total serum protein.

Table 6: Pairwise comparison analysis of differences in Serum albumin

Comparison	*p*-value
First trimester *vrs* Second trimester	0.900
First trimester *vrs* Third trimester	0.557
Second trimester *vrs* Third trimester	0.186
First-trimester *vrs* Non-pregnant	0.078
Second trimester *vrs* Non-pregnant	0.275
Third-trimester vrs Non-pregnant	0.002*

*significant at p ≤ 0.05, *vrs* = versus.

There was a reduction in serum albumin levels during normal pregnancy with a significant

reduction noticed during the third trimester compared to the non-pregnant state. Serum albumin levels showed no significant inter-trimester change.

Discussion

Normal pregnancy is associated with a reduction in systemic vascular resistance secondary to vasodilatation from reproductive hormones such as oestrogen and progesterone [32], [33]. Both systolic and diastolic blood pressures (DBP) have been reported to decrease in normal pregnancy. The reduction in DBP is reportedly greater than the reduction in systolic blood pressure (SBP). We observed a non-significant reduction in systolic blood pressures but a significant reduction in diastolic blood pressure especially in second and third trimesters compared to the non-pregnant state, with no significant inter-trimester change in DBP, a finding consistent with those of other similar studies [32], [34], [35]. The reduction in blood pressure noted in these studies starts in the first trimester and nadirs in the second trimester, with a rise towards the pre-pregnancy levels in the third trimester just as noted in our study. The DBP in the third trimester in this study was still significantly lower compared to the non-pregnant state.

Other studies have reported a non-significant change in blood pressures [36] while others have also noted a progressive rise in blood pressure throughout pregnancy [34], [37], [38]. These studies analysed the blood pressures based on patients' weight before pregnancy or total weight gained during pregnancy, factors which were not considered in this study and may, therefore, account for the differences in our results

We observed a non-significant reduction in total serum calcium levels during normal pregnancy (p = 0.779). This observation is supported by those of Standley et al., [22] and Olatunbosum et al., [39] but contrast those of Bassam Hanna [40] and Sultana et al., [41]. Studies by Bassam Hanna and Sultana et al., argued that the low serum total calcium observed in late normal pregnancy was due to increased foetal demand and physiological haemodilution secondary to increased intravascular volume as a result of significantly reduced serum albumin levels (which is the main calcium binding plasma protein) [40], [41]. In this study, we observed significantly reduced total serum proteins (p = 0.012) and albumin (p = 0.002) in the third trimester compared to the non-pregnant state as observed by Sultana et al.,

However, total serum calcium levels were not significantly reduced. Thus changes in total serum calcium levels in pregnancy may be unrelated to alterations in serum protein levels as noted by Oberst and Plass [42]. The differences in results may be attributed to factors that were not considered in this study but which are known to influence total serum calcium levels including pre-analytical factors such as alterations in blood pH, exercise, postural changes, increased ventilation and diurnal variation, [8], [43], [44] and dietary habits [8], [45], [46]. Another factor that could have affected the total serum protein and albumin levels and therefore total serum calcium levels which were not considered in this study is proteinuria [47], [48]. Differences in seasons and climate (differences in exposure to sunlight and therefore vitamin D synthesis), as well as racial differences, could also account for the differences in results [49], [50], [51].

We also observed a non-significant reduction (p = 0.566) in serum magnesium during the progression of normal pregnancy compared to non-pregnant women as noted by Zohreh and Sara [52] and Deeper V Kanagal et al., [53]. Although a lot of studies have reported a fall in serum magnesium levels during normal pregnancy [3], [28], [54], [55] and has attributed this to foetal demand, haemodilution especially in late pregnancy and urinary loss, Newman (1957) and Archari et al., (1961) reported a non-significant change in serum magnesium levels in both pregnant and non-pregnant women as a result of a wider range of serum magnesium levels in both pregnant and non-pregnant women [30], [31]. In this study, there was a wider range of serum magnesium levels in both non-pregnant and pregnant women. This could, therefore, account for our finding of a non-significant difference in serum magnesium levels in non-pregnant and pregnant women. The dietary history to estimate calcium and magnesium intake of the women in our study was not done. Genetic differences concerning dietary absorption, utilisation and demand by the foetuses, as well as urinary excretion of calcium and magnesium may account for differences between our findings and those of others.

In conclusion, we observed a non-significant reduction in systolic blood pressures but a significant reduction in diastolic blood pressure especially in second and third trimester compared to the non-pregnant state, with no significant inter-trimester change in DBP. There was no significant difference in total serum calcium and magnesium levels in normal pregnancy compared to non-pregnant women in Ghana. There was a reduction in total serum protein, and albumin levels during pregnancy with a significant reduction noticed during the third trimester compared to the non-pregnant state.

Further longitudinal research is however needed to determine the normal ranges of serum magnesium and total calcium levels as well as the influence of dietary intake and the urinary excretion of these electrolytes on serum levels of both pregnant and non-pregnant Ghanaian women.

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References

1. Dutta DC. Textbook of Obstetrics,; New Central Book agency (P) Ltd., 2011; 81:7381-142.

2. Allen LH. Introduction (please, complete the reference).

3. Luke B. Nutritional influences on fetal growth. Clinical obstetrics and gynecology. 1994; 37(3):538-49. https://doi.org/10.1097/00003081-199409000-00007 PMid:7955642

4. King JC. Determinants of maternal zinc status during pregnancy–. The American journal of clinical nutrition. 2000; 71(5):1334S-43S. https://doi.org/10.1093/ajcn/71.5.1334s PMid:10799411

5. Scholl TO, Reilly T. Anemia, iron and pregnancy outcome. The Journal of nutrition. 2000; 130(2):443S-7S. https://doi.org/10.1093/jn/130.2.443S PMid:10721924

6. Brar HS, Rutherford SE. Classification of intrauterine growth retardation. In Seminars in perinatology. 1988; 12(1):2-10. PMid:3287628

7. Zilva JF, Pannall PR, Mayne PD. Clinical chemistry in diagnosis and treatment. London: Lloyd-Luke, 1975. PMid:15637929 PMCid:PMC475734

8. Beckett G, Walker S, Rae P. Peter Ashby Clinical biochemistry (Lecture notes), 2005.

9. Pitkin RM, Reynolds WA, Williams GA, Hargis GK. Calcium metabolism in normal pregnancy: a longitudinal study. American Journal of Obstetrics & Gynecology. 1979; 133(7):781-90. https://doi.org/10.1016/0002-9378(79)90115-7

10. Dahlman T, Sjöberg HE, Bucht E. Calcium homeostasis in normal pregnancy and puerperium: a longitudinal study. Acta obstetricia et gynecologica Scandinavica. 1994; 73(5):393-8. https://doi.org/10.3109/00016349409006250 PMid:8009970

12. Black AJ, Topping J, Durham B, Farquharson RG, Fraser WD. A detailed assessment of alterations in bone turnover, calcium homeostasis, and bone density in normal pregnancy. Journal of Bone and Mineral Research. 2000; 15(3):557-63. https://doi.org/10.1359/jbmr.2000.15.3.557 PMid:10750571

13. Naylor KE, Iqbal P, Fledelius C, Fraser RB, Eastell R. The effect of pregnancy on bone density and bone turnover. Journal of Bone and Mineral Research. 2000; 15(1):129-37. https://doi.org/10.1359/jbmr.2000.15.1.129 PMid:10646122

14. Bidlack WR. Interrelationships of food, nutrition, diet and health: the National Association of State Universities and Land Grant Colleges White Paper. Journal of the American College of Nutrition. 1996; 15(5):422-33. https://doi.org/10.1080/07315724.1996.10718620 PMid:8892167

15. Khastgir G, Studd JW, King H, Abdaila H, Jones J, Carter G, Alaghband-Zadeh J. Changes in bone density and biochemical markers of bone turnover in pregnancy-associated osteoporosis. BJOG: An International Journal of Obstetrics & Gynaecology. 1996; 103(7):716-8. https://doi.org/10.1111/j.1471-0528.1996.tb09845.x

16. Pitkin RM, Gebhardt MP. Serum calcium concentrations in human pregnancy. American Journal of Obstetrics & Gynecology. 1977; 127(7):775-8. https://doi.org/10.1016/0002-9378(77)90256-3

17. Cunningham FG, Gant NF, Leveno KJ, Larry C. Williams obstetrics. The Journal of Midwifery & Women's Health. 2003; 48(5):369. https://doi.org/10.1016/S1526-9523(03)00291-5

18. Kovacs CS. Calcium and bone metabolism in pregnancy and lactation. The Journal of Clinical Endocrinology & Metabolism. 2001; 86(6):2344-8. PMid:11397820

19. Kahn DA, Koos BJ. Maternal physiology during pregnancy. Current Diagnosis and Treatment Obstetrics and Gynecology. 2007; 10:155.

20. Givens MH, Macy IG. The chemical composition of the human fetus. Journal of Biological Chemistry. 1933; 102:7-17.

21. Trotter M, Hixon BB. Sequential changes in weight, density, and percentage ash weight of human skeletons from an early fetal period through old age. The anatomical record. 1974; 179(1):1-8. https://doi.org/10.1002/ar.1091790102 PMid:4821360

22. Standley CA, Whitty JE, Mason BA, Cotton DB. Serum ionized magnesium levels in normal and preeclamptic gestation. Obstetrics & Gynecology. 1997; 89(1):24-7. https://doi.org/10.1016/S0029-7844(96)00380-8

23. Pedersen EB, Johannesen P, Kristensen S, Rasmussen AB, Emmertsen K, Møller J, Lauritsen JG, Wohlert M. Calcium, parathyroid hormone and calcitonin in normal pregnancy and preeclampsia. Gynecologic and obstetric investigation. 1984; 18(3):156-64. https://doi.org/10.1159/000299073 PMid:6489849

24. Shaikh MK, Devrajani BR, Soomro AA, Ali Shah SZ, Devrajani T, Das T. Hypomagnesemia in Patients with Diabetes mellitus. World Applied Sciences Journal. 2011; 12(10):1803-6.

25. Indumati V, Kodliwadmath MV, Sheela MK. The role of serum electrolytes in pregnancy induced hypertension. J Clin Diagnostic Res. 2011; 5(1):66-9.

26. Schouten LJ, Goldbohm RA, van den Brandt PA. Height, weight, weight change, and ovarian cancer risk in the Netherlands cohort study on diet and cancer. American journal of epidemiology. 2003; 157(5):424-33. https://doi.org/10.1093/aje/kwf224 PMid:12615607

27. Hantoushzadeh S, Jafarabadi M, Khazardoust S. Serum magnesium levels, muscle cramps, and preterm labor. International Journal of Gynecology & Obstetrics. 2007; 98(2):153-4. https://doi.org/10.1016/j.ijgo.2007.04.009 PMid:17574257

28. Takaya J, Yamato F, Kaneko K. Possible relationship between low birth weight and magnesium status: from the standpoint of "fetal origin" hypothesis. Magnesium research. 2006; 19(1):63-9. PMid:16846102

29. Makrides M, Crosby DD, Bain E, Crowther CA. Magnesium supplementation in pregnancy. Cochrane Database of Systematic Reviews. 2014(4).

30. Newman RL. Serum electrolytes in pregnancy, parturition, and puerperium. Obstetrics & Gynecology. 1957; 10(1):51-5. PMid:13441219

31. Achari G, Mishra KC, Achari K, Ramkissun R, Upadhyay SN. Serum magnesium in women in the normal state and in certain conditions. Journal of the Indian Medical Association. 1961; 36:93-5. PMid:13681198

32. Mahendru AA, Everett TR, Wilkinson IB, Lees CC, McEniery CM. A longitudinal study of maternal cardiovascular function from preconception to the postpartum period. Journal of hypertension. 2014; 32(4):849-56. https://doi.org/10.1097/HJH.0000000000000090 PMid:24406777

33. Gilligan DM, Badar DM, Panza JA, Quyyumi AA, Cannon RO. Effects of estrogen replacement therapy on peripheral vasomotor function in postmenopausal women. American Journal of Cardiology. 1995; 75(4):264-8. https://doi.org/10.1016/0002-9149(95)80033-O

34. Grindheim G, Estensen ME, Langesaeter E, Rosseland LA, Toska K. Changes in blood pressure during healthy pregnancy: a longitudinal cohort study. Journal of hypertension. 2012; 30(2):342-50. https://doi.org/10.1097/HJH.0b013e32834f0b1c PMid:22179091

35. Sanghavi M, Rutherford JD. Cardiovascular physiology of

pregnancy. Circulation. 2014; 130(12):1003-8. https://doi.org/10.1161/CIRCULATIONAHA.114.009029 PMid:25223771

36. Penaz J, Voigt A, Teichmann W. Contribution to the continuous indirect blood pressure measurement. Zeitschrift fur die gesamte innere Medizin und ihre Grenzgebiete. 1976; 31(24):1030-3. PMid:1020409

37. Nama V, Antonios TF, Onwude J, Manyonda IT. Mid-trimester blood pressure drop in normal pregnancy: myth or reality?. Journal of hypertension. 2011; 29(4):763-8. https://doi.org/10.1097/HJH.0b013e328342cb02 PMid:21178781

38. Gaillard R, Bakker R, Willemsen SP, Hofman A, Steegers EA, Jaddoe VW. Blood pressure tracking during pregnancy and the risk of gestational hypertensive disorders: the Generation R Study. European heart journal. 2011; 32(24):3088-97. https://doi.org/10.1093/eurheartj/ehr275 PMid:21821845

39. Olatunbosun DA, Adeniyi FA, Adadevoh BK. Serum calcium, phosphorus and magnesium levels in pregnant and non-pregnant Nigerians. BJOG: An International Journal of Obstetrics & Gynaecology. 1975; 82(7):568-71. https://doi.org/10.1111/j.1471-0528.1975.tb00688.x

40. Hanna B. The role of calcium correction during normal pregnancy at third trimester in Mosul. Oman medical journal. 2009; 24(3):188. https://doi.org/10.5001/omj.2009.37

41. Sultana MS, Begum R, Akhter QS, Lovely NS, Akhter S, Islam MT. Serum calcium and phosphate level in normal pregnant women. Bangladesh Journal of Medical Science. 2012; 11(3):217-20. https://doi.org/10.3329/bjms.v11i3.11732

42. Oberst WF, Plass ED. The variations in serum calcium, protein, and inorganic phosphorus in early and late pregnancy, during parturition and the puerperium, and in non-pregnant women. The Journal of clinical investigation. 1932; 11(1):123-7. https://doi.org/10.1172/JCI100395 PMid:16694022 PMCid:PMC435802

43. Dominiczak MH. Tietz Textbook of Clinical Chemistry. By CA Burtis and ER Ashwood, editors. Clinical Chemistry and Laboratory Medicine. 1999; 37(11-12):1136.

44. Winkel P, Statland BE, Bokelund H. The effects of time of venipuncture on variation of serum constituents: Consideration of within-day and day-to-day changes in a group of healthy young men. American journal of clinical pathology. 1975; 64(4):433-47. https://doi.org/10.1093/ajcp/64.4.433 PMid:1239188

45. Arneson WL, Brickell JM. Clinical Chemistry: a laboratory perspective. FA Davis, 2007.

46. Walker BR, Colledge NR. Davidson's Principles and Practice of Medicine E-Book. Elsevier Health Sciences, 2013.

47. Harewood WJ, Gillin A, Hennessy A, Armitstead J, Horvath JS, Tiller DJ. The effects of the menstrual cycle, pregnancy and early lactation on haematology and plasma biochemistry in the baboon (Papio hamadryas). Journal of medical primatology. 2000; 29(6):415-20. https://doi.org/10.1111/j.1600-0684.2000.290606.x PMid:11168833

48. Arneson WL, Brickell JM. Clinical Chemistry: a laboratory perspective. FA Davis, 2007.

49. Lips PT, Chapuy MC, Dawson-Hughes B, Pols HA, Holick MF. An international comparison of serum 25-hydroxyvitamin D measurements. Osteoporosis International. 1999; 9(5):394-7. https://doi.org/10.1007/s001980050162 PMid:10550457

50. Sayre RM, Dowdy JC, Shepherd J, Sadig I, Bager A, Kollias N. Vitamin D production by natural and artificial sources. Orlando, Florida, Photo Medical Society Meeting; 1998; 3:1.

51. Mull JW, Bill AH. Variations in serum calcium and phosphorus during pregnancy: I. Normal variations. American Journal of Obstetrics & Gynecology. 1934; 27(4):510-7. https://doi.org/10.1016/S0002-9378(34)90732-8

52. Tavana Z, Hosseinmirzaei S. Comparison of maternal serum magnesium level in pre-eclampsia and normal pregnant women. Iranian Red Crescent Medical Journal. 2013; 15(12). https://doi.org/10.5812/ircmj.10394 PMid:24693379 PMCid:PMC3955494

53. Kanagal DV, Rajesh A, Rao K, Devi UH, Shetty H, Kumari S, Shetty PK. Levels of serum calcium and magnesium in pre-eclamptic and normal pregnancy: A study from Coastal India. Journal of clinical and diagnostic research: JCDR. 2014; 8(7):OC01. https://doi.org/10.7860/JCDR/2014/8872.4537

54. De Swiet M. The respiratory system, in Clinical physiology in obstetrics, G. Chamberlain and F. Broughton Pipkin, Editors. Blackwell Science Ltd: Oxford, 1998:111-128.

55. Susser M. Maternal weight gain, infant birth weight, and diet: causal sequences. The American journal of clinical nutrition. 1991; 53(6):1384-96. https://doi.org/10.1093/ajcn/53.6.1384 PMid:2035466

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Basic Science

Association between Frequency of Prosthesis Cleaning and the Discharge Characteristics and the Tear Film in Subjects with Anophthalmic Socket after Eviceration with Dermis Fat Graft

Tari Adrian, Rodiah Rahmawaty Lubis*, T Siti Harilza Zubaidah

Department of Ophthalmology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

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***Correspondence:** Rodiah Rahmawaty Lubis. Department of Ophthalmology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. E-mail: rahma.lubis@yahoo.com

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Abstract

AIM: To analyze the associations between frequency of prosthesis cleaning with the discharge characteristics and the tear film in subjects with anophthalmic socket post evisceration with dermis fat graft.

SUBJECTS AND METHODS: This study is an analytic observational with cross sectional design study with control. The subjects of the study were unilateral acquired anophthalmic socket after evisceration with dermis fat graft at University of Sumatera Utara General Hospital which amounts to 30 subjects or 60 eyes (30 unilateral anophthalmic sockets, 30 contralateral eyes). Data was obtained from April 2018 to May 2018.

RESULT: There is significant association between frequency of prosthesis cleaning and the frequency of discharge (P = 0.001) and tear film quantity (P = 0.024). There is also significant association between the tear film quantity and the frequency of discharge (P = 0.024).

CONCLUSION: There was a significant association between frequency of prosthesis cleaning with the frequency of discharge and the tear film in subjects with Acquired Unilateral anophthalmic socket post-evisceration with dermis fat graft.

Introduction

After enucleation or evisceration is done, the main goal is to rehabilitate the patient to look normal and live a stress-free life. An ideal ocular prosthesis can be placed 4 to 8 weeks after evisceration or enucleation so that the patient feels comfortable and satisfied cosmetically [1], [2], [3], [4].

Discharge is the second problem after normal eye health, affecting 93% of patients with anophthalmic sockets and having various discharge characteristics. Dry eyes are also a problem in anophthalmic socket patients associated with prosthesis intolerance [5], [6].

Each patient individually has different intervals to remove and clean the prosthesis. Recommendations from the ophthalmologist for the frequency of cleaning of ocular prosthesis also vary. The American Society of Ocularists recommends the removal and cleaning of the prosthesis once a month, but must be inserting immediately after socket irrigation [1], [4].

Subjects and Methods

This is an analytic observational study with cross sectional design with control. The study subjects were all patients who admitted to the Oculoplasty Oncology and Reconstruction Division of University of Sumatera Utara General Hospital Medan who was diagnosed with unilateral acquiredanophthalmic socketpost-evisceration with dermis fat graft from April to May 2018. The sample size was 30 people with age over 18 years. First, the patient identity record that

meets the sample selection criteria and then checks the anterior segment with slit lamp. Followed by schirmer I test were performed for both eyes, the anophthalmic socket side and normal eye. Patients were asked to complete the questionnaire, that was a modification of Pine and associates questionnaire (under the supervision of the researcher). All results are recorded and analyzed.

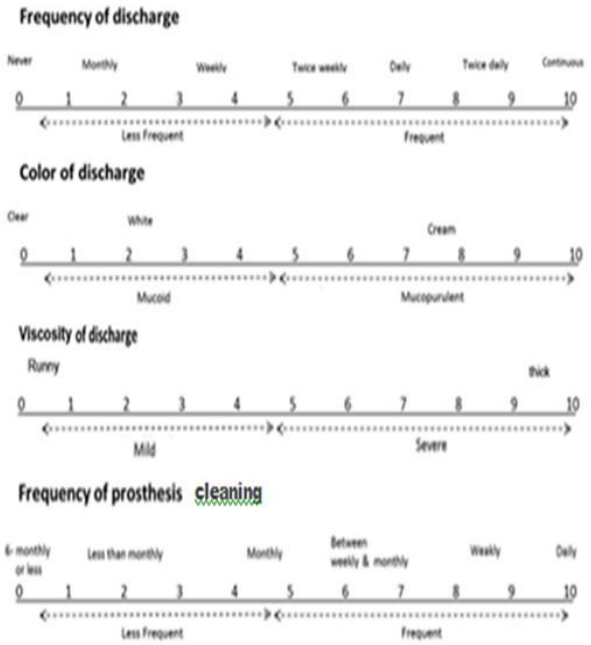

Figure 1: Questionnaire with visual analog scale (10 is the most severe) for self-reporting of the discharge characteristics (frequency, color, and volume) and frequency of prosthesis removal in subjects

Results

There were 30 consecutively recruited subjects with acquired unilateral anophthalmic socket post-evisceration with dermis fat graft, which they were drawn although with a higher ratio of men. The median age of participants was 46, and the youngest participant was 18.

Table 1: Characteristics of Subjects with Acquired Unilateral Anophthalmic Socket Post-Evisceration with Dermis Fat Graft

Characteristic	Study participants, n =30 (100%)
Gender	
Male	21 (70%)
Female	9 (30%)
Median age	
Male	46 years (range 22 - 75) (70%)
Female	50 years (range 18 - 60) (30%)
Median age at eye loss	
Male	34 years (range 10 - 65) (70%)
Female	26 years (range 5 - 57) (30%)
Anophthalmic side	
Left	17 (56.7%)
Right	13 (43.3%)
Reason for eye loss	
Accident	16 (53.3%)
Medical	14 (46.7%)
Median time since prosthesis fitted	12 years (range 1 - 35) (100%)

All the subjects scored 1 or more for all the items in the questionnaire with regard to the discharge characteristics (frequency, color, volume) and frequency of prosthesis cleaning (Table 2). Table 2 shows that of 30 subjects with acquired unilateral anophthalmic socket post-evisceration with dermis fat graft, most frequency of discharge were frequen in 18 people (60%), the color of discharge was mucopurulent in 21 people (70%), and the viscosity of discharge was thicker in 19 people (63.3%).

Table 2: Discharge Characteristics and Frequency of Prosthesis Cleaning Among 30 Subjects with Acquired Unilateral Anophthalmic Socket Post-Evisceration with Dermis Fat Graft

Discharge	Frequency (n) (%)
Frequency	
Less frequent	12 (40%)
Frequent	18 (60%)
Color	
Mucoid	9 (30%)
Mucopurulent	21 (70%)
Viscosity	
Runny	11 (36,7%)
Thick	19 (63,3%)
Frequency of prosthesis cleaning	
Less frequent	12 (40%)
Frequent	18 (60%)

The anophthalmic socket side, as compared to the normal side, showed a significantly lower Schirmer value, it can be concluded that there is a significant difference in the quantity of tear film between anophthalmic socket side and normal side (Table 3).

Table 3: Comparing the ResultsofSchirmer I TestBetween Anophthalmic Socket and Normal Eye of 30 Subjects With Acquired Unilateral Anophthalmic Socket Post-Evisceration with Dermis Fat Graft

	Anophthalmic Socket (30)	Normal (30)	P value
Mean (SD)[range] Schirmer I Test, mm	8 (2,31) [5-12]	15 (3,64) [10-25]	0.001

There was a significant associations between frequency of prosthesis cleaning with discharge and the frequency of discharge in the subjects with acquired unilateral anophthalmic socket post-evisceration with dermis fat graft, P = 0.001 (P <0.05) ,where the frequency of discharge was frequent in subjects who more frequently cleaning the prosthesis (Table 4).

Table 4: Associations Between Frequency of Prosthesis Cleaning withDischargeCharacteristics Among 30 Subjects with Acquired Unilateral Anophthalmic Socket Post-Evisceration with Dermis Fat Graft

Discharge	Frequency of Prosthesis Cleaning				Total		P value
	Less frequent		Frequent				
Characteristics	n	%	n	%	n	%	
Frequency							
Less frequent	12	40	0	0	12	100.0	0.001*
Frequent	0	0	18	60	18	100.0	
Color							
Mucoid	6	66.7	3	33.3	9	100.0	0.062
Mucopurulent	6	28.6	15	71.4	21	100.0	
Viscosity							
Runny	6	54.5	5	45.5	11	100.0	0.197
Thick	6	31.6	13	68.4	19	100.0	

There was a significant associations P =

0.024 (P < 0.05) between the frequency of prosthesis cleaning and the quantity of tear film in the the subjects with acquired unilateral anophthalmic socket post-evisceration with dermis fat graft (Table 5).

The 30 subjects with acquired unilateral anophthalmic socket post-evisceration with dermis fat graft, showed a significantly lower Schirmer valuein the subject with frequent prosthesis cleaning than subject with less frequent prosthesis cleaning (Table 5).

Table 5: Associations between Frequency of Prosthesis Cleaning with Tear Film Among 30 Subjects with Acquired Unilateral Anophthalmic Socket Post-Evisceration with Dermis Fat Graft

Characteristic	Frequency of Prosthesis Cleaning				Total		P value
	Less frequent		Frequent				
	mean	n	mean	n	n	%	
Schirmer I Test,mm	9.08	12	7.17	18	30	100.0	0.024*

A significant association (P = 0.024) between the quantity of tear film with the frequent of discharge, which the tear film quantity is lower than less frequency of discharge (Table 6).

Table 6: Associations between Tear Film with Discharge Characteristics Among 30 Subjects with Acquired Unilateral Anophthalmic Socket Post-Evisceration with Dermis Fat Graft

Discharge Characteristics	Schirmer I Test		P value
	mean	mm	
Frequency			
Less frequent	9.08	12	0.024*
Frequent	7.17	18	
Color			
Mucoid	9	9	0.100
Mucopurulent	7.48	21	
Viscosity			
Runny	8.27	11	0.551
Thick	7.74	19	

Discussion

The anophthalmic socket varies between individuals and their condition and shape are affected by loss of eyes, surgical techniques, types of implants that have accommodated a prosthetic eye and age. This problem willcertainly affect the quality of life of patients. This problem willcertainly affect the quality of life of patients. Problems that can be encountered in the patients anophthalmic socket are the presence of discharge, dry eye and poor prosthesis cosmetics such as not symmetrical with the other eye, the size of the prosthesis is too large or small can cause changes in the eyelid position (pseudoptosis or eyelid retraction), decreased motility prosthesis, discomfort and pain in the anophthalmic socket. Patients with anophthalmia sockets need rehabilitation to look normal and live a stress-free life [1], [2], [5].

Table 1 shows that the most from 30 subjects with acquired unilateral anophthalmic socket post-evisceration with dermis fat graftin males is 21 people (70%), while in women is 9 people (30%). The mean age of the study in males is 46 years and women are 50 years old. The mean ageat eye loss in males is 34 years and women are 26 years old. The anophthalmicsides in the right eye as 17 people (56.7%) and left eye is 13 persons (43.3%). The most reason for eye loss in the study is accident in 16 people (53.3%), while the medical reasonin 14 people (46.7%). The median time since prosthesis fitted is 12 years.

Discharge a common complaint of an anophthalmic socket patient that affects the quality of his life and there may be many underlying causes. Discharge is the glandular product of the bulbi conjunctiva released by goblet cells. The most common cause is giant papillary conjunctivitis. The pathogenesis is a combination of the immunological response to the mechanical trauma of the prosthesis [6], [7].

Using a visual analog scale for doing self-reporting of discharge characteristics (frequency, rare or frequent), color (mucoid or mucopurulent), and viscosity (runny or thick) [7], [8].

Table 2 shows subject with frequent dischargeis 18 people (60%), while less frequent dischargeis 12 people (40%). The most color of discharge is mucopurulent in 21 people (70%), while mukoid in 9 people (30%). The most viscosityof discharge is thick in 19 people (63.3%), while the runnyin 11 people (36.7%). Subject with frequent prosthesis cleaningis 18 people (60%) have ahigher frequency of discharge than subjects with less frequent prosthesis cleaningis 12 people (40%).

Losing of eyeball is accompanied by a rearrangement of the conjunctiva and lacrimal apparatus. Furthermore, following the fitting of an ocular prosthesis, cytological features of the conjunctiva undergo a change, as does the nature of tears. After enucleation or evisceration, the loose conjunctival lining of the newly formed socket adjusts as it heals and there is an inevitable loss of conjunctiva area [1], [2].

The provision of a prosthetic eye restores the fornices, which may have temporarily fore shortened, and returns the eyelids to their original positions where they resume their normal function. The presence of a prosthesis is necessary for basic tear distribution and drainage to resume although it may not operate as efficiently as previously. Lacrimal system efficacy in the anophthalmic socket (with structures intact) greatly depends upon the fit of the prosthesis. The prosthesis entering the anophthalmic socket will be contact with the conjunctiva, the eyelid will moisten the prosthesis with ocular fluid and rest on the surface of the sediment with ocular fluid and collecting precipitate on its surface. Protesa intolerance, tear delivery impeded by conjunctival scarring or lack of sensory reflex impulse to the

lacrimal gland of the ocular surface is often associated as a cause of dry eye in the anophthalmic socket patient [8], [9], [10], [11], [12].

In Table 3 showed that the results of the Independent t-Test can be concluded that there is a significant difference in the quantity of the tear film between the anophthalmic socket with the normal eye, where the quantity of the tear film in the anophthalmic socket is lower than the eye in the normal eye. It is in line with study by Allen at al who reported that the tear volume of the anophthalmic socket is not the same as the normal eye, as the tear volume on the anophthalmia socket have been less than in the normal eye. However, unlike previous study by Kim et al., showed that no statistically significant difference in Schirmer test results between anophthalmic socket with normal eye [12].

Recommendations for the cleaning of ocular prosthesis are no more than 1 month and no less than six months. A monthly cleaning of the prosthesis to remove the precipitate from the prosthesis surface and the ability of the conjunctiva to increase lubricating fluid on the socket reduces mechanical irritation resulting from friction of the prosthesis with conjunctiva and reduces the production of secretions. Mechanical irritation may be caused by the removal of the prosthesis, exposure to foreign materials or bacteria during the cleaning of the prosthesis so that it enters the socket. The presence of sediment is associated with fewer conjunctival inflammations and discharge, and that the sediment is not due to conjunctival inflammation in patients who do not frequently clear the prosthesis. All patients should clean their prosthesis at least six months, since the amount of sediment accumulated during this time may be sufficient before the occurrence of conditions required for GPC or for deposits begin to disrupt the interpalpebral zone where the precipitate dries and physically disrupts the conjunctiva when it flashes [1], [9].

It is assumed that there is an irritating or disruptive effect on the conjunctival anophthalmic socket associated with the removal and reinsertion of the prosthesis during cleaning of the prosthesis. Little is known about the severity of this effect or how long it lasts, it takes further research to determine the severity of conjunctival inflammation to measure inflammation before and after remove and reinsert the prosthesis [7]. Rodiah and Monica, the using contact lens may cause conjunctival inflammation and causeddry eye syndrome [13]. The Chi-Square test shows subjects with frequent prosthesis cleaning had a significantly higher frequency of discharge than subjectswith less frequent prosthesis cleaning,(P = 0.001), whereas color (P = 0.062) and viscosity of discharge(P = 0.197) were not significantly different between these2 groups.

The previous study by Kashkouli MB et al., also found that frequent prosthesis removal had a significantly higher frequency of discharge than subjects with less frequent prosthesis removal, whereas color and volume of discharge were not significantly different between these 2 groups. It is in line with study by Pine KR et al., who reported thatassociations were found between discharge frequency and cleaning regimes with more frequent cleaning accompanying more frequent discharge. No associations were found between color of discharge and cleaning regimes, but viscosity was associated with cleaning regimes and years of wearing with more frequent cleaning accompanying more viscous discharge. Different with previous research by Kim et al., and Chang WJ et al., compared inflammatory conjunctival of anophthalmic socket with contralateral eyes, showed no association between conjunctival inflammation and aspects of prosthesis use, including the frequency cleaning of the prosthesis [7]. Many studies have been explained the etiology of discharge most common and disturbing patient anophthalmic socket. There are two theories about the etiology of discharge: 1) growth of bacterial, specific of the infection process; and 2) reduced tear production [9].

In Table 5 with the Independent t-Test showedsignificant association (P = 0.024) between the frequency of cleaning prosthesis with the quantity of tear film in subject with anophthalmic socket, where the tear film quantity is lower in the subject withfrequent prosthesis cleaning than subject with less frequent prosthesis cleaning. It is in line with study by Kim et al, specimens from patients who cleaned their prosthesis once a day showed significantly less goblet cell density and greater nucleus to cytoplasm ratios at the superior tarsal conjunctiva than those who cleaned less often. The results of Kim et al., did not agree with the earlier results of an investigation by Chang et al., which found no statistical difference in goblet cell density or epithelial cell morphology in 12 anophthalmic patients with giant papillary conjunctivitis.

In Table 6, shows the results of Independent t-Test is a significant association (P = 0.024) between the quantity of tear film with the frequent of discharge, which the tear film quantity is lower than less frequency of discharge, whereas color (P = 0.100) and viscosity of discharge (P = 0.551) were not significantly different between these 2 groups. Allen et al., or Fett et al., directly linked low basic tear production or the use of prosthetic lubrication with the discharge problem.

References

1. American Academy of Ophthalmology: Orbit, Eyelid, and Lacrimal System, section 7, Basic and Clinical Science Course, 2016 – 2017:111 – 119.

2. Goldstein S.M, Lane K, Kherani F. Management of the

Congenital and Acquired Anophthalmic Socket. Ophthalmology Insight Engine. Philadelphia, 2016.

3. Gupta RK, Padmanabhan TV. Prosthetic rehabilitation of a post evisceration patient with custom made ocular prosthesis: A case report. The Journal of Indian Prosthodontic Society. 2012; 12(2):108-12. https://doi.org/10.1007/s13191-012-0115-z PMid:23858284 PMCid:PMC3382363

4. Kashkouli MB, Zolfaghari R, Es' haghi A, Amirsardari A, Abtahi MB, Karimi N, Alemzadeh A, Aghamirsalim M. Tear Film, Lacrimal Drainage System, and Eyelid Findings in Subjects With Anophthalmic Socket Discharge. American journal of ophthalmology. 2016; 165:33-8. https://doi.org/10.1016/j.ajo.2016.02.016 PMid:26930225

5. Rodiah RL. Post-Enuclean Socket Syndrome. 2018. Available at: http://repository.usu.ac.id/handle/123456789/52274. Accesed on 21 May 2018

6. Vaughen Daniel G, Asburi Tailor, Eva-Paul: Oftalmologi Umum, Edisi 17, Widia Medika, Jakarta, 2010:91–95.

7. Kabat AG, Sowka JW. Care for the Anophthalmic Patient,Ophthal Plast Reconstr Sur. 2012.

8. Pine KR, Sloan B, Stewart J, Jacobs RJ. The response of the anophthalmic socket to prosthetic eye wear. Clinical and Experimental Optometry. 2013; 96(4):388-93. https://doi.org/10.1111/cxo.12004 PMid:23336714

9. Pine. K.R, Franzco. B.S, Stewart. J. Protocol for managing mucoid discharge associated with prosthetic eye wear. Clin Experiment Ophthalmol. Australia. 2013.

10. Marcelo. M.C.T et all. Tear and Ocular Surface Profile in Adult Anophthalmic Sockets. Philippine Academy of Ophthalmology. Manila, Philippines. 2012; 37:104-110.

11. Khurana AK. in ComprehensiveOphthalmology, 4rdEdition, New Age InternationalLimited, Publisher, New Delhi, 2007:352 – 53.

12. Kim JH, Lee MJ, Choung HK, Kim NJ, Hwang SW, Sung MS, et al. Conjunctival cytologic features in anophthalmic patients wearing an ocular prosthesis. Ophthalmic Plastic & Reconstructive Surgery. 2008; 24(4):290-295. https://doi.org/10.1097/IOP.0b013e3181788dff PMid:18645434

13. Rodiah RL, Monica THG. The Correlation between Daily Lens Wear Duration and Dry Eye Syndrome. Open Access Maced J Med Sci. 2018; 6(5):829-834.

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Clinical Science

Moxifloxacin in the Outpatient Treatment of Moderate Exacerbations of Chronic Obstructive Pulmonary Disease

Jordan Minov[1*], Sasho Stoleski[1], Tatjana Petrova[2], Kristin Vasilevska[3], Dragan Mijakoski[1], Jovanka Bislimovska-Karadzhinska[1]

[1]Institute for Occupational Health of Republic of Macedonia-WHO Collaborating Center and GA2LEN Collaborating Center, Skopje, Republic of Macedonia; [2]Department of Pharmacy Practice, Chicago State University, Chicago, IL, USA; [3]Institute for Epidemiology and Biostatistics, Skopje, Republic of Macedonia

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Keywords: Antibiotic; Bacterial infection; Chronic obstructive pulmonary disease; Clinical success; Exacerbation; Side effects

***Correspondence:** Jordan Minov. Institute for Occupational Health of Republic of Macedonia-WHO Collaborating Center and GA2LEN Collaborating Center, Skopje, Republic of Macedonia. E-mail: minovj@hotmail.com

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Abstract

BACKGROUND: Bacterial infections are involved in more than a half of the exacerbations of chronic obstructive pulmonary disease (COPD).

AIM: To evaluate the efficacy and safety of moxifloxacin in the outpatient treatment of moderate exacerbations of bacterial origin in the COPD patients.

METHODS: We performed a prospective, observational study including 64 COPD patients with moderate exacerbation of bacterial origin empirically treated with moxifloxacin. In 31 of them, moxifloxacin was used as an initial antibiotic (Group 1), whereas in 33 of them moxifloxacin was used after treatment failure with another antibiotic (Group 2). All patients have treated 7 days with moxifloxacin 400 mg once daily per os, and they were followed up for 20 days, with an intermediate visit at 3, 5 and 7 days at which the duration of symptoms and the side effects of the drug were evaluated.

RESULTS: We registered high clinical success rate, i.e. the complete resolution of the symptoms or their return to the baseline severity, similar in both groups (84.3% in all study subjects, 83.9% in the Group 1 and 84.8% in the Group 2). The mean time to complete resolution of the cardinal symptoms or their return to the baseline severity was 5.2 ± 1.1 days. Also, the mean time to complete resolution of the certain cardinal symptoms (increased dyspnea, increased sputum volume and increased sputum purulence) or their return to the baseline severity is given 4.9, 4.7 and 4.2 days, respectively. The incidence of adverse effects during the treatment with moxifloxacin in all study subjects was 10.9%, 9.6% in Group 1 and 12.1% in Group 2. There was no serious adverse effect that required discontinuation of the treatment. Relapse during a 20 days follow-up period was registered in 7.4% of the all study subjects with complete resolution of the cardinal symptoms or their return to the baseline severity, i.e. in two patients from both Group 1 and Group 2 (7.6% and 7.1%, respectively).

CONCLUSION: Our findings suggest high efficacy and good tolerability of moxifloxacin in the treatment of moderate COPD exacerbations of bacterial origin.

Introduction

Exacerbations of chronic obstructive pulmonary disease (COPD) are important events in the course of the disease because they greatly contribute to the rates of hospitalisation and readmission, disease progression and mortality. According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2017 Report, COPD exacerbations are defined as an acute worsening of respiratory symptoms that result in additional therapy [1]. Respiratory infections, environmental and occupational exposures, discontinuation of the regular treatment, and worsening of the comorbid conditions are considered as causes of exacerbations, but in up 20% of the cases, the cause of exacerbation remains unknown [2] [3].

The goals of treatment for COPD exacerbations are to minimise the negative impact of the current exacerbation and prevent the development

of subsequent events. According to their severity and management, COPD exacerbations are classified as mild (treated with short-acting bronchodilators), moderate (treated with short-acting bronchodilators plus antibiotics and oral corticosteroids) and severe (requiring hospitalisation or visit to the emergency room). Depending on the severity of an exacerbation and/or the severity of the underlying disease, an exacerbation can be managed in an outpatient or inpatient setting. More than 80% of COPD exacerbations are managed in an outpatient setting with pharmacological treatment including short-acting bronchodilators, antibiotics and/or corticosteroids [1].

Respiratory infections account for up to 80% of exacerbations, of which bacterial infections are involved in around 50-70%. The predominant bacteria recovered from the lower airways in patients with chronic bronchitis (CB) and COPD exacerbations are *Streptococcus pneumoniae*, *Haemophilus influenza,* and *Moraxella catarrhalis.* Atypical bacteria, e. g. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* are implicated in up to 10% of exacerbations [4] [5]. The criteria of Anthonisen i.e. increased dyspnea, sputum volume and purulence, are still the most important classification system to identify patients likely to be infected with bacterial pathogens based on the presentation of clinical symptoms [6] [7] [8]. Current treatment guidelines recommend antibiotic therapy in patients with Anthonisen criteria of type I (all cardinal symptoms) or II (two cardinal symptoms) if increased purulence of sputum is one of the two symptoms and in patients who require mechanical ventilation (invasive or non-invasive). The choice of the antibiotic is based on the local bacterial resistance pattern. Usually, initial empirical treatment is an aminopenicillin with clavulanic acid, macrolide, or doxycycline. The recommended length of antibiotic therapy is 5-7 days. Resolution of exacerbation is considered as a complete resolution of cardinal symptoms or their return to the baseline severity [1] [9] [10].

Moxifloxacin is a fourth generation fluoroquinolone with a broad spectrum of activity against a wide range of the microorganisms, including Gram-positive and Gram-negative bacteria, atypical pathogens, and anaerobic bacteria, i.e. against nearly all treatable bacteria associated with COPD exacerbations, including *Streptococcus pneumoniae*, *Haemophilus influenza*, *Moraxella catarrhalis*, most *Staphylococcus aureus*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, etc. Also, moxifloxacin may be regarded as the most excellent tissue ability. Common side effects associated with the use of moxifloxacin are abdominal discomfort, nausea, vomiting, diarrhoea, headache, dizziness, blurred vision, anxiety, and skin itching. Serious side effects of moxifloxacin which occur rarely include severe diarrhoea, acute allergic reactions, connective tissue problems (tendon rupture and joint problems), muscle pain, confusion, agitation, depression, and prolonged QT heartbeat interval [11] [12] [13].

The present study aims to evaluate the efficacy and safety of moxifloxacin in the outpatient treatment of moderate bacterial exacerbations in COPD patients.

Material and Methods

A prospective, observational study (a real life-study) including 64 COPD patients with moderate exacerbation with clinical presentation suggesting bacterial origin, 39 males and 25 females, aged 43 to 77 years. The diagnosis of COPD in all patients was established according to the actual GOLD criteria [1]. All participants were informed about the study, and their written consent was obtained.

The study was carried out in the period August 2017-November 2017 at the Institute for Occupational Health of R. Macedonia, Skopje-WHO Collaborating Center. The study of the effects of various antimicrobial regimens on the clinical course of exacerbations of chronic bronchitis and COPD carried out by Miravittles et al., [14] was used as a model.

Including criteria was the presence of a moderate exacerbation of probably bacterial origin which can be managed on an outpatient basis. The diagnosis of bacterial exacerbation was defined by the patient's symptoms, using the criteria described by Anthonisen et al., [6]. Also, all patients underwent clinical examination, spirometry, blood gas measurements, ECG, and laboratory analysis. Chest X-ray was performed in a part of the patients by indications.

Excluding criteria were mild and severe exacerbations, patients with exacerbation type III (one cardinal symptom) or type II if increased purulence of sputum was not one of the two symptoms, patients with asthma, cystic fibrosis, malignancy, pneumonia and pulmonary embolism and patients with known hypersensitivity to moxifloxacin.

All study subjects were treated 7 days with moxifloxacin 400 mg once daily per os. In about a half of the patients enrolled in the study (31/64) moxifloxacin was given as an initial empirical treatment (Group 1), whereas in the rest of the study subjects (33/64) moxifloxacin was given after treatment failure with other antibiotic (aminopenicillin with clavulanic acid, clarithromycin, or doxycycline) (Group 2). The study subjects were advised to continue the regular treatment of stable COPD, as well as to use short-acting bronchodilators when needed. All study subjects had intermediate visits at 3, 5 and 7 days at which they were evaluated about the duration of symptoms and the side-effects of the drug.

The course of exacerbation was evaluated as a function of the resolution of the symptoms, and the treatment was considered to be successful if complete resolution of cardinal symptoms or their return to the baseline severity was achieved. In addition, spirometric parameters, i.e. forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), FEV_1/FVC ratio, mean expiratory flow at 50% of FVC (MEF_{50}), mean expiratory flow at 75% of FVC ($MEF7_{75}$), and mean expiratory flow at 25-75% of FVC (MEF_{25-75}), at the first visit and at the end of the treatment were compared. Relapse rates were registered during a 20 days follow-up period in the patients with remission of the symptoms.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 for Windows. Chi-square test was used for testing difference in the prevalence. Comparison of the mean time to relief of the symptoms and the mean FEV_1 values was performed by independent-samples *t*-test. A *P*-value less than 0.05 was considered as statistically significant.

Results

The characteristics of the study subjects are shown in Table 1.

Table 1. Characteristics of the study subjects

Variable	Study subjects (n = 64)
Males	39 (60.9%)
Mean age (years)	53.4 ± 9.3
Mean duration of COPD (years)	12.1 ± 5.7
COPD severity*	
GOLD 2 (50% ≤ FEV_1 < 80% predicted)	34 (53.1%)
GOLD 3(30% ≤ FEV_1 < 50% predicted)	30 (46.9%)
Type of exacerbation**	
Type I	35 (54.7%)
Type II	29 (45.3%)
Increased dyspnea	53 (82.8%)
Increased sputum volume	47 (73.4%)
Increased sputum purulence	64 (100%)
Smoking status***	
Current smokers	18 (28.1%)
Pack-years smoked	11.7 ± 4.3
Ex-smokers	21 (32.8%)
Passive smokers	14 (21.8%)
Comorbidities	
Arterial hypertension	23 (35.9%)
Depression	10 (15.6%)
Diabetes mellitus type 2	7 (10.9%)

Numerical data are expressed as a mean value with standard deviation; the frequencies as a number and percentage of examinees with a certain variable. COPD: chronic obstructive pulmonary disease: GOLD: Global Initiative for Chronic Obstructive Lung Disease; FEV_1: forced expiratory volume in 1 second. * COPD severity was defined according to the severity of airflow limitation [1]; ** Type of exacerbation was defined according to the Anthonisen et al., [6] classification; *** Classification of smoking status was done according to the World Health Organization recommendations [15].

Moxifloxacin was not discontinued prematurely in any patient enrolled in the study. The percentage of clinical success (i.e. complete resolution of the cardinal symptoms or their return to the baseline severity) in all study subjects was 84.3% (54/64) being similar in the study subjects of both Group 1 (83.9%, i.e. 26/31) and Group 2 (84.8%, i.e. 28/33) (Figure 1).

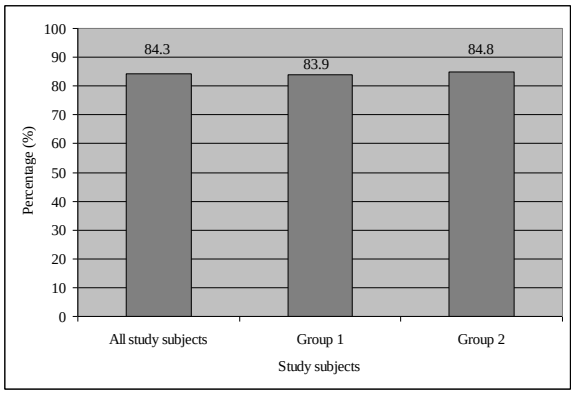

Figure 1: the Clinical success of the treatment with moxifloxacin

The mean time to complete resolution of the cardinal symptoms or their return to the baseline severity was 5.2 ± 1.1 days. The mean time to complete resolution of the certain cardinal symptoms or their return to the baseline severity is given in Figure 2.

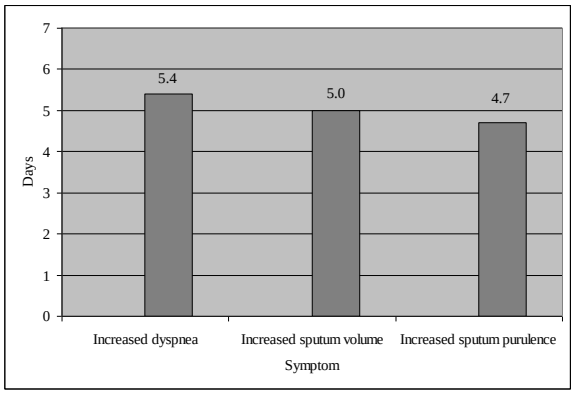

Figure 2: Mean time to complete resolution of the certain cardinal symptoms or their return to the baseline severity

Mean values of the measured spirometric parameters after the treatment with moxifloxacin were significantly higher as compared to their pre-treatment mean values (Table 2).

Table 2: Pre-and post-treatment mean values of the spirometric parameters

Spirometric parameter	Pre-treatment mean value (% pred)	Post-treatment mean value (% pred)	*P*-value
FVC	69.4 ± 8.7	74.3 ± 9.1	0.0023
FEV_1	50.1 ± 7.4	54.7 ± 6.7	0.0003
FEV_1/FVC ratio	0.57 ± 0.05	0.61 ± 0.03	0.0000
MEF_{50}	43.5 ± 9.7	49.2 ± 10.2	0.0015
MEF_{75}	34.1 ± 11.3	38.3 ± 9.4	0.0239
MEF_{25-75}	62.4 ± 10.8	68.1 ± 11.2	0.0040

% pred: % of predicted value; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 second; MEF_{50}: mean expiratory flow at 50% of FVC; MEF_{75}: mean expiratory flow at 75% of FVC; MEF_{25-75}: mean expiratory flow at 25-75% of FVC.

The incidence of adverse effects during the treatment with moxifloxacin in all study subjects was

7.8% (5/64), 6.5% in the Group 1 (2/31) and 9.1% in the Group 2 (3/33) (Figure 3). Registered side effects were mild and self-limited, i.e. there was no serious adverse effect that required discontinuation of the treatment. Dyspeptic problems (nausea, vomiting, and epigastric pain), headache and dizziness were the most frequent adverse events.

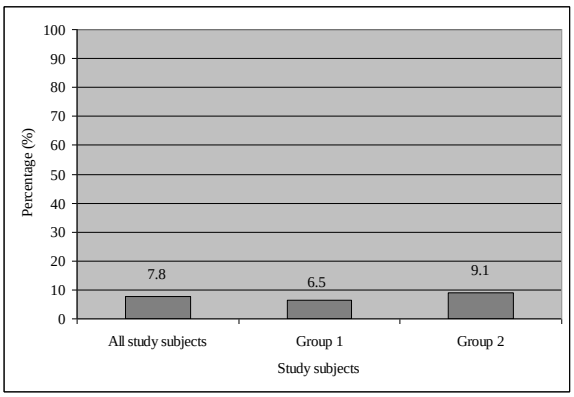

Figure 3: Incidence of adverse effects during the treatment with moxifloxacin

Relapse during a 20 days follow-up period was registered in 7.4% of the all study subjects with complete resolution of the cardinal symptoms or their return to the baseline severity (4/54), i.e. in two patients from both Group 1 and Group 2 (7.6% and 7.1%, respectively) (Figure 4).

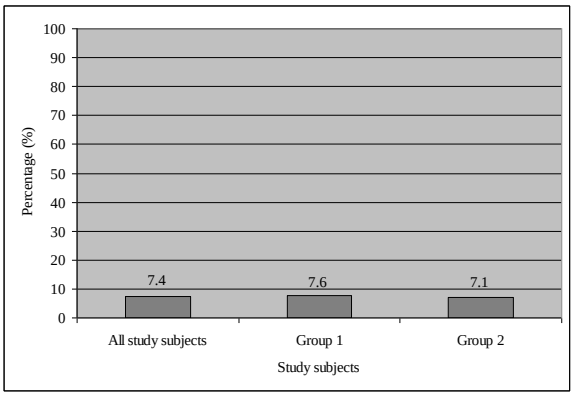

Figure 4: Incidence of relapses after treatment with moxifloxacin during 20 days follow-up period

Discussion

COPD is one of the leading causes of morbidity and mortality worldwide that induces substantial and increasing economic and social burden. Also, exacerbations are the most frequent cause of hospitalisation and death in patients with COPD, so their treatment and prevention have great importance in the management of the disease.

In the present study, we assessed the efficacy and safety of moxifloxacin in the outpatient treatment of moderate COPD exacerbations with clinical manifestations indicating bacterial origin. The diagnosis of exacerbation was based on the presence of clinical symptoms and lung function measurements without microbiological evaluation of the sputum. According to the actual recommendations, sputum analysis should be reserved for patients with frequent exacerbations and for chronic purulent sputum in whom the presence of more virulent and/or more resistant bacteria is likely [3] [9] [16]. Moxifloxacin was an initial treatment in about a half of the study subjects, whereas in the rest it was given following treatment failure with amoxicillin with clavulanic acid, clarithromycin, or doxycycline. We found a large proportion of active and passive smokers among examined subjects that was similar to its prevalence in adults documented in our previous studies [17] [18].

Results of the present study indicated a high clinical success rate (84.3%) and time-course of the recovery (5.2 days). Similar results were obtained in our study on efficacy and tolerability of eight antimicrobial regimens in the outpatient treatment of exacerbations of COPD performed in 2013. Namely, the clinical success rate for moxifloxacin in that study was 80.9%, while the mean time for complete resolution of the symptoms of exacerbation or their return to the baseline severity was 5.7 days [19] [20]. Furthermore, results indicating a high efficacy of moxifloxacin in the treatment of COPD exacerbations were obtained in several other studies. In the meta-analysis of 11 randomized controlled studies Kai-Xiong et al. found that moxifloxacin was as clinically equivalent and bacteriologically superior to the antibiotic regimens routinely used in patients with exacerbations of CB and COPD and concluded that therapy with moxifloxacin might be a promising and safe alternative to empirical treatment for CB and COPD exacerbations [21]. Also, in the comparison of the efficacy of oral moxifloxacin versus other intravenous antibiotics in patients with COPD exacerbations requiring urgent admission, Juan Pator et al., found that *ab initio* oral moxifloxacin is an effective alternative to other intravenous antibiotics (amoxicillin with clavulanic acid, ceftriaxone, or levofloxacin) [22].

In a prospective observational study conducted in eight Eastern European countries including 2,536 patients older than 35 years with CB or COPD exacerbation treated with moxifloxacin for 5, 7 and 10 days, Chuchalin et al., reported *very good* or *good* efficacy in 97.7% of the treated patients, *sufficient* in 1.8%, and *insufficient* in 0.5% [23]. In the Moxifloxacin in Acute Exacerbations of Chronic Bronchitis Trial (MAESTRAL), Wilson et al., found significantly lower clinical failure rates in the patients with COPD exacerbations treated with moxifloxacin as compared to the patients treated with amoxicillin with clavulanic acid [24].

There is consistent evidence that the most

commonly associated side effects of antibiotics used in the treatment of COPD exacerbations are gastrointestinal and that the risk of *Clostridium difficile*-associated diarrhoea may be increased with antibiotic use [25]. In the present study, we found a low incidence of mild side effects during the moxifloxacin treatment (7.8%) that did not require its discontinuation. Similar incidence and severity of the adverse effects during moxifloxacin therapy were found in our study performed in 2013 (7.1%) [19]. The incidence of side effects ranging from 4 to 12% is reported in several studies on the safety of moxifloxacin during treatment of COPD exacerbations [21] [24] [26]. In the study performed by Chuchalin et al. reported incidence of serious adverse effects during moxifloxacin treatment (atrial fibrillation, acute myocardial infarction, diplopia, allergic oedema, amnesia, and skin reaction) considered to be drug-related was 0.15% [23].

One of the main goals of treatment of COPD exacerbations is to prevent the development of subsequent events as exacerbations can cluster in time, and once a COPD patients experience an exacerbation, they will show increased susceptibility to another event [1] [27]. In the present study, the incidence of relapses during the follow-up period was 7.4%. Several studies indicated a low incidence of relapses after the treatment of COPD exacerbations with moxifloxacin and a prolonged time to the next exacerbation [28] [29] [30].

The present study must be interpreted within the context of its limitations. First, the results obtained should be viewed with caution, since the study was neither blinded nor randomised and, therefore, can be a subject to possible selection bias. On the other hand, the study design may be its strength, as it is documented by other real life-studies. Second, a relatively small number of the study subjects could have certain implications on the data obtained and its interpretation. Third, the short follow-up period could also have certain implications on the data obtained and its interpretation.

In conclusion, in a prospective, observational study on efficacy and tolerability of moxifloxacin in the treatment of moderate exacerbations of COPD in the outpatient setting, we found high clinical success rate and low incidence of side effects indicating good tolerability. Our findings suggest that moxifloxacin can be used as a first choice antibiotic in the outpatient treatment of moderate bacterial exacerbations of COPD.

Ethical Approval

The Ethical Committee of the Institute of Occupational Health of R. Macedonia, Skopje – WHO Collaborating Center and GA^{2}LEN Collaborating Center approved for performing the study and publishing the results obtained (0302-619-04.09.2017).

Authors Participations

JM participated in the study design, writing the protocol, data collection, managing the analyses of the study, and writing all versions of the manuscript. JKB participated in the study design, writing the protocol, managing the analyses of the study, as well as writing all versions of the manuscript. TP managed the literature searches and participated in managing the analyses of the study. KV performed the statistical analysis and participated in the managing of the analyses of the study. SS and DM participated in the data collection. All authors read and approved the final manuscript.

References

1. Tan WC, Bourbeau J, Aaron SD, Zhou G, Maltais F, Hernandez P, Fitzgerald JM, Marciniuk DD, Walker BL, Sin DD. Global Initiative for Chronic Obstructive Lung Disease 2017 Classification and Lung Function Decline in Chronic Obstructive Pulmonary Disease. American journal of respiratory and critical care medicine. 2018; 197(5):670-3. https://doi.org/10.1164/rccm.201706-1154LE PMid:28858570

2. Bhowmik A, Seemungal TA, Sapsford RJ, Wedzicha JA. Relation of sputum inflammatory markers to symptoms and lung function changes in COPD exacerbations. Thorax. 2000; 55:114-120. https://doi.org/10.1136/thorax.55.2.114 PMid:10639527 PMCid:PMC1745686

3. Miravittles M, Niederman M. Lectures in Respiratory Tract Infections: Acute Exacerbations of Chronic Bronchitis.

4. Ball P, Chodosh S, Grossman R, et al. Causes, epidemiology, and treatment of bronchial infections. Infect Med. 2000; 17:186-198.

5. Sethi S, Wrona C, Grant BJB, Murphy TF. Strain-specific immune response to Haemophilus influenzae in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2004; 169:448-453. https://doi.org/10.1164/rccm.200308-1181OC PMid:14597486

6. Anthonisen NR, Menfreda J, Warren CP, et al. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. Ann Intern Med. 1987; 106(2):196-204. https://doi.org/10.7326/0003-4819-106-2-196 PMid:3492164

7. Woodhead M, Blasi F, Ewig S, et al. Guidelines for the management of adult lower respiratory tract infections. Eur Respir J. 2005; 26:1138-1180. https://doi.org/10.1183/09031936.05.00055705 PMid:16319346

8. Celli BR, MacNee W. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur Respir J. 2004; 23(6):932-946. https://doi.org/10.1183/09031936.04.00014304

9. Auwaerter PG, Barlett JG. Chronic bronchitis, Acute exacerbations. Available at: https://www. hopkinsguides.com/ (Accessed 14.08.2017).

10. Miravitlles M. Epidemiology of chronic obstructive pulmonary disease exacerbations. Clin Pulm Med. 2002; 9:191-197. https://doi.org/10.1097/00045413-200207000-00001

11. Balfour JA, Lamb HM. Moxifloxacin: a review of its clinical potential in the management of community-acquired respiratory tract infections. Drugs. 2000; 59:115-139. https://doi.org/10.2165/00003495-200059010-00010 PMid:10718103

12. Soman A, Honeybourne D, Andrews J, et al. Concentrations of moxifloxacin in serum and pulmonary compartments following a single oral dose in patients undergoing fibre-optic bronchoscopy. J Antimicrob Chemother. 1999; 44:835-838. https://doi.org/10.1093/jac/44.6.835 PMid:10590288

13. Yoshida K, Okimoto N, Kishimoto M, et al. Efficacy and safety of moxifloxacin for community-acquired bacterial pneumonia based on pharmacokinetic analysis. J Infect Chemother. 2011; 17:678-685. https://doi.org/10.1007/s10156-011-0282-6 PMid:21847518

14. Miravittles M, Llor C, Naberan K, et al. Effect of various antimicrobial regimens on the clinical course of exacerbations of chronic bronchitis and chronic obstructive pulmonary disease in primary care. Clin Drug Invest. 2004; 24(2):63-72. https://doi.org/10.2165/00044011-200424020-00001

15. World Health Organization. Guidelines for controlling and monitoring the tobbaco epidemic. Geneva: WHO, 1998.

16. Stockley RA, O'Brien C, Pye A, Hill SL. Relationship of sputum color to nature and outpatient management of acute exacerbations of COPD. Chest. 2000; 117:1638-1645. https://doi.org/10.1378/chest.117.6.1638 PMid:10858396

17. Minov J, Karadzinska-Bislimovska J, Vasilevska K, Stoleski S. Smoking status in exposed and unexposed workers. Mak med pregled. 2006; 60(68):128.

18. Minov J, Karadzinska-Bislimovska J, Vasilevska K, et al. Exposure to environmental tobacco smoke in the workplace in Macedonia: Where are we now? Arh Hig Rada Toksikol. 2008; 59:103-109. https://doi.org/10.2478/10004-1254-59-2008-1870 PMid:18573747

19. Minov J, Karadzinska-Bislimovska J, Petrova T, Vasilevska K, Stoleski S, Mijakoski D, Risteska-Kuc S. Efficacy and tolerability of eight antimicrobial regimens in the outpatient treatment of exacerbations of chronic obstructive pulmonary disease. Open Access Maced J Med Sci 2014; 2(3):519-524. https://doi.org/10.3889/oamjms.2014.093

20. Acevski S, Minov J, Sterjev Z, et al. Cost effectiveness analysis of antibiotic regimens used in outpatient treatment of exacerbations of chronic obstructive pulmonary disease (COPD). Adv Pharmacoepidemiol Drug Saf. 2016; 5:5.

21. Kai-Xiong L, Bing X, Wang J, et al. Efficacy and safety of moxifloxacin in acute exacerbations of chronic bronchitis and COPD: a systematic review and meta-analysis. J Thorac Dis. 2014; 6(3):221-229.

22. Juan Pastor A, Llopia Roca F, Masuet Aumatell C, et al. COPD exacerbations treated with oral moxifloxacin versus other intravenous antibiotics in a short-stay emergency outpatient clinic: a comparative study. Emergencias. 2007; 19:65-69.

23. Chuchalin A, Zakharova M, Dokic D, et al. Efficacy and safety of moxifloxacin in acute exacerbations of chronic bronchitis: a prospective, multicenter, observational study (AVANTI). BMC pulmonary Medicine. 2013; 13:5. https://doi.org/10.1186/1471-2466-13-5 PMid:23343427 PMCid:PMC3560260

24. Wilson R, Anzueto A, Miravitlles M, et al. Moxifloxacin versus amoxicillin/clavulonic acid in outpatient acute exacerbations of COPD: MAESTRAL results. Eur Respir J. 2012; 40:17-27. https://doi.org/10.1183/09031936.00090311 PMid:22135277 PMCid:PMC3393767

25. Manalan K, Rashid T, Singanayagam A. Antibiotic treatment in exacerbations of chronic obstructive pulmonary disease: recent trial results. Clin Invest. 2015; 5(2):189-204. https://doi.org/10.4155/cli.14.113

26. Mensa J, Trilla A. Should patients with acute exacerbation of chronic bronchitis be treated with antibiotics? Advantages of the use of fluoroquinolones. Clin Microbiol Infect. 2006; 12(3):42-54. https://doi.org/10.1111/j.1469-0691.2006.01396.x PMid:16669928

27. Hurst JR, Vestbo J, Anzueto A, et al. Susceptibility to exacerbations in chronic obstructive pulmonary disease. N Engl J Med. 2010; 363(12):1128-1138. https://doi.org/10.1056/NEJMoa0909883 PMid:20843247

28. Adams SG, Melo J, Luther M, et al. Antibiotics are associated with lower relapse rates in outpatients with acute exacerbations of COPD. Chest. 2000; 117:1345-1352. https://doi.org/10.1378/chest.117.5.1345 PMid:10807821

29. Wilson R, Allegra L, Huchon G, et al. Short-term and long-term outcomes of moxifloxacin compared to standard antibiotic treatment in acute exacerbations of chronic bronchitis. Chest. 2004; 125:953-964. https://doi.org/10.1378/chest.125.3.953 PMid:15006954

30. Miravitlles M. Moxifloxacin in the management of exacerbations of chronic bronchitis and COPD. Int J Chron Obstruct Pulmon Dis. 2007; 2(3):191-204. PMid:18229559 PMCid:PMC2695197

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Clinical Science

The Prevalence of Risk Factors for the Development of Bacteraemia in Children

Sayed Yousef Mojtahedi[1], Aliakbar Rahbarimanesh[2], Leila Khedmat[3], Anahita Izadi[2*]

[1]Department of Pediatric Nephrology, Bahrami Hospital, Tehran University of Medical Sciences, Tehran, Iran; [2]Department of Pediatric Infection Disease, Bahrami Hospital, Tehran University of Medical Sciences, Tehran, Iran; [3]Health Management Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

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Keywords: Children; Risk factor; Septicemia; Septic shock; Systemic inflammatory response Syndrome

***Correspondence:** Anahita Izadi. Department of Pediatric Infection Disease, Bahrami Hospital, Tehran University of Medical Sciences, Tehran, Iran. E-mail: dr.anahita.izadi@gmail.com

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Abstract

AIM: The objective of this study was to evaluate the frequency of risk factors for bacteremia in children less than 15 years of age was determined in Bahrami Hospital during 2013-2016.

METHODS: This study conducted on 84 children aged 3 months' to15 years old, who hospitalised in the pediatrics ward and the PICU in Bahrami Hospital from 2012 to 2016. Our study consisted of 46 boys (54.2%) and 38 girls. Moreover, 24.1% of subjects (20 patients) were entered in the study as young as three months old, followed by three months to three years (49.4 %; 41 subjects), and 3 to 15 years of age (26.5%; 22 individuals).

RESULTS: The average hospitalization duration was determined to be 15.30 ± 8.75 days. Moreover, our results revealed that a history of blood transfusion in 11.2% of patients. On the other hand, 35.7% of cases were determined to be positive for blood cultures. The microorganisms reported from positive blood cultures include *Enterobacter* (81.48%), *Escherichia coli* (11.11%) and *Klebsiella* (3.70%). Also, 50% of patients were hospitalised in the internal ward, 12% received immunosuppressive drugs, and 96.4% of the patients had a history of vaccination.

CONCLUSION: Pediatric severe sepsis remains a burdensome public health problem, with prevalence, morbidity, and mortality rates similar to those reported in critically ill adult populations. International clinical trials targeting children with severe sepsis are warranted.

Introduction

Systemic Inflammatory Response Syndrome (SIRS) is an inflammatory state initiated by the host in response to infectious or noninfectious insult. *Sepsis* is known to be a life-threatening complication, which can be referred to SIRS due to a suspected or confirmed infection [1]. Bacteremia diseases one of the most serious infectious diseases in children, which can be potentially life-threatening. Bacteremia can be caused by a wide range of gram-negative and gram-positive microorganisms and can be manifested with or without a specific site of infection, such as pneumonia or meningitis. Some bacteremia is a *transient self-limiting* [2] [3]. An epidemiological study of sepsis has shown that in the period from 1979 to 2000, Gram-positive infections have dominated [4], although recent studies of 14,000 patients in 75 countries have suggested that the *cases composed* predominantly of gram-negative bacteria (62%), followed by gram-positive (47%) and fungi (19%) [5].

The incidence of bacteremia in children in the hospital and outpatient department has been investigated. The most common microorganisms in healthy children over a month of age are pneumococcal bacteremia, *Haemophilus influenzae* type B, and *Neisseria meningitidis*, which is characterised by moderate to severe pathogenesis. *Staphylococcus aureus*, Salmonella and Streptococcus A have been reported in children and also in children who suffer from pyelonephritis or

diarrhoea due to Gram-negative bacteria [5].

The underlying disease in children reduces the host's response to the infection and may cause bacteremia to spread by the same microorganisms. In this group of children, especially when hospitalised, *Enterobacteriaceae*, *Staphylococcus aureus*, Staphylococcus coagulase negative and fungi are the most important organisms that are commonly isolated from the blood cultures [7] [8]. The use of intravascular and urethral catheters, endotracheal tubes, and other extraneous materials will make children with immunodeficiency disease more susceptible to infections.

The prevalence of septicemia detected over the years has increased, partly due to the advancement of medical technology and an increase in the number of people with a life-threatening immunodeficiency, where they can be survived [9]. The most common cause of catheter-related sepsis is gram-positive bacteria, especially *coagulase-negative staphylococci* (CoNS), and gram-negative bacteria [6]. Children's bacteremia can lead to complications such as severe sepsis, septic shock, and ultimately death.

The severity of *mortality* rate from *septic shock* depends on the following factors, such as the location of infection, bacterial pathogens, and multifocal dysfunction and host immune responses.

It should be noted that severe sepsis is still one of the leading causes of death in children. Recent data indicate therapeutic strategies at *improving sepsis* outcomes in children with a mortality rate of about 10%. Infants, especially those who are shown as having a *low birth weight* and children with chronic medical conditions are at the highest risk of severe sepsis [7] [8] [9]. The mortality rate of the child's patients who undergo bone marrow transplantation is higher than that of other patients with septic shock. It should be taken into consideration that severe sepsis encompasses a large national cost, where the median admission period is 31 days for children; while for infants, the median admission period is 53 days. The highest incidence of severe sepsis was observed among infants and the lowest among children aged 5to14 [10].

Despite the advances in drugs and vaccinations, sepsis can usually lead to septic shock and eventually death. This disease remains a problem of pediatric medicine. Regarding the importance of sepsis, the present study was designed to identify the most common risk factors for reducing morbidity and mortality and treatment costs. The frequency of risk factors for bacteremia in children under 15 years of age was determined in Bahrami Hospital during 2013-2016.

Material and Methods

This descriptive and cross-sectional study was conducted to determine the frequency of risk factors for bacteremia in children under the age of 15 in Bahrami Hospital. Patient information was collected based on the evidence in hospital records. All the necessary specifications were recorded in information forms, which included age, gender, gestational age, weight, the cause of hospitalisation, hospitalisation date, discharge date or death, blood and urine culture results, cerebrospinal fluid, a graph of the chest, Positive clinical signs and routine blood tests.

This retrospective study was conducted on patients diagnosed with bacteremia in Tehran's Bahrami Hospital in Tehran during the years 2013 to 2016. It should be noted that Bahrami Hospital is classified in the third level of care and many of its patients are referenced from other medical centres in Tehran and other cities. Patients are admitted to the intensive care unit at the age of 1 month to 15 years. The possibility of non-invasive monitoring and mechanical ventilation is provided for all patients.

In this study, patients (*aged* > 15 months < *15 years*) were diagnosed with septic sepsis or septic shock at the time of admission. Exclusion criteria included the history of surgery during hospitalisation or having underlying conditions such as malignancy or *immune compromised* patient with bacteremia. All patients were evaluated for many factors such as underlying variables, duration of the disease, previous medical history, and previous use of the drug, symptoms based on the definitions of sepsis, as well as the initial infection site, tests, positive culture responses, therapeutic response and the ultimate fate. Of all children referring to the treatment centre, 84 samples were examined. The number of patients in this study was determined by the census. Declaration of *Helsinki* was applied for all patients as a *statement* of ethical *principles* for m*edical research* . Furthermore, no additional financial or spiritual burden was imposed on patients, and all information was remained confidential.

All the analyses were performed using EpiInfo (version 6, Centers for Disease Control and Prevention, Atlanta, GA, U.S.A.). Mean, and proportions were compared by Student t-test and chi-squared. $P < 0.05$ was considered to be statistically significant.

Results

The children studied consisted of 46 boys (54.2%) and 38 girls (45.8%). In terms of age, 24.1%of subjects (20 patients) were enrolled as young

as *one* to three months old, 49.4% (41 subjects) were three months to three years, and the remaining 26.5% (22 individuals) included children with 3 to 15 years of age (Figures 1 and 2). The average length of stay in hospitals (ALOS) was calculated to be 15.30 ± 8.75.

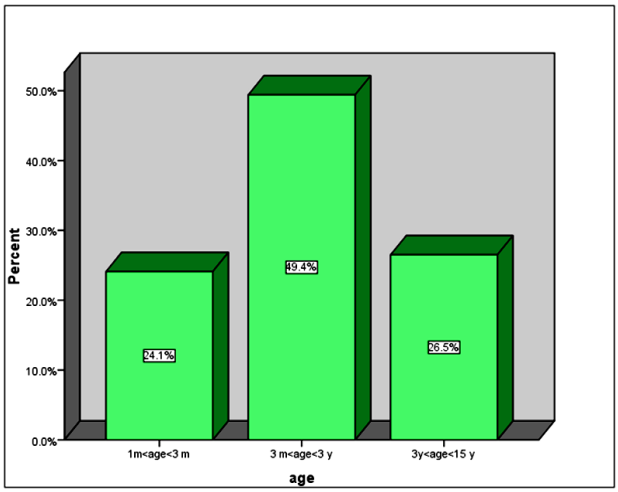

Figure 1: Frequency of different age groups in this study

Table 1 and Figure 2 show the list patient with bacteremia.

Table 1: Frequency of underlying illness in patients

Underlying disease	Number	Per cent
Malignancy	10	12.2
Kidney	2	2.4
Cardiac	4	4.9
Respiratory	3	3.7
Digestive	2	2.4
Endocrine and Metabolic	3	3.7
Nervous	28	34.1
No underlying illness	32	36.6
Total	84	100

The results revealed that 63.6% of the patients had an underlying disease, followed by neurological diseases (34.1%) and malignancies (12.2%), and less than one-quarter of the patients exhibited growth impairment, which growth fell *below 5*% of the growth *c*urve.

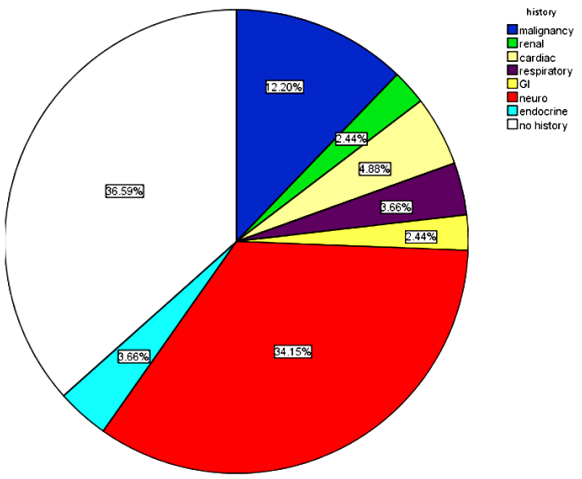

Figure 2: Frequency of underlying illness in patients

A peripheral venous catheter *(PVC)* was used for 52.4% of children, followed by nasogastric tube (7.7%), intubation (14.7%) and urinary catheter (4.9%), (Figure 3). A record of blood intake was found in 11.2% of the subjects.

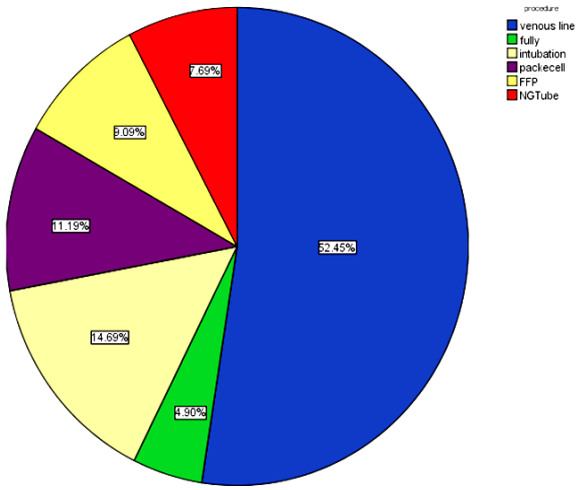

Figure 3: Frequency of methods used for blood intake

Positive cultures were reported in 35.7% of subjects. The frequency of microorganisms reported from positive blood cultures was as follows: *Enterobacter* was observed in 81.48% of positive cultures, followed by *Escherichia coli* (11.11%), *Klebsiella* (3.70%) and CoNS (3.70%). It is worth noting that 50% of patients were admitted to the general ward, followed by *infectious diseases section* and the pediatric intensive care unit (*PICU*). Regarding the positive results of blood cultures, a number of clinical symptoms were recorded in patients including fever (34.0%), respiratory symptoms (19.7%), coughing (12.8%), respiratory distress (6.9%), gastrointestinal symptoms (20.7%), diarrhea (7.9%), vomiting (12.8%), torpidity (12.8%), dermal signs (1.5%), (Figure 4)

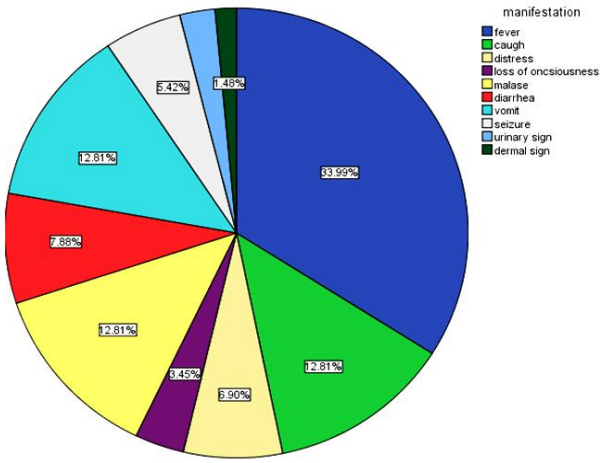

Figure 4: Frequency of clinical symptoms in patients

The results of the present study indicated that 23.5% of the cases received antibiotics, while 12% have taken an immunosuppressant drug the remaining 96.4% received the vaccine.

Given laboratory findings, WBCs changes reached to fewer than *3,000*/µl in 13.3% of subjects, 64% showed an increased WBCs level, ranging from 3,000 to 15,000. Moreover, the remaining 25.3% showed *WBC* count ≥ *15,000*/µL.

Our data revealed *platelet numbers* equal to *150,000 platelets* for 30.1% of the subjects, followed by platelets ranging from 150,000 to 500,000 (56.6%) and platelets *greater* than 500,000 (13.3%). Furthermore, 50% of patients revealed an *erythrocyte sedimentation rate* (*ESR*) > 30 mm/hr. The remaining 50% of the cases showed *ESR* less than 25 mm/hr. It should be noted that mortality in our patients was calculated at 37.8%.

The following information was obtained in the investigation of dead children; about 26.7% of the cases were male deaths. Also, the number of cases in age groups is as follows: between *1-3 months* of *age* (26.7%), 3 months to 3 years (48%), and between 3 - 15 years of age (25.3%). Regarding underlying illness, malignancy was seen in 11.3% of patients, followed by kidney complications (2.8%), neurological complications (39.4%), and no underlying disease (35.2%). On the other hand, based on the blood culture, 12.5% of the deaths showed *E. coli*, while most of them (79.2%) revealed enterobacter

Other findings based on the medical records of dead children included fever (84%), abnormal white blood cell (38.4%; leukocytosis, leukopenia), abnormal platelet (43.4%; thrombocytopenia, thrombocytosis), blood intake (1.3%), immunosuppressive drug (12.5%) and history of antibiotic use (24.7%).

Discussion

According to the results of this study, sepsis was more prevalent in the age group of 3 months to 3 years, and the disease in boys was about 10% higher than in girls, underlying disease was observed in 63.6% of cases and neurological diseases were also the most common *conditions*.

As other study indicated, sepsis was more common in infants from 1 month to 1 year old in Bahrami Hospital. Similar to our study, the prevalence of disease among boys was about 10% higher than girls. In the study above, malignancies and then kidney disease were the most common underlying disorders among patients [11]. While the current study demonstrated that neurological diseases are the most common underlying disorders that show changes in recent years among *those referred* to the centre because the location of the study in the two studies was the same.

A study reported sepsis prevalence in 2% of all hospitalised subjects. The mentioned study revealed that 83% of patients had underlying diseases and hematologic disorders or neoplasms has been found to be the most common underlying disorder. Furthermore, based on the study above, Gram-positive bacteria accounted for 68% of the septic-influencing organisms, with the most frequent occurrence of *Staphylococcus aureus* (18%). The mortality due to gram-negative bacteria was higher than that of gram-positive [12].

The largest epidemiological report of severe sepsis in children has been derived from cohort studies conducted in the United States, in which the annual incidence of severe sepsis in children (under age 20) is as follows: the incidence of sepsis in children under one year of age has been determined to be 9.7%, which is much higher in comparison with 0.25 to 0.5 in the age group of one year to 19 [13].

It has also been shown in the study above that incidence of severe sepsis in children with concomitant illnesses is more common than other children. However, despite the increasing trend in severe sepsis, the death rate has dropped from 10.3% to 8.9%. This reported data from 42 United States hospitals affirm the increasing prevalence of sepsis and a reduction in the risk of death [13].

Based findings of a previous study, *Klebsiella* B has been reported to be the most commonly isolated organism (43.75%) from children under the age of 10 years with sepsis, followed by *Staphylococcus aureus* (18.75%) [8]. They also stated that the highest prevalence was in children aged 10 to 5 years and male to female ratio was determined to be 1.28:1.0. Symptoms of bacteremia are different, where depending on the age and underlying disorders, the duration of the disease, and certain microorganisms. Children between 3 months and 3 years may have fever and evidence of an upper or lower respiratory tract infection, or an unlikely infectious site and bacteremia. Most studies show that the risk of bacteremia can develop with increasing body temperature, and when the body temperature reaches 41°C, approximately 25% of these patients may develop a broad array of bacteremia [9].

The incidence of invasive infections, sepsis and septic shock in children (0 to 15 years old) has increased in the Australian and New Zealand care units over a 12-year period. Severe infections are associated with about one-quarter of ICU deaths among children. In 2002-2008, the mortality rate from invasive and septic infections decreased by about one third, compared with 2008 to 2013, and septic shock mortality dropped by one fifth, which did not have a significant difference compared with the overall improvement in the overall survival of children

admitted to ICUs due to non-infectious causes [9] [10] [13].

Therefore, severe infections have an important impact on the health of children and impose significant pressure on health systems. As studies have shown, ICU admission due to sepsis and septic shock is costly, may also have adverse outcomes in the long term, such as neurological complications and disability and death [14].

The incidence of severe sepsis in the United States increased from 0.56 to 0.89 per 1,000 children between 2000 and 2005, while the mortality of sepsis remained unchanged at 9%. , this increase in the age group of newborns was more than other age groups, and this data was not consistent with our findings [15].

Base on a study, pediatric patients with hematologic and malignant diseases who had sepsis, included determining the risk factors for mortality after the development of sepsis. The results of the study above indicated that more than half (52.9%) of the isolates from sepsis-related death belonged to Gram-positive cocci resistant to β-lactam antibiotics. In mentioned study, it has been revealed that relapse history, history of the underlying disease, and high C reactive protein concentration at the onset of fever could be a key factor for mortality in patients with sepsis [16]. Sepsis is the leading cause of 5.6% of deaths in adult patients with trauma, but about 33% of brain damage has been shown a safe interrupt the neural axis. While TBI is a major cause of death in the pediatric population, it remains unclear whether children with neuroprotection similar to that of adults are at higher risk for sepsis. Therefore, a study was conducted to determine the relationship between traumatic brain injury (TBI) and sepsis in children under the age of 18 years between 2001 and 2012. Initial results of this study showed that 63.9% of children with any type of TBI were identified, and sepsis was reported in 1.0% of children, compared with 2.8% of children without this complication [17].

In our study, 34.1% of the studied population had sepsis with neurological disorders. These results are in line with our study of the prevalence of sepsis in children under the age of 18 in patients with a variety of neurological diseases. In a cross-sectional study, tachycardia, mental changes, abnormal condition and temperature were the highest regarding clinical and diagnostic symptoms of sepsis. The white blood cell count had the highest changes in laboratory tests [18].

In the present study, in 34.0% of cases, fever was observed in the clinical symptoms during admission. Respiratory symptoms (12.8% cough, 6.9% respiratory distress) were recorded in 19.7%, followed by gastrointestinal symptoms (20.7%, diarrhea 7.9%, vomiting 12.8%), lethargy (12.8%), and dermal signs (1.5%). According to laboratory finding, abnormal white blood cell was seen in 38.4% of subjects in the present study (leukocytosis, leukopenia), followed by abnormal platelet (43.4%; thrombocytopenia, thrombocytosis), blood intake (1.3%), immunosuppressive drug (12.5%), antibiotic use (24.7%) and history of vaccination (96.4%).

Severe sepsis remains very common in children and accounts for about 8% of all children with severe depression. Although the absolute number of cases of severe sepsis is 10-fold lower than adults [4] [19], the prevalence of 8.2% in PICU patients is highly similar to the proportion of adults suffering from severe sepsis. These results demonstrated that 16-bed PICU is probably to be treating one child suffering from severe sepsis at least. Also, hospital mortality, which is often much lower, was determined as 25% in this study and is higher than estimated prevalence in previous epidemiological studies, where examined hospital data retrospectively [20]. *Coexisting diseases*, immunosuppressive diseases, and kidney diseases have the highest mortality rate in previous studies among children. However, the present study has the highest mortality rate for neurological diseases, but it cannot be determined whether the death was attributed to sepsis or a condition associated with an underlying disorder. In one-third of children with developing the disease, progressive organ dysfunction and severe functional disability are common in nearly one-fifth of sepsis survivors [20] [21] [22] [23] [24].

In the previous study at the same centre in 2010-2011, most of the organisms isolated from blood culture were gram-positive bacteria (CoNS and *S. aureus*), [11]. In our study, the gram-negative bacteria Enterobacter and Escherichia coli were the most commonly isolated microorganisms isolated from blood culture in patients. Regarding the results, intravascular and urinary catheters were the most leading causes of sepsis in admitted children.

Urinary tract infections can be considered as causes of sepsis among these patients. The bacteria of the UTI are faecal microbial flora found in the perineum and periurethral, where enter the bladder through the urethra. In uncircumcised babies, pathogenic bacteria can enter the urinary tract from the fore skin flora. The sensitivity of girls to urinary tract infection is due to the shorter urinary tract and the closeness of the perina to the urethral duct and colonisation of the perineal region with intestinal organisms. Colonization with *E. coli* and enterococci decreases during the first year of life and is naturally negligible after 5 years of age. In early childhood, Enterobacteria and Enterococci are normal periurethral flora, which can also be opportunistic agents of infections. *E. coli* is the dominant gram-negative bacteria in young girls, while in boys*, E.coli* and Proteus have this condition [22] [23] [24].

Children are at risk of UTI due to the colonisation of E. coli, enterococci and Proteus in the surrounding area of ureter up to 5 years old. The use of urinary catheters increases the likelihood of UTI, which can justify an increase in infections reported

with *E. coli* and enterococci in the present study. To prove it, there is a need for other studies to review the positive blood cultures as well as to control the conditions when using the urinary catheter regarding sterilisation conditions. It can be concluded that the hospital mortality rate in the present study was 37.8%, which suggested a significant increase compared to the previous survey at the centre [11], which was 6.1%, indicating the importance of severe sepsis as a major public health problem, even in children. This finding contrasts with reports from epidemiological studies that report infectious death rates of children at about 10.4%. The results of this study were consistent with other studies but also differed in parts that could be explained by the nature of sepsis, its underlying factors, the population studied, and the health and diagnostic standards.

Our study was conducted in a General Pediatric Hospital, that the most common causes of sepsis were recorded in the age group of three months to three years old. Regarding underlying illnesses, neurological disorders were at the top and growth disturbances were estimated in more than a quarter of patients. This information will help us to pay special attention to these patients. Given dead children, the most common age range of the disease was found to be between 3 months and 3 years in the current study. Regarding underlying diseases and microorganisms, malignancies and Enterococcus were found to be the most common cause of disease, respectively, which similar results were obtained compared to the previous survey at the same centre [11].

Finally, it can be concluded that the evaluation of sepsis in each health centre is necessary to identify its risk factors to control and reduce the frequency of sepsis and to reduce mortality, morbidity and medical expenses.

The limitation of the current study was small sample size because only 84 patients had inclusion criteria.

Author's contributions

SYM, AR, LKH, and AI equally participated in experimental design, data collection, writing and revision of the manuscript.

References

1. Gray J, Gossain S, Morris K. Three-year survey of bacteremia and fungemia in a pediatric intensive care unit. The Pediatric infectious disease journal. 2001; 20:416-21. https://doi.org/10.1097/00006454-200104000-00009 PMid:11332667

2. Watson RS, Carcillo JA, Linde-Zwirble WT, Clermont G, Lidicker J, Angus DC. The epidemiology of severe sepsis in children in the United States. American journal of respiratory and critical care medicine. 2003; 167:695-701. https://doi.org/10.1164/rccm.200207-682OC PMid:12433670

3. Norton EB, Archibald LK, Nwanyanwu OC, et al. Clinical predictors of bloodstream infections and mortality in hospitalised Malawian children. The Pediatric infectious disease journal. 2004; 23:145-51. https://doi.org/10.1097/01.inf.0000109258.82988.40 PMid:14872181

4. Elward AM, Fraser VJ. Risk factors for nosocomial primary bloodstream infection in pediatric intensive care unit patients: a 2-year prospective cohort study. Infection Control. 2006; 27(06):553-60. https://doi.org/10.1086/505096 PMid:16755473

5. Pound CM, Johnston DL, Armstrong R, Gaboury I, Menon K. The morbidity and mortality of pediatric oncology patients presenting to the intensive care unit with septic shock. Pediatric blood & cancer. 2008; 51:584-8. https://doi.org/10.1002/pbc.21670 PMid:18623196

6. Aiken AM, Mturi N, Njuguna P, et al. Risk and causes of paediatric hospital-acquired bacteraemia in Kilifi District Hospital, Kenya: a prospective cohort study. Lancet. 2011; 378:2021-27. https://doi.org/10.1016/S0140-6736(11)61622-X

7. Area CGftSoSiPiB. Clinical study on sepsis in 2 pediatric intensive care units in Beijing. Zhonghua er like za zhi Chinese journal of paediatrics. 2012; 50:178. PMid:22801197

8. Tiwari DK, Golia S, Sangeetha K, Vasudha C. A study on the bacteriological profile and antibiogram of bacteremia in children below 10 years in a tertiary care hospital in Bangalore, India. Journal of clinical and diagnostic research: JCDR. 2013; 7:2732. PMid:24551625 PMCid:PMC3919345

9. Bass JW, Steele RW, Wittler RR, et al. Antimicrobial treatment of occult bacteremia: a multicenter cooperative study. The Pediatric infectious disease journal. 1993; 12:466-73. https://doi.org/10.1097/00006454-199306000-00003 PMid:8345978

10. McCarthy P. Controversies in pediatrics-what tests are indicated for the child under 2 with fever. Pediatrics. 1979; 64:PR51-PR6.

11. Rahbarimanesh A, Mobedi M, Alizade Taheri P. Sepsis risk factors in children: a brief report. Tehran University Medical Journal, 2012; 70:264-269.

12. Oda K, Matsuo Y, Nagai K, Tsumura N, Sakata Y, Kato H. Sepsis in children. Pediatrics International. 2000; 42:528-33. https://doi.org/10.1046/j.1442-200x.2000.01281.x PMid:11059544

13. Thompson GC, Kissoon N. Sepsisin Canadian children: A national analysis using administrative data. Clinical epidemiology. 2014; 6:461. https://doi.org/10.2147/CLEP.S72282 PMid:25525390 PMCid:PMC4266244

14. Schlapbach LJ, Straney L, Alexander J, et al. Mortality related to invasive infections, sepsis, and septic shock in critically ill children in Australia andNew Zealand, 2002–13: a multicentre retrospective cohort study. The Lancet Infectious Diseases. 2015; 15:46-54. https://doi.org/10.1016/S1473-3099(14)71003-5

15. Hartman ME, Linde-Zwirble WT, Angus DC, Watson RS. Trends in the epidemiology of pediatric severe sepsis. Pediatric Critical Care Medicine. 2013; 14:686-93. https://doi.org/10.1097/PCC.0b013e3182917fad PMid:23897242

16. Sano H, Kobayashi R, Iguchi A, et al. Risk factors for sepsis-related death in children and adolescents with hematologic and malignant diseases. Journal of Microbiology, Immunology and Infection. J Microbiol Immunol Infect. 2017; 50:232-38. https://doi.org/10.1016/j.jmii.2015.04.002 PMid:26055687

17. Thompson GC. Sepsis in Children Admitted to Hospital Following Traumatic Brain Injury. In2014 AAP National Conference and Exhibition 2014. American Academy of Pediatrics.

18. Thompson GC, Macias CG. Recognition and Management of Sepsis in Children: Practice Patterns in the Emergency Department. The Journal of Emergency Medicine. 2015; 49:391-9. https://doi.org/10.1016/j.jemermed.2015.03.012 PMid:26093939

19. Ellison AM, Ota KV, McGowan KL, Smith-Whitley K. Epidemiology of bloodstream infections in children with sickle cell disease. Pediatr Infect Dis J. 2013; 32:560-3. https://doi.org/10.1097/INF.0b013e318286c75b PMid:23340560

20. Weiss SL, Fitzgerald JC, Pappachan J, et al. Global epidemiology of pediatric severe sepsis: thesepsis prevalence, outcomes, and therapies study. Am J Respir Crit Care Med. 2015; 191:1147-57. https://doi.org/10.1164/rccm.201412-2323OC PMid:25734408 PMCid:PMC4451622

21. Cruz AT, Perry AM, Williams EA, Graf JM, Wuestner ER, Patel B. Implementation of goal-directed therapy for children with suspected sepsis in the emergency department. Pediatrics. 2011; 127(3):e758-e66. https://doi.org/10.1542/peds.2010-2895 PMid:21339277

22. Larsen GY, Mecham N, Greenberg R. An emergency department septic shock protocol and care guideline for children initiated at triage. Pediatrics. 2011; 127:e1585-92. https://doi.org/10.1542/peds.2010-3513 PMid:21576304

23. Paul R, Neuman MI, Monuteaux MC, Melendez E. Adherence to PALS sepsis guidelines and hospital length of stay. Pediatrics. 2012; 130(2):e273-e80. https://doi.org/10.1542/peds.2012-0094 PMid:22753559

24. Weiss SL, Fitzgerald JC, Balamuth F, et al. Delayed antimicrobial therapy increases mortality and organ dysfunction duration in pediatric sepsis. Critical care medicine. 2014; 42:2409. https://doi.org/10.1097/CCM.0000000000000509 PMid:25148597 PMCid:PMC4213742

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Clinical Science

The Effects of Filgrastim on Complications of Patients with Cerebral Hemorrhage Due To Head Trauma

Morteza Qaribi[1], Arash Yazdanbakhsh[1], Koorosh Ahmadi[2], Mohammad Reza Maghsoudi[2], Vagefe Farshin[1], Ali Ahmadi[3], Abolfazl Jokar[1*]

[1]Department of Emergency Medicine, Arak University of Medical Sciences, Arak, Iran; [2]Department of Emergency Medicine, Alborz University of Medical Sciences, Karaj, Iran; [3]Iran University of Medical Sciences, Iran

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Keywords: Filgrastim; Brain Trauma; Complications

***Correspondence:** Abolfazl Jokar. Department of Emergency Medicine, Arak University of Medical Sciences, Arak, Iran. E-mail: Dr.a.jokar@gmail.com

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Abstract

BACKGROUND: Filgrastim, a neopogen brand, is a blood-forming agent and a natural protein in the body that plays a role in stimulating the growth of white blood cells and protecting them against infectious agents. To the best of knowledge, human and animal specimens have shown the effect of Filgrastim on treating brain injuries regarding bone marrow transfusion into the blood, neuroprotection, stimulation of neurons for forming new neural networks and reducing the risk of bacterial infections.

AIM: This study aimed to investigate the effect of Filgrastim on the prognosis of a cerebral haemorrhage in patients with traumatic brain injury.

METHODS: This study was conducted as a clinical trial, in which the initial diagnosis of patients with cerebral haemorrhage due to head trauma was performed with a clinical examination and CT scan. After the patient arrives at the emergency room, the patient's initial examination is performed, and blood tests are taken from the patient. Moreover, CBC values (Hb, Platelet, Hematocrit) were checked and recorded in the checklist. The intervention group received 150 mcg/day Filgrastim injected subcutaneously for 4 days. Furthermore, patients in the control group received the same amount of sterile water. At the end of the treatment period, blood tests were performed again in all patients, and their results were then recorded. All data were analysed by SPSS v.21 software package.

RESULTS: Our findings revealed that the mean volume of bleeding in the intervention group based on CT scan was significantly reduced after four days as compared to the control group. Moreover, the mean score of consciousness and muscular strength of patients in the intervention group was significantly higher than the control group. Also, WBCs in the intervention group exhibited a significant increase after four days of intervention, while platelet and hematocrit levels in the intervention group decreased significantly compared to the control group.

CONCLUSION: Regarding the results, the therapeutic application of filtration is considered to be effective. Given the lack of serious complications of the proposed dosages, the use of this drug can be suggested.

Introduction

Brain trauma and its complications, such as cerebral haemorrhage results from a blow or jolt to the head and have led to the loss of high-yielding years in the community, which is a leading cause of mortality. Traumatic brain injury is one of the key factors in public health, where is considered to be one of the most important causes of death among *young people* in rich countries.

Given the growing growth of motor vehicles, the rate of brain injury in the world is increasing. It is estimated that more than 10 million brain injury occurs annually worldwide in need of hospital care. It is worth noting that 9% of all deaths in the world are due to traumatic brain injury. Surviving patients are likely to be affected by permanent or temporary disabilities. The financial burden of traumatic brain injury has been estimated to be more than $ 60 billion annually (only in the United States). Many traumatic brain damage develops during hospitalisation and increases the need for surgery or the degree of complications and mortality of patients [1]. Trauma is the most common cause of death for *people* ages *1* to *44*

years. Head trauma is the most common cause of hospitalisation and death of traumatic patients (40 to 50%), [2]. The traumatic brain haemorrhages are divided into *delayed* and early traumatic intracerebral *haemorrhages*. Early haemorrhages are related to patients who have been diagnosed with haemorrhage in a CT scan for up to six hours after the trauma. However, haemorrhage is likely to appear after 6 hours with CT scans for various reasons, where such haemorrhage is called delayed intracerebral haemorrhage. Delayed traumatic intracerebral hematoma (*DTICH*) is one of the most important treatable complications of secondary brain damage in patients with brain trauma [3].

Since CT scan has become commonplace as a good diagnostic tool, the DTICH incidence in all patients with brain damage is reported to be between 0.6% and 7.4%. DTICH can include all kinds of cerebral haemorrhages, including intraparenchymal haemorrhage (IPH), *subdural haemorrhage* (SDH), and extradural *haemorrhage* (EDH) or *epidural haemorrhage* (*EDH*). Although DTICH may be present in a patient who has already had a history of brain damage, it can occur in patients with primary normal CT scan results. The death rate for DTICH is more than 35% to 40% [2]. Nevertheless, various studies have shown that mortality rates are significantly reduced in the event of timely diagnosis and rapid treatment [3]. In the absence of timely diagnosis and treatment, the mortality rate may even be higher than 50% [4]. Studies show that DTICH can occur in each age group with varying degrees of trauma and with different levels of patient alertness, even in patients with GCS of 15, as well as patients with a completely normal CT scan or without a skull fracture [5].

Granulocyte colony stimulator (G-CSF) is a glycoprotein that generates a hematopoietic cell colony in the culture of bone marrow cells. G-CSF is an important factor in neutrophil-based immune defence system due to a regulatory role in growth, differentiation, survival and activation of neutrophils and precursors [6].

Filgrastim is a blood-forming agent and a natural protein called Neupogen, which stimulates the growth of white blood cells in the body and protects these cells against infection. Filgrastim is used for several therapeutic purposes, including neutropenia, the loss of some white blood cells from cancer, bone marrow transplantation, chemotherapy, or other conditions. Filgrastim is considered to be the synthetic form of G-CSF (Granulocyte Stimulator), a natural protein that promotes the production of white blood cell production. It should be taken into consideration that G-CSF deficiency increases the risk of bacterial infections.

This drug induces bone marrow to produce white blood cells and thereby reduce the risk of infection. Therefore, bone marrow cells enter the bloodstream to make bone marrow transplantation easy [7]. Recent studies on human and animal specimens have revealed the effect of filgrastim on the treatment of brain injuries for several functions, such as the entrance of bone marrow cells into the blood, neuroprotection, stimulation of neural cells for forming new neural networks and reducing the risk of bacterial infections [2] [8].

Despite extensive research in recent years, there is still no drug that can protect against the effects of brain damage and subsequent bleeding from brain cells. Therefore, this study aimed to investigate the effect of Filgrastim on the prognosis of a cerebral haemorrhage *in patients with traumatic brain injury*.

Material and Methods

This randomised, double-blind clinical study was conducted among patients referred to the emergency department of Vali-e-Asr Hospital in Arak for head trauma. Patients were selected based on the initial diagnosis with a clinical examination and CT scan. All patients with inclusion criteria were entered into intervention and control groups at the time of arrival using a random number table. After the patient's arrival, the patient's primary examination was performed and the patient's background information, including age, sex, and the cause of the trauma, were recorded. Blood tests were performed from patients, and the complete blood count (CBC) (Hct and PLt, Hb) were checked and recorded on the checklist. Patients in the case group (34 patients) received a subcutaneous injection of 150 mg Filgrastim weekly for 4 days. Before and during treatment with this drug, vital signs, haemoglobin and electrolytes were carefully checked.

On the other hand, patients in the control group (34 patients) received the same amount of distilled water. It should be noted that the patient was not aware of the type of treatment received. Patients were monitored for the type of brain injury, CBC, vital signs, and coagulation tests, as well as some electrolytes. At the end of the course of treatment, blood tests were repeated in all patients. If the laboratory factors were normal, response to the treatment was recorded for patients.

Finally, the data were analysed by SPSS v.21 software using SPSS software. To evaluate the results, indicators such as mean, standard deviation, standard error, frequency percentage were employed. For analytical analysis, the covariance test, Chi-div test and Independent T-test or its nonparametric equivalents were used to compare the mean. Meanwhile, values of $p < 0.05$ were considered as significant levels.

Entry criteria included: 1) aged 18 years and

above; 2) patients with head trauma, 3) consciousness (GCS) between 9 and 13; 4) Filling out the informed consent.

Exclusion criteria included: 1) Inclination to participate in the study; 2) Alcohol, and drug use, or any factor other than brain trauma that reduces the level of consciousness in the patient; 3) Acute hypersensitivity to the drug; 4) history of hypertension, diabetes, embolism and DVT; 5) Alertness below 9 and above 13; 6) Patient's death 72 hours after entering the emergency room.

In all stages of the project, ethical considerations such as informed consent for participating in the study and withdrawal were voluntarily, and the confidentiality of the information was observed. This research project was approved by the ethical committee of Arak University of Medical Sciences (No. 1173 and the code of ethics: IR.ARAKMU.REC.1395.100).

Results

Most of the patients in the experimental group (76.7%) were male, and the remaining was female. Furthermore, most of the patients in the control group (83%) belonged to men and 17% of the remaining was women. To examine the homogeneity of the two groups, the Chi-square test was applied. The findings revealed that there was no significant difference in sex between the two groups (P = 0.22). In the test group, the majority of patients (46.6%) were in the age group of 21-30 years and the lowest (10%) in the age group of 20 to 20 years old. Furthermore, most of the patients in the control group (50%) were in the age group 40, and the lowest (6%) belonged to the age group of 40-31 years old (Figure 1).

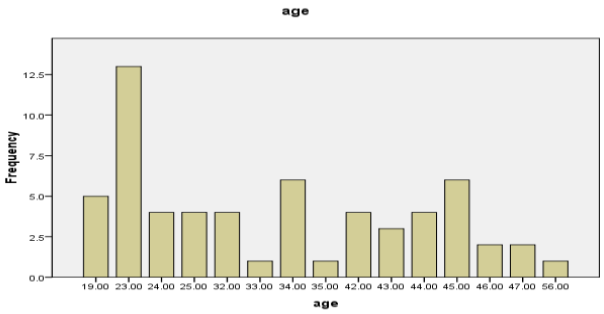

Figure 1: Age distribution in the intervention and control group

It should be taken in to account that the Fisher test was employed to check the homogeneity of the two groups. Based on the results, there was no significant difference between the two groups regarding age (P = 0.42). Chi-square test exhibited no significant difference regarding height and weight between the two groups (Table 1).

Table 1: Frequency distribution of patients in two groups of intervention and control regarding height and weight

Sex	Height	Weight
Intervention	32.166 ± 89.10	96.72 ± 43.11
Control	2.168 ± 55.10	3.72 ± 58.10
P –value	0.580	0.420

Based on the results of t-test presented here, the level of consciousness of both groups did not show any significant difference (P = 0.65) and also after the intervention, no significant difference was found in consciousness level of both groups (P = 0.05) based on the use of t-test (Table 2).

Table 2: Frequency distribution of patients in two groups of intervention and control regarding the level of consciousness

GCS	Intervention	controls	P –value
GCS0	80.10 ± 12.1	76.10 ± 16.1	0.650
GCS4	56.11 ± 22.1	36.10 ± 24.1	0.05

The t-test demonstrated that the mean scores of vital signs before and after intervention in both groups were not statistically significant (P > 0.05; Table 3).

Table 3: Frequency distribution of patients in two groups of intervention and control regarding vital signs

Variables	Intervention	Control	P-value T-test
SBP before intervention	3.130 ± 3.73	12.137 ± 41.14	0.150
SBP after intervention	23.132 ± 19.8	6.132 ± 70.9	0.430
DBP before intervention	93.77 ± 93.6	76.81 ± 41.7	0.770
DBP after intervention	10.75 ± 44.5	86.75 ± 78.6	0.390
PR before intervention	43.100 ± 55.5	03.94 ± 8.5	0.300
PR after intervention	76.936 ± 90.5	40.96 ± 26.6	0.710
RR before intervention	4.18 ± ½	1.19 ± 17.2	0.880
RR after intervention	3.17 ± .91	9.16 ± 1.1	0.780
T before intervention	1.37 ± .36	08.37 ± .3	0.380
T after intervention	1.37 ± .3	1.37 ± .37	0.550

As shown in Table 4, the mean scores of hospitalisation days in both groups were statistically significant (P = 0.000).

Table 4: Distribution of hospitalisation days in two groups of intervention and control

Variable	Intervention	Control	Test
hospitalisation days	7±0 .90	53.9±32.1	0.000

Based on the data presented in Table 5, independent t-test indicated no significant difference regarding the mean blood volume in both groups (P = 0.3), while it was statistically significant after four days (P = 0.000).

Table 5: Computed tomography from the first to fourth days

Variable	Intervention (cm3)	Control (cm3)	T-test P-value
Scan the first day	83.14±52.2	50.15±93.2	0.003
Scan the second day	82.10±88.1	60.14±86.1	0.000
Scan the third day	36.9±37.1	06.13±70.1	0.000
Scan the fourth day	66.7±.86	06.11±59.1	0.000

Moreover, we found that the mean of muscle strength of the first day was not statistically different in both groups (P = 0.3), while its values were

statistically significant after four days in two groups (P = 0.000) (Table 6).

Table 6: Muscle strength on days 1 to 4 in both groups

Variable	Intervention	Control	T-test P-value
Muscle strength the first day	2.1±71.0	20.2±56.0	0.300
Muscle strength the second day	36.2±61.0	30.2±53.0	0.06
Muscle strength the third day	26.3±73.0	70.2±65.0	0.30
Muscle strength the fourth day	3.3±86.0	96.2±55.0	0.005

The mean of HCT, WBC and PLT in both groups did not show a significant difference by t-test before the intervention (P > 0.05), while the mean values of these variables in both groups were not significantly different after four days of intervention (P >0.05) (Table 7).

Table 7: Frequency distribution of CBC in the control and intervention groups

Variable	Intervention	Control	T-test P-value
Hematocrit before intervention	76.43±78.3	52.42±52.3	0.30
Hematocrit after intervention	83.41±21.4	26.45±71.3	0.10
WBC before intervention	95.10±41.2	83.10±66.1	0.90
WBC after intervention	36.20±42.4	50.15±39.3	0.000
Platelet before intervention	27.302±23.83	53.295±05.71	0.080
Platelet after intervention	53.241±52.83	23.306±89.85	0.020

Based on t-test, mean PT and PTT before and after intervention were not significantly different in both groups(P > 0.05; Table 8). However, after four days of intervention, mean PT in both intervention and control groups exhibited a significant difference (P < 0.05). Where the PTT coagulation test did not show a significant difference after four days, as a comparison of the two groups (P = 0.4).

Table 8: Frequency distribution of patients in two groups of intervention and control according to coagulation tests

Variable	Intervention	Control	T-test P-value
PT before intervention	7.13±2 .97	9.14±52.2	0.09
PT after intervention	03.15±96.2	03.16±.96	0.05
PTT before intervention	13.20±25.2	13.20±65.1	0.01
PTT after intervention	30.20±.83	13.20±03.1	0.04

Discussion

This study aimed to investigate the effects of filgrastim on the complications of patients with a head traumatic brain haemorrhage. The present study showed that the majority of patients in the intervention group (46.6%) were in the age group of 21-30 years old and the lowest number of patients (10%) was in the age group of 10 to 20 years old.

In the control group, the majority of patients (50%) were in the 40-year-old age group, while fewer patients (6%) were in the age group of 31-40 years...

According to the results presented in this study, most of the patients (76.7%) belonged to men. These statistics vary in different societies, but the most common age group with traumatic brain injury has been reported to be between 20-29 years old in most countries, including Taiwan. In concurring with the present study, various international and regional studies have reported that the prevalence of head injuries in men was higher than in women [9].

The results of the current study demonstrated that the level of consciousness in both groups was not significantly different on the first day; however, after 4 days of administration, the level of consciousness in the intervention group based on Glasgow criteria was significantly higher than that of the control group.

To the best of our knowledge, a similar study was not found on the effects of filgrastim and consciousness levels, but Zareian et al., have revealed that using Epigallocatechin-3-gallate over 7 days has been able to increase the level of consciousness in the intervention group compared to the control group, which is consistent with our study [10]. Furthermore, our findings emphasised that the number of hospital admissions days in the intervention group was significantly lower than those in the control group. One of the important issues that have always been addressed by the managers of hospitals and health centres is the length of stay that is both economically and organizationally important.

The length of hospitalisation can be employed as a factor in assessing the efficiency and effectiveness of hospital services. These criteria can be applied for various purposes such as healthcare management, quality control of hospital services [11].

It should be noted that the use of filtration in controlling the complications of head trauma patients has been able to reduce the length of hospitalisation. The location, volume of bleeding and the amount of pressure on the brain tissue are important factors for hematoma. CT scan is a tool that, in addition to being able to perform in emergency situations, has the ability to detect the exact location of the hematoma, the volume of the hematoma, and the amount of pressure on one part of the brain (shifts), so it is very helpful in determining the therapeutic strategies. To diagnosis the volume of hematoma, Epperson and Peterson criteria can be employed, which is found by multiplying the length, width and height of the hematoma in 0.5 [12]. Based on the results presented here, using filgrastim drug significantly reduced the volume of bleeding in CT scan on days 3 and 4, as compared to the patients in the control group. In other words, filgrastim has been able to reduce the volume of bleeding in patients with a brain hemorrhage, when comparing with the control group. Shabiri et al. measured the *hemorrhage volumes* on CT in the *bleeding trauma patients*, concluding that reducing the volume of cerebral hemorrhage is associated with an increase in the level of consciousness [13], which is consistent with our study outcomes. In addition, the results of this study revealed that the patients in both groups did not differ in muscle strength before the study, but the mean scores of muscle strength

increased significantly in the intervention group after four days of using filgrastim. Studies have emphasised that lower levels of brain injury and bleeding complications could be linked to an increase in muscle strength, which is directly related to the patient's level of consciousness [14].

Moreover, our findings suggested that after four days of taking filgrastim, the hematocrit levels, platelet counts in the intervention group were lower than the control group, were showed a statistically significant difference. Nevertheless, WBCs in the intervention group showed a significant increase after four days of intervention. We can point out that the extracted results of this study are consistent with the properties of Filgrastim (Neupogen).

The effects of filgrastim on the complications of patients with cerebral haemorrhage due to positive trauma were evaluated, where it was positively effective. Because there is no serious complication on the days recommended, the use of this medication can be suggested.

References

1. Margolick J, Dandurand C, Duncan K, Chen W, Evans DC, Sekhon MS, Garraway N, Griesdale DEG, Gooderham P, Hameed SM. A Systematic Review of the Risks and Benefits of Venous Thromboembolism Prophylaxis in Traumatic Brain Injury. Can J Neurol Sci. 2018; 13:1-13. https://doi.org/10.1017/cjn.2017.275

2. Youmans JR, Becher DP, Dunsker SB, Friedman WA, Hoffman HJj, Smith RR e, et al. Neurological surgery 4th ed. Philadelphia Sanders. 1996; 3:1557-8

3. Gopinath SP, Robertson CS, Contant CF, Narayan RK, Grossman RG, Chance B. Early detection of delayed traumatic intracranial hematomas using near-infrared spectroscopy. Journal of neurosurgery. 1995; 83(3):438-44. https://doi.org/10.3171/jns.1995.83.3.0438 PMid:7666220

4. Cooper P. Delayed traumatic intracerebral haemorrhage. Neurosurgery clinics of North America. 1992; 3(3):659-65. https://doi.org/10.1016/S1042-3680(18)30654-5

5. Iuvara-Bommeli A, de Tribolet N. [Delayed intracranial hematomas following cranio-cerebral trauma]. Schweizerische medizinische Wochenschrift. 1991; 121(18):646-52. PMid:2047825

6. Dale DC. Colony-stimulating factors for the management of neutropenia in cancer patients. Drugs. 2002; 62(1):1-15. https://doi.org/10.2165/00003495-200262001-00001 PMid:12479591

7. Welte K. G-CSF: filgrastim, lenograstim and biosimilars. Expert Opin Biol Ther. 2014; 14(7):983-93. https://doi.org/10.1517/14712598.2014.905537 PMid:24707817

8. Heard SO, Fink MP, Gamelli RL, Solomkin JS, Joshi M, Trask AL, et al. Effect of prophylactic administration of recombinant human granulocyte colony-stimulating factor (filgrastim) on the frequency of nosocomial infections in patients with acute traumatic brain injury or cerebral haemorrhage. Critical care medicine. 1998; 26(4):748-54. https://doi.org/10.1097/00003246-199804000-00027 PMid:9559614

9. Andelic N, Sigurdardottir S, Brunborg C, Roe C. Incidence of hospital-treated traumatic brain injury in the Oslo population. Neuroepidemiology. 2008; 30(2):120-8. https://doi.org/10.1159/000120025 PMid:18334828

10. Effect of Epigallocatechin-3-Gallate Supplementation on Glasgow Coma Score Scale of Patients with Traumatic Brain Injury. The-Neuroscience-Journal-of-Shefaye-Khatam. 2016; 4(4):61-6. https://doi.org/10.18869/acadpub.shefa.4.4.61

11. Ribah Adnan; Adeleh Hashemi fard; Seyyed Ehsan Saffari. The Effective Factors on the number of hospitalization days for MI patients in Vasei hospital of Sabzevar in 2012 using regression models. Journal of Sabzevar University of Medical Sciences. 2014; 20(4):447-456.

12. Hardemark HG, Wesslen N, Persson L. Influence of clinical factors, CT findings and early management on outcome in supratentorial intracerebral hemorrhage. Cerebrovascular diseases (Basel, Switzerland). 1999; 9(1):10-21. https://doi.org/10.1159/000015890 PMid:9873158

13. Shabiri E, Saeidi Bourojeni HR, Rezaei M, Jahanbakhshi A. Relationship of CT scan findings with consciousness, surgical findings and the fate of patients with traumatic intracranial hemorrhage. Journal of kermanshah university of medical sciences. 2014; 18(3):165-172.

14. Von Eisenhart-Rothe RM, Jäger A, Englmeier K-H, Vogl TJ, Graichen H. Relevance of arm position and muscle activity on three-dimensional glenohumeral translation in patients with traumatic and atraumatic shoulder instability. The American Journal of Sports Medicine. 2002; 30(4):514-22. https://doi.org/10.1177/03635465020300041101 PMid:12130406

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Clinical Science

Predictors of Glucose Control in Children and Adolescents with Type 1 Diabetes: Results of a Cross-Sectional Study in Khartoum, Sudan

Zainab Taha[1*], Zeinab Eltoum[2], Sidiga Washi[2†]

[1]Department of Health Sciences, Zayed University, Abu Dhabi, United Arab Emirates; [2]School of Health Sciences, Ahfad University for Women, Omdurman, Sudan

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***Correspondence:** Zainab Taha. Department of Health Sciences, Zayed University, Abu Dhabi, United Arab Emirates. E-mail: ztaha8@gmail.com

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Abstract

BACKGROUND: Type 1 diabetes mellitus (T1DM) is a rapidly growing problem in Sudan as well as other African countries. Children and adolescents with type 1 diabetes have previously been found to have poor glycemic control. Strict glycemic control reduces the incidence and progression of chronic complications.

AIM: This study aimed to identify the factors associated with glycemic control among children and adolescents.

METHODS: The study was a health-centre based descriptive cross-sectional study. Data on socioeconomic, demographic, disease history, and diabetes-specific variables was obtained. Glycemic control was assessed by measuring glycosylated haemoglobin (HbA1C). Linear regression analysis was done to determine factors associated with glycemic control.

RESULTS: One hundred Sudanese children with T1DM aged from (1-18) years were recruited for the study (63 % females). Most of the study children (80%) had high random blood glucose levels. Less than half (40%) suffered from the presence of glucose in their urine and one-quarter of them have urine ketones. Also, Glycosylated haemoglobin (HbA1c) level of the study children showed that more than three-quarters (76%) had poor glycemic control. It was found that there is no relationship between nutritional status and glycemic control. However, there is a relationship between socioeconomic status and glycemic control (P = 0.025)

CONCLUSION: To improve metabolic control, more frequent BGM should be encouraged among children and adolescents with T1DM. Emphasis needs to be put on providing families with children with diabetes with the medical, financial and social support for better control of their diabetes.

Introduction

Type 1 diabetes mellitus (T1DM) is one of the most significant and serious chronic diseases targeting children and adolescents worldwide. It is an autoimmune disease with a strong genetic component [1] [2]. While it may target any age group, it tends to develop during childhood [3].

More than half a million children (542,000) worldwide have T1DM and the number of newly diagnosed cases each year is 86,000 [4]. Prevalence of cases in children under 15 is expected to rise by 70% in the coming years until 2020 [5] [6].

Type 1 Diabetes is a major health problem in Sudan and other African countries and is a leading cause of morbidity and mortality [7]. The incidence was found to range between 4.4/100,000 in Algeria to 20/100,000 in Morocco [8]. The prevalence of T1DM among Sudanese young people is increasing. Old studies showed an increase in incidence from 9.5/100,000 in 1991 to 10.3/100,000 in 1995 [9]. In Sudan, T1DM prevalence is 10.1 per 100,000 children, and the overall annual increase is estimated to be around 3% [10].

Diabetic children are expected, with proper nutrition and care, to acquire normal nutritional status [11] [12]. However, several studies describe growth impairment and poor nutritional status to be well-

known complications of T1DM [13] [14]. It is also associated with poor glycemic control [13].

A previous study in children with T1DM in Sudan found the glycemic control to be poor in 86% of the patients, where the pre-meal blood glucose level was 9 mmol/l or higher [15]. It has been well documented and supported by the Diabetes Control and Complication Trial (DCCT) that strict glycemic control could reduce the long-term complications of T1DM [16]. The general recommendations, as evidenced by the DCCT, are to maintain the glycosylated haemoglobin (HbA1c) below 7 %. Nevertheless, to protect children from hypoglycemia, the American Diabetes Association (ADA) provides recommendations for children and adolescents which vary. A level of < 8% is recommended for children between 6 and 12 years, and a level of < 7.5% for those > 12 years [17]. In Sudan, a high prevalence of chronic complications has been described, and they are associated with poor glycemic control, low quality of life, and particularly with morbidity [15].

A study carried out on children and adolescents aged 5 to 18 years in Khartoum showed acute complications of diabetes, as evidenced by ketone bodies in urine, reported in 46% of the children, and hypoglycemia that needed special attention had occurred in 37% of the patients [18]. There was no correlation between the parents' incomes and glycemic control, nor was there is a difference in diabetes control between children attending private and public clinics. Hypoglycemia requiring special attention had occurred in 37% of the patients, and 57% had been admitted at least once to the hospital within the last year; the main causes of admission being diabetic ketosis (72%), hypoglycemia (6%), malaria (11%) or other medical disorders or surgical interventions (9%) [18].

Various risk factors and challenges have been described that are associated with glycemic control. Some of these include socio-demographic variables, such as the age of the child, socioeconomic status, and family structure [19] [20]. Other diabetes-related factors, such as duration of diabetes, adherence and caregiver involvement in the child's care, have also been significantly associated with glycemic control [21] [22]. There was a negative correlation between the mother's educational level and the fasting blood glucose level of children with diabetes. Most of these studies were done in Europe and North America, and very little data exists about risk factors in low resource settings in sub-Saharan Africa.

This study aimed to identify the factors associated with glycemic control in children and adolescents with T1DM in Khartoum. This will help to plan and implement effective intervention programs that focus on improving diabetes control in children and adolescents and to prevent chronic complications.

Methods

This study was a Health-center based descriptive cross-sectional study. We recruited 100 Sudanese children with T1DM aged from (1-18) years attending Sudan Childhood Diabetes Centre in Khartoum after obtaining consent from parents.

The study subjects were diagnosed with T1DM for at least 1 year with or without complications, attended the centre during the study period from October 2017 to March 2018. We excluded any child with T1DM whose age was below or above the age group (1-18) years and those diagnosed with T1DM for less than one year.

The study was approved by the Ahfad University for Women Research Ethics Committee. Additional clearance was obtained from the Sudan Childhood Diabetes Centre, who enabled the data collected from the patients. Informed consent was taken from respondent families before the enrolment of participants in the study. Privacy and confidentiality were maintained throughout the study period by excluding personal identifiers during data collection.

Primary data was collected using a pretested questionnaire that was initially developed in English and then translated into Arabic using a cross-translation technique. The questionnaire includes questions about demographics and disease history of the study subjects, food intake using a standard food frequency questionnaire, and nutritional habits. Biochemical data (blood and urine test results) were obtained from the patients' records. Anthropometric measurements, including weight and height, were measured using standard procedures.

All analyses were performed using IBM SPSS Statistics version 14, and the results were presented in the form of tables of frequencies and percentages. Chi-square test was used to test the relationship between nutritional status, socioeconomic status, and glycemic control. The nutritional status was assessed using the BMI-for-age (Z-score) Child Growth Reference 0-2, 2-5 and 5-19 years [23]. In the abstract, you mentioned using linear regression analysis.

Results

A total of 100 children and adolescents aged up to 18 years with T1DM were recruited for the study. The mean age was 12.5 ± 2.7 years (median: 12.5, range: 7-18 years). The majority of the children (89%) were in the age group (7-18) years, and the females were more (63%) than males (37%). Over half of the

study subjects (58%) were in basic school, and one quarter (26%) at secondary school (Table 1).

Table 1: Child Characteristics (n = 100)

Parameters	Description	Frequency	Percentage
Gender	Female	63	63
	Male	37	37
Age	1-3	3	3
	4-6	8	8
	7-9	15	15
	10-12	27	27
	13-15	29	29
	16-18	18	18
Child's Education	No Schooling	10	10
	Pre-school	5	5
	Basic Education	58	58
	Secondary	26	26
	University	1	1
	Total	100	100
Child's Position	1st child	26	26
	2nd child	18	18
	3rd child	21	21
	4th child	19	19
	Above	16	16
	Total	100	100

The family demographics are shown in Table 2. Most of the children's families (77%) have incomes less than 1500 (SDG) per month. While child's birth order in the family shows that one-quarter of the children (26%) were the first child, about half (53%) of the children were coming from family members of 6-8 (Table 2).

Table 2: Family demographics (n = 100)

Parameters	Description	Frequency	Percentage (%)
Mother's Age	20-30	13	13
	Above 30	87	87
Mother's Education	Illiterate	8	8
	Primary	24	24
	Middle	12	12
	Secondary	32	32
	University	16	16
	Postgraduate	8	8
Mother's Occupation	Housewife	80	80
	Worker	2	2
	Employee	8	8
	Self-employed	10	10
Father's Education	Illiterate	9	9
	Primary	20	20
	Middle	12	12
	Secondary	28	28
	University	25	25
	Postgraduate	6	6
Father's Occupation	Retired	3	3
	Unemployed	11	11
	Worker	10	10
	Employee	16	16
	Self-employed	60	60
Origin	North Sudan	27	27
	East Sudan	13	13
	West Sudan	17	17
	Center of Sudan	40	40
	Outside Sudan	3	3
Residence	Omdurman	20	20
	Khartoum	40	40
	Bahri	28	28
	Aljazira Villages	12	12
Family Members	3-5	29	29
	6-8	53	53
	9-11	18	18
	Above 11	0	0
Income/Month(SDG)	Less than 1500	77	77
	1500 - 2500	19	19
	More than 2500	4	4

Most of the children had normal weight (70%). About 88% were using insulin mixtures, while none of them was using insulin pumps. Most of the children (80%) had had a history of hospital admission with DKA. Of the 71% of the children who reported a regular Self-Monitoring Blood Glucose (SMBG), 31% do it on a daily basis. Seventy-nine percent of those who didn't do regular SMBG claimed the cost of tests to be the main obstacle. Twenty one percent of the children have other family members with diabetes, where one-third of them (33.3%) were their mothers (Table 3).

Table 3: Nutritional status and diabetes history (n = 100)

Parameters	Description	Frequency	Percentage (%)
Nutritional Status	Overweight	10	10
	Obesity	3	3
	Sever thinness	4	4
	Thinness	13	13
	Normal	70	70
Insulin Regimen	Basal/bolus	12	12
	Mixtures	88	88
	Insulin pump	0	0
	Total	100	100
History of DKA	Yes	80	80
	No	20	20
	Total	100	100
Regular SMBG	Yes	71	71
	No	29	29
If yes, frequency (n=71)	On a daily basis	22	31
	Three times a week	4	5.6
	Twice a week	41	57.7
	Once a week	2	2.8
	Once a month	2	2.8
	Total	71	100
If no, the reason (n=29)	Cost of test	23	79.3
	Damaged device	3	10.3
	Doesn't know the importance of the test	3	10.3

Most of the study children (80%) had high random blood glucose levels. Less than half of them (40%) suffered from the presence of glucose in their urine and one-quarter of them had urine ketones. Also, Glycosylated haemoglobin (HbA1c) level of the children showed that more than three-quarters of them (76%) had poor glycemic control and less than one quarter (24%) have a good glycemic control (Table 4).

Table 4: Biochemical Data (n = 100)

Parameters	Description	Frequency	Percentage (%)
Random blood glucose level	Normal	20	20
	High	80	80
	Total	100	100
Urine glucose level	Normal	60	60
	Present	40	40
	Total	100	100
Urine ketones level	Normal	75	75
	Present	25	25
	Total	100	100
Glycosylated haemoglobin (HbA1c) level	Good control	24	24
	Poor control	76	76
	Total	100	100

It was found that there is no relationship between nutritional status and glycemic control, while there is a relationship between socioeconomic status and glycemic control (P = 0.025) (data not shown).

Discussion

In this cross-sectional study, most of the children (80%) had a history of hospital admission

with DKA. Similar results have been recently reported in Sudan as (81%) of the children diagnosed with T1DM were presented to hospitals with DKA [18]. According to WHO, the highest rates of DKA are found in low- and middle-income countries and therefore, our findings might be associated with the cost of test that, resulting in a low frequency of SMBG [24]. Among the children who reported a regular SMBG, only one third of them performed the test on daily basis. The cost of the test was given as the main factor for the majority (79.3%) for not following a regular SMBG. The finding of the association between the SMBG and the cost is of great importance, as it will affect the control of diabetes. A massive study of 26723 children with T1DM and similar age to our study's children found that increasing the SMBG frequency was significantly associated with better metabolic control and reduced frequency of DKA. Only (21%) of the children have other family members with diabetes, where one-third of them (33.3%) were the children' mothers. This might prove that T1DM is a form of the disease that has no known aetiology and low role of heredity associated with it [25].

Diabetic complications were reported among our study children, where (11%) of the children have eye problems and (2%) had kidney problems. Another study in Sudan has revealed the association between T1DM in children with poor glycemic control, the high prevalence of complications, low quality of life, and particularly with morbidity. Regardless of the importance of consistent glycemic control for protection from chronic diabetes complications that has been well documented, adhering to a diabetes regimen is particularly difficult for young children. This has ultimately led to more frequent hospitalisations and medical complications among children [26]. In the current study, (8%) of the children had celiac disease, and only (2%) had thyroid problems. This might be in adherence with the reported figures in the Krause's food & the nutrition care process whereby celiac disease affects 1-16% of patients compared with 0.3-1% in the general population, and autoimmune thyroid disease occurs in 17-30% of people with T1DM [27].

The management of diabetes in childhood has implications for later development of complications which have been linked to poor glycemic control and the duration of the disease [28]. Children with T1DM should be targeted to achieve an HbA1c ≤ 7.0% to reduce the risks of diabetic complications [29]. In the current study, the biochemical data of the children revealed poor results. Their Glycosylated haemoglobin (HbA1c) levels show that most of them (76%) had poor glycemic control. Also, the majority of the children (80%) had high random blood glucose levels, more than one-third (40%) suffered from the presence of glucose in urine and a quarter (25%) had urine ketones. Similar results of poor glycemic control were reported in Sudan among children with T1DM [18]. Other studies have been conducted in Africa and have also documented poor glycemic control among children with T1DM [30] [31]. Regardless of the poor glycemic control of the children, no significant effect was detected on their growth. Most children have a normal weight, and no significant association was found between their nutritional status and glycemic control (P = 0.168). This result contrasts with the findings of other studies where children with poor metabolic control were reported to have a significantly lower growth velocity than those with adequate metabolic control [32].

The major finding of our study is the significant association between the children's socioeconomic status and their glycemic control (P = 0.025). In contrast to our study, Eliadarous, 2017 was not able to detect any correlation between the parents' incomes and glycemic control of diabetic children in Sudan [18]. Several reasons may stand behind the poor glycemic control of those children, such as high illiteracy rates amongst both mothers and fathers. Besides the direct effect of illiteracy on good health care, illiteracy may also affect the father's income capacity to provide for the family including health care and hence, hamper good financial support to children with diabetes. Nevertheless, this poor glycemic control increases the children's risk of diabetic complications and reduces the quality of their lives.

In conclusion, we found that the metabolic control of our diabetic children is very poor. No significant correlation was found between the children nutritional status and glycemic control (P = 0.168) and most of the study subjects had normal weight. However, a significant association was revealed between their socioeconomic status and glycemic control (P = 0.025).

To improve metabolic control, more frequent BGM should be encouraged among children and adolescents with T1DM. Emphasis needs to be put on providing families with diabetic children with the medical, financial and social support for better control of their diabetes. The Sudanese healthcare should emphasise continuous educational programs for parents and caregivers on the important practices that aim for metabolic control and proper management. Close follow up of the children is needed as this group is the most vulnerable to develop complications.

Further research is needed to evaluate the effectiveness of teaching children and adolescents with T1DM and their family members about the glycemic index of foods consumed in the context of different insulin treatment regimens.

References

1. Noble JA, Erlich HA. Genetics of type 1 diabetes. Cold Spring Harbor perspectives in medicine. 2012; 2(1):a007732. https://doi.org/10.1101/cshperspect.a007732PMid:22315720 PMCid:PMC3253030

2. Steck AK, Rewers MJ. Genetics of type 1 diabetes. Clinical chemistry. 2011; 57(2):176-85. https://doi.org/10.1373/clinchem.2010.148221 PMid:21205883 PMCid:PMC4874193

3. Marigliano M, Tadiotto E, Morandi A, Sabbion A, Contreas G, Avossa F, et al. Epidemiology of type 1 diabetes mellitus in the pediatric population in Veneto Region, Italy. Diabetes research and clinical practice. 2015; 107(3):e19-21. https://doi.org/10.1016/j.diabres.2014.12.009 PMid:25641011

4. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes research and clinical practice. 2017; 128:40-50. https://doi.org/10.1016/j.diabres.2017.03.024 PMid:28437734

5. Dabelea D, Bell RA, D'Agostino RB, Jr., Imperatore G, Johansen JM, Linder B, et al. Incidence of diabetes in youth in the United States. Jama. 2007; 297(24):2716-24. https://doi.org/10.1001/jama.297.24.2716 PMid:17595272

6. Vehik K, Hamman RF, Lezotte D, Norris JM, Klingensmith G, Bloch C, et al. Increasing incidence of type 1 diabetes in 0- to 17-year-old Colorado youth. Diabetes care. 2007; 30(3):503-9. https://doi.org/10.2337/dc06-1837 PMid:17327312

7. Noor SK, Elmadhoun WM, Bushara SO, Almobarak AO, Salim RS, Forawi SA, Awadallah H, Elwali ES, Ahmed MH. Glycaemic control in Sudanese individuals with type 2 diabetes: Population based study. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2017; 11:S147-51. https://doi.org/10.1016/j.dsx.2016.12.024 PMid:28034691

8. Majaliwa ES, Elusiyan BE, Adesiyun OO, Laigong P, Adeniran AK, Kandi CM, et al. Type 1 diabetes mellitus in the African population: epidemiology and management challenges. Acta bio-medica : Atenei Parmensis. 2008;79(3):255-9.

9. Elamin A, Ghalib M, Eltayeb B, Tuvemo T. High incidence of type 1 diabetes mellitus in Sudanese children, 1991-1995. Ann Saudi Med. 1997; 17(4):478-80. https://doi.org/10.5144/0256-4947.1997.478 PMid:17353609

10. Patterson C, Guariguata L, Dahlquist G, Soltesz G, Ogle G, Silink M. Diabetes in the young - a global view and worldwide estimates of numbers of children with type 1 diabetes. Diabetes research and clinical practice. 2014; 103(2):161-75. https://doi.org/10.1016/j.diabres.2013.11.005 PMid:24331235

11. Danne T, Kordonouri O, Enders I, Weber B. Factors influencing height and weight development in children with diabetes. Results of the Berlin Retinopathy Study. Diabetes care. 1997; 20(3):281-5. https://doi.org/10.2337/diacare.20.3.281 PMid:9051372

12. Demir K, Altincik A, Abaci A, Buyukgebiz A, Bober E. Growth of children with type 1 diabetes mellitus. Journal of clinical research in pediatric endocrinology. 2010; 2(2):72-7. https://doi.org/10.4274/jcrpe.v2i2.72 PMid:21274342 PMCid:PMC3005675

13. Marcovecchio ML, Heywood JJ, Dalton RN, Dunger DB. The contribution of glycemic control to impaired growth during puberty in young people with type 1 diabetes and microalbuminuria. Pediatric diabetes. 2014; 15(4):303-8. https://doi.org/10.1111/pedi.12090 PMid:24320564

14. Silverstein J, Klingensmith G, Copeland K, Plotnick L, Kaufman F, Laffel L, et al. Care of children and adolescents with type 1 diabetes: a statement of the American Diabetes Association. Diabetes care. 2005; 28(1):186-212. https://doi.org/10.2337/diacare.28.1.186 PMid:15616254

15. Elrayah H, Eltom M, Bedri A, Belal A, Rosling H, Ostenson CG. Economic burden on families of childhood type 1 diabetes in urban Sudan. Diabetes research and clinical practice. 2005; 70(2):159-65. https://doi.org/10.1016/j.diabres.2005.03.034 PMid:15919129

16. Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: Diabetes Control and Complications Trial. Diabetes Control and Complications Trial Research Group. The Journal of pediatrics. 1994; 125(2):177-88. https://doi.org/10.1016/S0022-3476(94)70190-3

17. 15. Diabetes Advocacy: Standards of Medical Care in Diabetes-2018. Diabetes care. 2018; 41(Suppl 1):S152-s3. https://doi.org/10.2337/dc18-S015 PMid:29222386

18. Eliadarous H. Exploring the impact of diabetes in Sudan:Out-of-pocket expenditure and social consequences of diabetes on patients and their familie. Stockholm, Sweden. : Karolinska Institutet; 2017.

19. Rosilio M, Cotton JB, Wieliczko MC, Gendrault B, Carel JC, Couvaras O, et al. Factors associated with glycemic control. A cross-sectional nationwide study in 2,579 French children with type 1 diabetes. The French Pediatric Diabetes Group. Diabetes care. 1998; 21(7):1146-53. https://doi.org/10.2337/diacare.21.7.1146 PMid:9653610

20. Araujo MB, Mazza CS. Assessment of risk factors of poor metabolic control in type 1 diabetic children assisted in a public hospital in Argentina. Pediatric diabetes. 2008; 9(5):480-7. https://doi.org/10.1111/j.1399-5448.2008.00388.x PMid:18761645

21. Hood KK, Peterson CM, Rohan JM, Drotar D. Association between adherence and glycemic control in pediatric type 1 diabetes: a meta-analysis. Pediatrics. 2009; 124(6):e1171-9. https://doi.org/10.1542/peds.2009-0207 PMid:19884476

22. Anderson B, Ho J, Brackett J, Finkelstein D, Laffel L. Parental involvement in diabetes management tasks: relationships to blood glucose monitoring adherence and metabolic control in young adolescents with insulin-dependent diabetes mellitus. The Journal of pediatrics. 1997; 130(2):257-65. https://doi.org/10.1016/S0022-3476(97)70352-4

23. World Health Organization. (WHO). (2007). Child Growth Reference 0-19 years. https://play.google.com/store/apps/details?id=com.LetsStart.GrowthChart.

24. WHOW. Global report on diabetes. 2016;World Health Organization 2016.

25. L. Kathleen Mahan MS RD CDE JLRMRC, Sylvia Escott-Stump MA RD LDN Krause's food & the nutrition care process. (13th ed.) ed: Elsevier, 2012.

26. Icks A, Rosenbauer J, Holl RW, Grabert M, Rathmann W, Giani G. Hospitalization among diabetic children and adolescents and the general population in Germany. German Working Group for Pediatric Diabetology. Diabetes care. 2001; 24(3):435-40. https://doi.org/10.2337/diacare.24.3.435 PMid:11289464

27. L. Kathleen Mahan JLR. Krause's Food & the Nutrition Care Process (Krause's Food & Nutrition Therapy) Elsevier; 2017.

28. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. Diabetes care. 2018; 41(Suppl 1):S13-s27. https://doi.org/10.2337/dc18-S002 PMid:29222373

29. Foundations of care: education, nutrition, physical activity, smoking cessation, psychosocial care, and immunization. Diabetes care. 2015; 38(Suppl):S20-30. https://doi.org/10.2337/dc15-S007 PMid:25537702

30. Ngwiri T, Were F, Predieri B, Ngugi P, Iughetti L. Glycemic Control in Kenyan Children and Adolescents with Type 1 Diabetes Mellitus. International journal of endocrinology. 2015; 2015:761759. https://doi.org/10.1155/2015/761759 PMid:26494998 PMCid:PMC4606130

31. Noorani M, Ramaiya K, Manji K. Glycaemic control in type 1 diabetes mellitus among children and adolescents in a resource limited setting in Dar es Salaam - Tanzania. BMC endocrine disorders. 2016; 16(1):29. https://doi.org/10.1186/s12902-016-0113-y PMid:27246505 PMCid:PMC4886407

32. Giannini C, Mohn A, Chiarelli F. Growth abnormalities in children with type 1 diabetes, juvenile chronic arthritis, and asthma. International journal of endocrinology. 2014; 2014:265954. https://doi.org/10.1155/2014/265954 PMid:24648838 PMCid:PMC3932221

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IGF1R Gene Alterations in Children Born Small for Gestitional Age (SGA)

Aleksandra Janchevska[1*], Marina Krstevska-Konstantinova[1], Heike Pfäffle[2], Marina Schlicke[2], Nevenka Laban[1], Velibor Tasic[1], Zoran Gucev[1], Kristina Mironska[1], Aleksandar Dimovski[3], Jürgen Kratzsch[2], Jürgen Klammt[2], Roland Pfäffle[2]

[1]Medical Faculty, Ss. Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]University of Leipzig, Leipzig, Germany; [3]Macedonian Academy of Sciences and Arts, Skopje, Republic of Macedonia

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***Correspondence:** Aleksandra Janchevska. Medical Faculty, Ss. Cyril and Methodius University in Skopje, Skopje, Macedonia. E-mail: dr.sasha1969@yahoo.com

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Abstract

BACKGROUND: Small for gestational age (SGA)-born children are a heterogeneous group with few genetic causes reported. Genetic alterations in the IGF1 receptor (IGF1R) are found in some SGA children.

AIM: To investigate whether alterations in *IGF1R* gene are present in SGA born children.

PATIENTS AND METHODS: We analysed 64 children born SGA who stayed short (mean -3.25 ± 0.9 SDS) within the first 4 years of age, and 36 SGA children who caught up growth (0.20 ± 1.1 SDS). PCR products of all coding IGF1R exons were screened by dHPLC followed by direct sequencing of conspicuous fragments to identify small nucleotide variants. The presence of IGF1R gene copy number alterations was determined by Multiplex Ligation-dependent Probe Amplification (MLPA).

RESULTS: The cohort of short SGA born children revealed a heterozygous, synonymous variant c.3453C > T in one patient and a novel heterozygous 3 bp in-frame deletion (c.3234_3236delCAT) resulting in one amino acid deletion (p.Ile1078del) in another patient. The first patient had normal serum levels of IGF1. The second patient had unusually low IGF1 serum concentrations (-1.57 SD), which contrasts previously published data where IGF1 levels rarely are found below the age-adjusted mean.

CONCLUSIONS: *IGF1R* gene alterations were present in 2 of 64 short SGA children. The patients did not have any dysmorphic features or developmental delay. It is remarkable that one of them had significantly decreased serum concentrations of IGF1. Growth response to GH treatment in one of the patients was favourable, while the second one discontinued the treatment, but with catch-up growth.

Introduction

Small for gestational age children (SGA; low birth weight and/or birth length) are a heterogeneous group both regarding clinical characteristics and the aetiology (fetal, maternal, placental, and/or genetic factors). Most SGA children normalise their stature by 2 yr. of age. Nevertheless, approximately 10-15% of SGA children do not achieve normal growth and height until adolescence and adulthood and remain short [1] [2]. In addition to the short stature SGA children have a reduced lean body mass, fat mass, skin folds, and body mass index (BMI) [3] [4] [5] [6] [7], as well as a lower calorie, fat, and carbohydrate intake [1] [3]. An impaired IGF1R function may lead to disturbed glucose homeostasis [8], which may partly explain the increased risk for diabetes in SGA adults.

IGF-I, the hormone ligand that binds to the IGF1R, is fundamental for prenatal and postnatal growth and development. Intrauterine and postnatal growth retardation, deafness, microcephaly, and mental retardation have been reported in homozygous deletion or mutation in the *IGF1* gene [1] [5]. The effects of IGF-I are mediated through the type 1 IGF receptor (IGF1R), which is a tyrosine kinase receptor encoded by the *IGF1R* gene [9]. Growth failure and microcephaly have been reported in patients with IGF1R defects.

We used dHPLC and Sanger sequencing and MLPA to detect small nucleotide variants (SNV) or copy number variants (CNV), respectively, to reveal

possible genetic alterations in the *IGF1R* gene as a cause of the observed phenotype in SGA children with or without catch-up growth.

Patients and Methods

SGA was defined as a birth length and/or weight < 2 standard deviation scores (SDS) for the gestational age. SGA children remaining short at age 4 (height > 2.00 SDS) were included in the study. All children had an uncomplicated perinatal and postnatal period. Exclusion criteria included endocrine disorders, skeletal abnormalities, chronic diseases and chromosomal abnormalities.

The study protocol was approved by the Medical Ethics Committee of the Medical Faculty Skopje, Macedonia.

Birth and growth data before the start of treatment were retrieved from records of nurseries, and general practitioners. Height and head circumference were expressed as SD scores [10]. Body mass index was calculated (weight in kg/height in meters^2) and expressed as SD scores for age and sex. Bone age was determined according to Greulich and Pyle [11]. The dysmorphological examination was performed by an experienced clinical geneticist.

GH pituitary reserve was assessed by L-dopa and clonidine GH tests. Serum samples were analysed for IGF-1 and IGFBP-3 by either chemiluminescent immunoassays (Mediagnost, Reutlingen, Germany), or by colourimetric ELISA (Mediagnost, Reutlingen, Germany).

IGF-1 inter- and intra-assay variation coefficients were 6.8 and 6.7%, respectively; IGFBP-3 inter- and intra-assay variation coefficients 6.30 and 4.51%, respectively. Serum GH was measured by a solid-phase, two-site, chemiluminescent immunoassay (ARUP, Salt Lake City, Utah, USA). Cortisol, testosterone and estrogens were measured by colourimetric ELISA (Diagnostic Products Corporation, Los Angeles, Calif., USA).

NA was extracted from peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). PCR products of all coding exons and adjacent intronic sequences of the *IGF1R* gene were generated and subjected to denaturing HPLC (dHPLC) pre-screening (WAVE System; Transgenomic, Glasgow, UK). PCR products with conspicuous chromatograms were further analysed by Sanger dideoxy-sequencing (ABI PRISM 310 Genetic Analyzer; Thermo Fisher Scientific, Waltham, MA). Primer sequences can be obtained upon request. Sequences were compared to the human reference genome (UCSC, version 19 (GRCh37)) and annotated according to the GenBank reference coding sequence of the *IGF1R* NM_000875 and UniProtKB protein reference P08069. Multiplex Ligation-dependent Probe Amplification (MLPA) to detect copy-number variants (CNV) in the *IGF1R* gene was performed according to the manufacturer's recommendations (SALSA MLPA P217; MRC Holland, Amsterdam, The Netherlands).

Results

All 64 short SGA children were investigated, and mutations in two patients were identified by dHPLC and direct sequencing.

Patient A is a boy who was born spontaneously after 37 weeks of gestation and after uneventful pregnancy and delivery. He is the second child of young, non-consanguineous parents. His brother had a normal birth size, and postnatal growth. The proband's birth weight was 1700 g (-3.36 SDS score) and birth length 41 cm (-3.66 SDS score). The parents' height was: father 166cm (-0.76 SDS score) and of his mother 154.5 cm (-1.9 SDS score), with a target height of 166.2 cm (-1.27 SDS score). His psychomotor development, sight and hearing were normal.

At 6.3 yr. of age, his height was 101.4 cm (-3.90 SDS score), weight 13.8 kg (-3.24 SDS weight for height), and head circumference was not available. L-dopa and clonidine stimulation tests were performed at age 5 years with a maximal GH response of 6.31 ng/ml and 10.8 ng/ml, respectively. At this age, his bone age was 4 yr. His IGF-I level was 52.3 ng/ml (-1.57 SDS score) and IGFBP-3 level 1.17 mg/liter (-1.63 SDS score). Ultrasound of the heart and kidneys were uneventful, antibodies for gliadin negative. MRI of the hypothalamic and pituitary region revealed normal size pituitary and no anomalies. Morphologic examination showed no anomalies. He attends to a regular primary school. His IQ score was 89. Since the age of 11.1 years, the GH treatment (37 μg/kg/day) was given for 18 months. The treatment resulted in catch-up growth, and he reached 144.6 cm (-0.21 SDS) at 12.7 years when the parents interrupted the treatment.

In patient A a heterozygous synonymous nucleotide transition, c.3453C > T (p.I1151 =), was found. Position 3435 is located 5 nucleotides upstream of the last nucleotide of exon 18. In silico analysis using MutationTaster (http://www.mutationtaster.org/; accessed April 2018) predicts pathogenicity due to a potential splice site change. The additional computational analysis suggests the introduction of a new exonic splicing silencer site while a potentially existing splicing enhancer site is broken by the nucleotide substitution (Human Splicing Finder 3.1;

http://www.umd.be/HSF3/; accessed April 2018). Aberrant splicing at the intron 18 splice donor site would presumably result in a severely disturbed IGF1R function because the affected amino acid residue(s) are part of the tyrosine kinase domain.

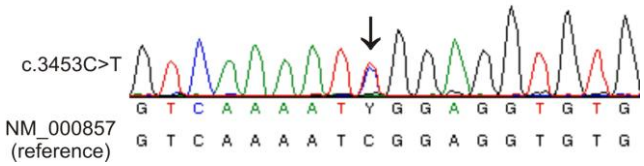

Figure 1: Genetic analysis of IGF 1R; a novel heterozygous variant (synonymous); c.3453C>T; a possible impact on splicing has to be verified

The 8.9 old boys was referred for pediatric endocrine evaluation because of short stature. He was born at 40 wk gestation, after uneventful pregnancy and delivery. The parents are young and non-consanguineous. His brother and sister had normal birth size and normal postnatal growth. His birth weight was 2300 g (-2.76 SDS), his birth length 46 cm (-2.14 SDS), the head circumference at birth was not available. The parents' height was: father 158 cm (-2.0 SDS score) and of his mother 158.7 cm (-1.26 SDS score), with a target height of 165.0 cm (-1.38 SDS score). His psychomotor development, sight and hearing were normal. At referral, the boy had a height of 114.6 cm (-3.08 SDS) and a weight of 22.2 kg (-1.2 SDS). His head circumference was 49.8 cm (normal). The Greulich and Pyle male standards bone age was 10 years. His sight, hearing and development were normal. He had average grades in the primary and secondary school. L- Dopa and clonidine tests of pituitary GH reserve were 16.4 ng/ml and 17.7 ng/ml respectively. Initially, IGF-1 was 468 ng/ml (reference 237-996) and IGFBP-3 levels were not available, but under GH treatment IGF-I was 205 ng/ml (reference 226–903), while IGF binding protein-3 (IGFBP)-3 were not available. At the age of 15.41 years, his height was 138.2 cm (-4.11 SDS), his weight 30.3 kg (-2.71 SDS), head circumference normal. T4, TSH, cortisol, renal function, hepatic analysis were normal. Since the age of 16.25 years (-3.77 SDS) the GH treatment (37 µg/kg/day) was initiated and lasted 3 years. This resulted in a final adult height of 160.5 cm (-2.5 SDS) which was within the parental target height range.

In patient B a 3-bp in-frame deletion, c.3234_3236delCAT, was identified. The deletion probably leads to removal of isoleucine at protein position 1078 (p.I1078del) and is predicted to be disease-causing (MutationTaster). The affected position maps to the tyrosine kinase domain of the IGF1R. Isoleucine 1078 is highly conserved among species and paralogues (insulin receptor, insulin receptor-related receptor) with only isoleucine or valine found at this position. Disturbance of the receptor's kinase activity can be assumed but has to be shown experimentally.

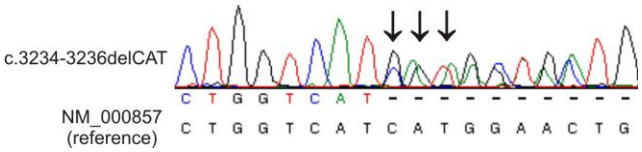

Figure 2: Genetic analysis of IGF 1R of Patient B; a novel heterozygous 3 bp deletion (c.3234_3236delCAT) resulting in a one amino acid deletion (p.Ile1078del)

Discussion

Mutations in the *IGF1R* gene resulting in IGF1 resistance underlie some cases of prenatal and postnatal growth failure [4]. Interestingly, there were three types of phenotypes reported. Some SGA children with IGF1R genetic alterations had microcephaly and short stature, others had only short stature without microcephaly, while some reports describe short stature and/or microcephaly and impaired glucose tolerance. Also, most reports describe elevated circulating levels of IGF1, consistent with the expectations when there is a receptor defect. It is of note that some patients were described with low normal levels of IGF1, as was the case in one of our patients with the novel heterozygous 3 bp deletion (c.3234_3236delCAT) resulting in one amino acid deletion (p.Ile1078del). There is one report that describes hypoglycemia found in a patient with a heterozygous mutation (c.94+1G > A, p.D1105E) affecting the splicing site of the *IGF1R* mRNA [12].

Several reports describe SGA with growth failure: a compound heterozygote for point mutations in exon 2 of the *IGF1R* gene bearing a p.R108Q mutation in 1 allele and a p.K115N mutation in the second [4]. Short stature and intrauterine growth retardation (IUGR) were found to be caused by a heterozygous mutation in the *IGF1R* gene, Arg709 to Gln (p.R709Q) [13], while the p.R481Q mutation was described in two family members with increased serum IGF-I levels and intrauterine and postnatal growth retardation [14]. Normal IGF-I, but short stature was found in a boy and several family members with a 19-nucleotide duplication within exon 18 of the *IGF1R* gene and consequently haploinsufficiency of IGF1R protein [15]. One of our patients had unusually low IGF1 serum concentrations (-1.5 SD), which contrasts with previous reports.

Prenatal and postnatal growth failure was found in patients with p.Y387X mutation [16] where the proband had high IGF-I levels, while he and his two paternal aunts had impaired glucose tolerance. SGA was also reported in a novel IGF1R mutation (p.Alal 40fsX20) [17], and intrauterine and postnatal growth retardation was found in a missense mutation

(p.R431L) [18]. Heterozygous nonsense mutations affecting the C-terminal region (p.Q1250X, p.W1249X) of IGF1R were described in two out of 55 analysed Japanese patients with SGA and growth failure [19].

A heterozygous mutation (p.C1248Y) in the IGF1R gene was found in two brothers with prenatal and postnatal growth retardation and their father [8]. It is of note that OGTT showed progressive impaired glucose tolerance, while the father was already treated for type 2 diabetes mellitus. In a child with a deletion on 15q26.2 intrauterine growth retardation, postnatal growth failure, and recurrent hypoglycemia there was only a single copy of the *IGF1R* gene [20].

Microcephaly is frequently associated with IGF1R genetic alterations. SGA, microcephaly, persistent postnatal growth retardation, and elevated IGF-I levels were reported in a 15-year-old girl with heterozygous deletion of 15q26.2-qter which included the IGF1R gene [21]. The p.R59X mutation was reported in two half-brothers with primary microcephaly, mild mental retardation, and intrauterine as well as postnatal growth deficits [4] [22]. Microcephaly, pre- and postnatal growth retardation were found in patients with heterozygous missense mutations in three unrelated patients, de novo p.R1256S, de novo p.N359Y and p.Y865C [23]. Also the c.1549A > T, the p.Y487F mutation was reported in a patient with microcephaly and prenatal and postnatal growth impairment [24]. GH treatment of a patient with short stature, microcephaly, dysmorphic features, developmental delay and a terminal deletion of 15q26.2q26.3 containing the *IGF1R* gene in addition to a terminal duplication of the 4q35.1q35.2 region resulted in a strong growth response [25]. It is of note that the GH treatment in our patients had mixed effects. The first patient did achieve height within the parental target range, while the second one discontinued the treatment without having a catch-up growth.

The compound heterozygous mutation p.E121K/E234K was reported as the cause of intrauterine growth retardation and severe postnatal growth failure [26]. The homozygous c.119G > T (p.R10L) was shown to be associated with dysmorphic features, severe IUGR, and insulin resistance [27].

In conclusion, *IGF1R* gene alterations are an important and relatively frequent cause for SGA. Microcephaly with prenatal or postnatal growth failure should alert the physician on a possible *IGF1R* defect. Increased IGF-I levels are also a major sign of IGF1R defects. It is of note that low normal serum IGF-I levels have also been reported, and therefore are not an argument not to test the *IGF1R* gene. A precise phenotype-genotype correlation is still lacking. The GH treatment in one of the patients did result in a height gain into the parental range. However, the second patient interrupted the treatment but induced a catch-up growth.

Statement

Written informed consent has been obtained from each patient or subject after full explanation of the purpose and nature of all procedures used.

References

1. Woods KA, Camacho-Hubner C, Savage MO, Clark AJ. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. N Engl J Med. 1996; 335:1363–1367. https://doi.org/10.1056/NEJM199610313351805 PMid:8857020

2. Klammt J, Kiess W, Pfäffle R. IGF1R mutations as a cause of SGA. Best Pract Res Clin Endo-crinol Metab. 2011; 25(1):191-206. https://doi.org/10.1016/j.beem.2010.09.012 PMid:21396585

3. Kiess W, Kratzsch J, Keller E, Schneider A, Raile K, Klammt J, et al. Clinical examples of disturbed IGF signaling: intrauterine and postnatal growth retardation due to mutations of the insulin-like growth factor I receptor (IGF-IR) gene. Rev Endocr Metab Disord. 2005; 6(3):183-187. https://doi.org/10.1007/s11154-005-3049-5 PMid:16151622

4. Abuzzahab MJ, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E, et al. Intrauterine Growth Retardation (IUGR) Study Group. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. N Engl J Med. 2003; 349:2211–2222. https://doi.org/10.1056/NEJMoa010107 PMid:14657428

5. Walenkamp MJ, Karperien M, Pereira AM, Hilhorst-Hofstee Y, van Doorn J, Chen JW, et al. Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. J Clin Endocrinol Metab. 2005; 90:2855–2864. https://doi.org/10.1210/jc.2004-1254 PMid:15769976

6. Walenkamp MJ, van der Kamp HJ, Pereira AM, Kant SG, van Duyvenvoorde HA, Kruithof MF, et al. A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor I receptor. J Clin Endocrinol Metab. 2006; 91:3062–3070. https://doi.org/10.1210/jc.2005-1597 PMid:16757531

7. Wietske AE, van Duyvenvoorde HA, de Wit CC, Broekman AJ, Ruivenkamp CAL, Govaerts LCP et al. Two Short Children Born Small for Gestational Age with Insulin-Like Growth Factor 1 Receptor Haploinsufficiency Illustrate the Heterogeneity of Its Phenotype. J Clin Endocrinol Metab. 2009; 94:4717–4727. https://doi.org/10.1210/jc.2008-1502 PMid:19864454

8. Burkhardt S, Gesing J, Kapellen TM, Kovacs P, Kratzsch J, Schlicke M, et al. Novel heterozygous IGF1R mutation in two brothers with developing impaired glucose tolerance. J Pediatr Endocrinol Metab. 2015; 28(1-2):217-25. https://doi.org/10.1515/jpem-2014-0132 PMid:25153223

9. Abbott AM, Bueno R, Pedrini MT, Murray JM, Smith RJ. Insulin-like growth factor I receptor gene structure. J Biol Chem. 1992; 267:10759–10763. PMid:1316909

10. Kromeyer-Hauschild K, Wabitsch M, Kunze D, Geller F, Geiß HC, Hesse V, von Hippel A, Jaeger U, Johnsen D, Korte W, Menner K. Perzentile für den Body-mass-Index für das Kindes-und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. Monatsschrift Kinderheilkunde. 2001; 149(8):807-18. https://doi.org/10.1007/s001120170107

11. Greulich WW, Pyle SI, Todd TW. Radiographic atlas of skeletal development of the hand and wrist. Stanford: Stanford university press, 1959.

12. Solomon-Zemler R, Basel-Vanagaite L, Steier D, Yakar S, Mel E, Phillip M, et al. A novel heterozygous IGF-1 receptor mutation

associated with hypoglycemia. Endocr Connect. 2017; 6(6):395-403. https://doi.org/10.1530/EC-17-0038 PMid:28649085 PMCid:PMC5551424

13. Kawashima Y, Kanzaki S, Yang F, Kinoshita T, Hanaki K, Nagaishi J, et al. Mutation at cleavage site of insulin-like growth factor receptor in a short-stature child born with intrauterine growth retardation. J Clin Endocrinol Metab. 2005; 90:4679–4687. https://doi.org/10.1210/jc.2004-1947 PMid:15928254

14. Inagaki K, Tiulpakov A, Rubtsov P, Sverdlova P, Peterkova V, Yakar S, et al. A familial insulin-like growth factor-I receptor mutant leads to short stature: clinical and biochemical characterization. J Clin Endocrinol Metab. 2007; 92:1542–1548. https://doi.org/10.1210/jc.2006-2354 PMid:17264177

15. Fang P, Schwartz DI, Johnson BD, Derr MA, Roberts CT, Hwa V, et al. Familial Short Stature Caused by Haploinsufficiency of the Insulin-Like Growth Factor I Receptor due to Nonsense-Mediated Messenger Ribonucleic Acid Decay. The Journal of Clinical Endocrinology & Metabolism. 2009; 94 (5):1740-1747. https://doi.org/10.1210/jc.2008-1903 PMid:19240156

16. Mohn A, Marcovecchio ML, de Giorgis T, Pfaeffle R, Chiarelli F, Kiess W. An insulin-like growth factor-I receptor defect associated with short stature and impaired carbohydrate homeostasis in an Italian pedigree. Horm Res Paediatr. 2011; 76(2):136-143. https://doi.org/10.1159/000324957 PMid:21811077

17. Choi JH, Kang M, Kim GH, Hong M, Jin HY, Lee BH, et al. Clinical and functional charac-teristics of a novel heterozygous mutation of the IGF1R gene and IGF1R haplo-insufficiency due to terminal 15q26.2->qter deletion in patients with intrauterine growth retardation and postnatal catch-up growth failure. J Clin Endocrinol Metab. 2011; 96(1):E130-4. https://doi.org/10.1210/jc.2010-1789 PMid:20962017

18. Kawashima Y, Higaki K, Fukushima T, Hakuno F, Nagaishi J, Hanaki K, et al. Novel missense mutation in the IGF-I receptor L2 domain results in intra-uterine and postnatal growth retardation. Clin Endocrinol (Oxf). 2012; 77(2):246-54. https://doi.org/10.1111/j.1365-2265.2012.04357.x PMid:22309212

19. Fujimoto M, Kawashima Sonoyama Y, Hamajima N, Hamajima T, Kumura Y, Miyahara N, Nishimura R, Adachi K, Nanba E, Hanaki K, Kanzaki S. Heterozygous nonsense mutations near the C-terminal region of IGF1R in two patients with small-for-gestational-age-related short stature. Clinical endocrinology. 2015; 83(6):834-41. https://doi.org/10.1111/cen.12791 PMid:25866162

20. Okubo Y, Siddle K, Firth H, O'Rahilly S, Wilson LC, Willatt L, et al. Cell proliferation activities on skin fibro-blasts from a short child with absence of one copy of the type 1 insulin-like growth factor receptor (IGF1R) gene and a tall child with three copies of the IGF1R gene. J Clin Endocrinol Metab. 2003; 88:5981–5988. https://doi.org/10.1210/jc.2002-021080 PMid:14671200

21. Walenkamp MJ, de Muinck Keizer-Schrama SM, de Mos M, Kalf ME, van Duyvenvoorde HA, et al. Successful long-term growth hormone therapy in a girl with haploinsufficiency of the insulin-like growth factor-I receptor due to a terminal 15q26.2->qter deletion detected by multiplex ligation probe amplification. J Clin Endocrinol Metab. 2008; 93:2421–2425. https://doi.org/10.1210/jc.2007-1789 PMid:18349070

22. Raile K, Klammt J, Schneider A, Keller A, Laue S, Smith R, et al. Clinical and functional cha-racteristics of the human Arg59Ter insulin-like growth factor I receptor (IGF1R) mutate-on: implications for a gene dosage effect of the human IGF1R. J Clin Endocrinol Metab. 2006; 91:2264–2271. https://doi.org/10.1210/jc.2005-2146 PMid:16569742

23. Juanes M, Guercio G, Marino R, Berensztein E, Warman DM, Ciaccio M, et al. Three novel IGF1R mutations in microcephalic patients with prenatal and postnatal growth impair-ment. Clin Endocrinol (Oxf). 2015; 82(5):704-711. https://doi.org/10.1111/cen.12555 PMid:25040157

24. Labarta JI, Barrio E, Audí L, Fernández-Cancio M, Andaluz P, de Arriba A, et al. Familial short stature and intrauterine growth retardation associated with a novel mutation in the IGF-I receptor (IGF1R) gene. Clin Endocrinol (Oxf). 2013; 78(2):255-262. https://doi.org/10.1111/j.1365-2265.2012.04481.x PMid:22738321

25. Mahmoud R, Naidu A, Risheg H, Kimonis V. Response to Growth Hormone Treatment in a Patient with Insulin-Like Growth Factor 1 Receptor Deletion. J Clin Res Pediatr Endocrinol. 2017; 9(4):380-386. https://doi.org/10.4274/jcrpe.4456 PMid:28720553 PMCid:PMC5785648

26. Fang P, Cho YH, Derr MA, Rosenfeld RG, Hwa V, Cowell CT. Severe short stature caused by novel compound heterozygous mutations of the insulin-like growth factor 1 receptor (IGF1R). J Clin Endocrinol Metab. 2012; 97(2):E243-247. https://doi.org/10.1210/jc.2011-2142 PMid:22130793

27. Gannagé-Yared MH, Klammt J, Chouery E, Corbani S, Mégarbané H, Choucair N, Pfäffle R, Mégarbané A. Homozygous mutation of the IGF1 receptor gene in a patient with se-vere pre- and postnatal growth failure and congenital malformations. Eur J Endocrinol. 2012; 168(1):K1-7. https://doi.org/10.1530/EJE-12-0701 PMid:23045302

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A Comparison of the Effects of Dexmedetomidine and Propofol in Controlling the Hemodynamic Responses after Intubation: A Double-Blind, Randomized, Clinical Trial Study

Alireza Kamali[1*], Mehrdad Taghizadeh[2], Mohtaram Esfandiar[2], Amin Shams Akhtari[2]

[1]Department of Anesthesiology, Arak University of Medical Sciences, Arak, Iran; [2]Department of Emergency, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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***Correspondence:** Alireza Kamali. Department of Anesthesiology, Arak University of Medical Sciences, Arak Iran. E-mail: Dr.kamali@arakmu.ac.ir

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Abbreviations: MAP = Mean arterial blood pressure; HR = Heart rate; PPV = Positive pulmonary ventilation; PEEP = Positive end-expiratory pressure; CVA = Cerebrovascular accident; DAP = Diastolic arterial pressure

Abstract

AIM: This study aimed to compare the effects of dexmedetomidine and propofol in controlling the hemodynamic response following intubation of patients' candidate intubation in the emergency department

METHODS: A total of 114 patients were randomly assigned to one of 2 groups to receive one of the following treatments: dexmedetomidine 0.4 μg/kg (Group D, n = 57) and propofol 1–1.5 mg/kg/h (Group P, n = 57). Hemodynamic data such as the systolic blood pressure, diastolic blood pressure, arterial oxygen saturation and heart rate (HR) were recorded from the entrance to operation room to 5, 10 and 15 min after tracheal intubation

RESULTS: Compared with group D, group P exhibited increases in mean arterial blood pressure (MAP), and systolic blood pressure significantly at all times and immediately after the endotracheal intubation. Moreover, the mean diastolic blood pressure changes due to tracheal intubation in group P were significantly more than group D immediately after the intubation. Furthermore, the mean heart rate changes immediately and 5 min after tracheal intubation was significantly higher in group P

CONCLUSION: Our data suggest that the benefits of dexmedetomidine more than propofol in hemodynamic stability because propofol was associated with more variability in systolic/diastolic blood pressure, HR and MAP after endotracheal intubation.

Introduction

Laryngoscopy and tracheal intubation can cause tachycardia, high blood pressure, heart rate abnormalities, increased catecholamines concentration, myocardial ischemia, increased myocardial oxygen demand and increased intracranial pressure. On the other hand, it has been determined that stimulation is very severe due to laryngoscopy and intubation, as well as cutting the skin by the surgeon and can cause sympathetic stimulation, increased blood pressure and tachycardia in people whose sympathetic responses are not sufficiently slowed. These factors may cause potentially deadly risks such as cardiac ischemia, myocardial infarction and hemorrhagic cerebrovascular accident (CVA), so these issues enhance the importance of prescribing in patients [1]. Many drugs have been used to block hemodynamic responses such as opioids [2], vasoconstrictor drugs [3], beta receptor blockers [4] alpha receptor blockers [5] and benzodiazepines [6]. General anaesthesia includes those that may reduce blood pressure and reduce myocardial contractions, vasodilatation, and weaken the activity of the autonomic nervous system [7] [8] [9]. The unwanted side effects of intubation into the trachea cause such reactions as increased blood pressure and tachycardia and dis-arrhythmia [10] [11].

Dexmedetomidine is a highly selective alpha-2 adrenoreceptor agonist [12]. This drug is used as an adjuvant in general anaesthesia with central supportive effects, stabilises the hemodynamic state

of the patient and has anaesthetic and analgesic effect [13] [15], which reduces the need for opioids and their complications [16] and decreases Stress response and quality recovery [17]. One of the ways to reduce hemodynamic changes, in addition to muscle anaesthetic and paralysis, is to provide sufficient depth of anaesthesia with fast-acting intravenous drugs that can provide quick and easy anaesthesia in the short term [18]. One of the major concerns in general anaesthesia is the hemodynamic stability in the patient is optimally based on the type and technique of surgery [19]. Compared to other anaesthetic drugs, Propofol has a faster return on alertness to the patient with minimal effects on the central nervous system, which inhibits increased heart rate and blood pressure in response to airway induction due to tracheal intubation, and thus changes can be made. Hemodynamically reduces the intubation [7]. In both of our medications, we are confronted with a drop in blood pressure and bradycardia, and both drugs control the effects of intubation on hemodynamic in large measure, in the meantime, we were looking for better drug combinations with fewer effects on hemodynamics in patients. And while controlling the hemodynamic responses induced by intubation, preventing excessive hypertension in the patient & apos;s heart and preventing hemodynamic imbalance.

Considering that so far no study has been done with this aim, we decided to conduct a study comparing the effect of propofol and dexmedmotidine on hemodynamic changes in patients undergoing intubation in the emergency department.

Methods

This study included a double-blind, randomised, clinical trial on emergency patient candidates in the emergency department of Vali-e-Asr Hospital. The clinical trial study data dated from 2015 to 2017. The clinical trial began in 2016 until the present. This research was performed at the Department of Emergency at our institution, and the registration number from the Iranian Registry of Clinical Trials is IRCT2016102520258N14 with the date of registration (31-10-2016). All procedures were done by the Helsinki declaration 1964, and its later amendments or comparable ethical standards in the emergency department of Vali-e-Asr Hospital and all experimental protocols were approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences. All eligible patients provided written informed consent before entering this study.

Inclusion criteria: All patients who are candidates for intubation for one of the following reasons:

1. Protection pulmonary

2. ↓PaO_2/FiO_2 (↓SpO_2)

3. Pulmonary toileting

4. Positive pulmonary ventilation (PPV)

5. Positive end-expiratory pressure (PEEP)

Exclusion criteria:

1. All patients who have been referred to the emergency department for suicide (due to drug interactions);

2. All patients who have been attempted more than twice for intubation.

3. Patients with multiple fractures of the upper and lower jaw and the possibility of severe intubation and kidney of patients with a probability of intubation, which is likely to be given several times a laryngoscopy.

4. All patients with a specific drug allergy to propofol and doxedemotomidine.

Sampling method: Sampling method according to the type of clinical study. The purposeful sampling method was used. Using random numbers, 114 patients were randomly divided into two equal groups of 57 (Propofol and Dexmedetumidine), respectively. All patients who had the inclusion criteria were using a random number table they were completely randomized into two groups of 57 patients with dimethomidine propofol (taking into account paired and random numbers).

A group of 114 patients were randomly divided into two groups of Propofol and Dexmedetomidine. For all patients, 2-3 micrograms/kg of fentanyl plus 2-3 mg/kg of lidocaine 2% were given intravenously as well as 2-3 mg of midazolam at first. Also, in both groups succinylcholine was given at a dose of 1 mg it was also given on kilograms. Then, for group 1 (group dexmedmotidine) 0.4 µg/kg dx dimethomidine and group 2 (propofol group), 1 to 1.5 mg/kg propofol was given to control hemodynamic responses of patients and prepared for intubation. Syringe volume in two groups was 10 Then, for both groups, the tracheal tube was selected and sprayed with lidocaine and after injection of the drugs by the resident responsible for the design and preparation for intubation of the patient by a resident of the largesic and intubate design, and finally the fixation tube And connected to the ventilator. The drugs were injected to patients for intravenous administration (dimethomidine and propofol) in syringes that were prepared by the researcher of Emergency Medicine in charge of the plan, and the volume was the same (10 cc). Then, according to the above explanations, the patients with Intubation candidates were contacted by emergency room researcher at the emergency department of Vali Asr Hospital. All of these patients had cryptography for inclusion in the study and had no exits from the

study, the hemodynamic symptoms of the patients before and after the intubation (hemodynamic symptoms of patients including systolic blood pressure and diastolic blood pressure, and arterial blood pressure and heart rate and oxygen saturation) Resident co-registered.

This study was a double-blind study and patients were blinded to intubation and were not aware that the drugs were already prepared by the Resident of Emergency Medicine, and each of the syringes with the same volumes The cover was covered in a fridge and injected into the syringes during the injection of drugs in accordance with Nos. 1 and 2, and the resident researcher of the project, who was completely harmed by drugs and groups, informed the patients and developed a questionnaire for patients including their hemodynamic and demographic questions Completed. All the information obtained from the patients in the questionnaire of the project was registered and completed by the resident researcher.

Calculating sample size and number:

$$N = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 (S_1^2 + S_2^2)}{(\mu_1 - \mu_2)}$$

$Z_{1-\frac{\alpha}{2}}$= 1.96 Z1-β = 2.33 μ2=40.7 1μ=33.2 S_1= 16.7 S_2 =2.9

N = 57 individuals in each group-the total number of samples was 114. (S1, S2, μ1, μ2 were calculated according to reference number 18); Z1-α/2 = 1.96, Z1-β = 2.33.

Finally, the information obtained from the questionnaires was analysed by statistical software SPSS 20 and analysed in tables, and diagrams Statistical analysis was performed and the effect of two drugs dexmedmotidine and propofol on the control of hemodynamic response in patients after intubation was analysed.

Results

According to Table 1, there was a significant difference between the two groups in terms of average changes in MAP at different times, so that according to P = 0.00001, MAP changes in the Propofol group were significantly more than in the group of dexmodutomidine, and more simply in patients In the dexmodutomidine group, they were more stable after intubation.

Table 1: Comparison of MAP changes in patients with emergency intubation candidates in two groups: Peropofol and Dexmedmotidine

Average MAP changes	Peropofol group	Dexmedmotidine group	P-value
Immediately after intubation	6.46 ± 4.6	9.4 ± 1.1	P = 0.00001
5 min after intubation	4.22 ± 1.5	1.2 ± 2.0	P = 0.00001
10 min after intubation	1.24 ± 5.4	7.9 ± 1.2	P = 0.00001
15 min after intubation	5.21 ± 8.3	1.8 ± 7.1	P = 0.0001

According to Table 2, there was a significant difference between the two groups regarding mean SBP changes at different times, so that according to P = 0. 001, SBP changes were significantly more in the propofol group than in the dexmedmotidine group. In other words, patients in the dexmedmotidine group were more stable after intubation.

Table 2: Comparison of mean systolic pressure changes (SBP) in patients with an emergency intubation candidate in two groups of peropofol and dexmedmotidine

Average SBP changes	Peropofol group	Dexmedmotidine group	P-value
Immediately after intubation	7.25 ± 8.5	1.13 ± 1.4	P = 0.001
5 min after intubation	2.14 ± 1.6	8.4 ± 1.2	P = 0.001
10 min after intubation	3.23 ± 2.4	8.13 ± 1.5	P = 0.01
15 min after intubation	9.21 ± 9.3	1.12 ± 8.4	P = 0.01

There was a significant difference between the two groups in terms of average diastolic arterial pressure (DAP) changes immediately after intubation, and DAP changes were significantly higher in the propofol group after intubation than in the dexmodutomidine group (P = 0.01), but at other times after intubation 5, 10 and 15 minutes later), no significant difference was observed between the two groups (P ≥ 0.05) (Table 3).

Table 3: Comparison of changes in mean diastolic pressure (DAP) in patients with emergency intubation candidates in two groups of peropofol and dexmedetomidine

Average DAP changes	Peropofol group	Dexmedmotidine group	P-value
Immediately after intubation	8.15 ± 8.4	3.10 ± 1.4	P = 0.01
5 min after intubation	1.1 ± 58.0	07.1 ± 64.0	P ≥ 0.05
10 min after intubation	3.2 ± 97.0	1.2 ± 1.1	P ≥ 0.05
15 min after intubation	9.7 ± 1.3	1.7 ± 2.4	P ≥ 0.05

In terms of mean heart rate, there was a significant difference between the two groups immediately and 5 minutes after intubation, and the changes in heart rate immediately and 5 minutes after intubation in the propofol group were significantly higher than that of the dexamethymidine group (P = 0.0001), but in other times after intubation (10 and 15 minutes later), there was no significant difference between the two groups (P ≥ 0.05) (Table 4).

Table 4: Comparison of mean heart rate changes (PR) in patients with emergency intubation candidates in two groups: Peropofol and Doxedemotomidine

Average PR changes	Peropofol group	Dexmedmotidine group	P-value
Immediately after intubation	6.22 ± 6.5	3.2 ± 4.1	P = 0.0001
5 min after intubation	9.11 ± 4.4	7.0 ± 12.0	P = 0.001
10 min after intubation	1.13 ± 8.3	8.12 ± 7.2	P ≥ 0.05
15 min after intubation	8.8 ± 7.3	4.9 ± 9.2	P ≥ 0.05

There was no significant difference between the two groups regarding the mean oxygen saturation percentage at different times before and after intubation. Both groups are identical (P ≥ 0.05) (Table 5).

Table 5: Comparative of changes in mean oxygen saturation (SPO_2) in patients with emergency intubation candidates in two groups of Peropofol and Dexmedetomidine

Average SpO_2 changes	Peropofol group	Dexmedmotidine group	P-value
Immediately after intubation	58.0 ± 23.0	49.0 ± 13.0	P ≥ 0.05
5 min after intubation	1.1 ± 85.0	4.1 ± 27.0	P ≥ 0.05
10 min after intubation	4.1 ± 56.0	02.1 ± 45.0	P ≥ 0.05
15 min after intubation	3.1 ± 33.0	40.1 ± 28.0	P ≥ 0.05

There was no significant difference between the two groups regarding mean age and percentage of sexual intercourse. Both groups are identical (P ≥ 0.05) (Table 6).

Table 6: Comparison of the mean age and gender prevalence of patients with emergency intubation candidates in the two groups of peropofol and dexmedmotidine

Groups	Peropofol group	Dexmedmotidine group	P-value
Mean age	5.45 ± 2.1	2.48 ± 8.1	P ≥ 0.05
Male frequency	57.1%	56.4%	P ≥ 0.05

Discussion

Achieving appropriate drug combinations for intubating patients with emergency intubation is one of the goals of emergency medicine and anesthesiologists. An appropriate drug combination that prevents tachycardia and dysrhythmia and hypertension caused by laryngoscopy and endotracheal intubation and it has always been the attention of the experts [1] [2]. Laryngoscopy and endotracheal intubation can cause tachycardia, hypertension, dysrhythmia, increased concentrations of catecholamines and even myocardial ischemia. In many cases, intubation of emergency patients has been accompanied by an increase in ICP. Therefore, the drug compounds that can control these changes are very important and considerable [1] [2] [3] [6]. These drugs include opioids, BZDs, barbiturates and alphabucers [1] [2] [3] [4] [6], while occasionally the administration of these drugs to endotracheal intubation leads to hemodynamic changes in patients [1] [6] [7] [9].

In this study, we compared the effects of dexmedmotidine and propofol on the control of hemodynamic responses after intubation of patients who were candidates for emergency intubation. The results of this study were that the mean of MAP changes immediately after intubation and at different times after intubation was significantly higher in the propofol group than in the dexmedmodyntidine group. Also, changes in systolic and diastolic blood pressure and heart rate at different times after intubation in the propofol group were greater than that of the Dexmedmotidine group. Therefore, these results indicate that the hemodynamic changes in patients with emergency intubation in the propofol group were significantly higher than that of the dexmedmodine group. In previous studies, the results are similar to those of our study. In a study by Chalam et al., in 2015, it was found that there was a significant difference between the two groups regarding MPA changes in systolic blood pressure And diastolic and heart rate was absent. In this study, patients were compared in the two groups of 50 patients with dimethomidine and propofol, which showed no significant difference between the hemodynamic changes of the two unrecognised, but better patients in the dexamethymidine tolerance group and better airway maintenance and autonomic ventilation in patients [20]. However, the results obtained in this study were not consistent with our study. In our study, hemodynamic changes were significantly lower in the dexedotomidine group than in the propofol group. But changes in SpO_2 in our study, like the above study, did not show a significant difference between the two groups. The reason for this difference may be that in our study, hemodynamic changes after intubation have been investigated in patients undergoing emergency intubation. In the study of Jalam et al., This comparison was performed in patients in the operating room in the form of awaked intubation and with airway block. Another study by Blokoglo and colleagues in 2013 compared the effects of dexmeditomidine plus propofol and doxedmotomidine with autoimide and doxedmotidine with thiopental on intubation of patients without loosening. 76 patients were divided into three groups and compared to each other. The results of this study showed that there was no significant difference in the hemodynamic changes between the 3 groups (P > 0.005). However, the need for muscle relaxant after intubation in the group consisting of dimethomidine and propofol was less than the other two groups which seem to be better than the two above-mentioned doses of dimethomidine and propofol [21].

The results obtained in this study were not consistent with our study, so in our study, hemodynamic changes in the dexmedmodyntidine group were less than that of the prophylaxis group. The reason for the difference in the results of these two studies is that in the study of Blokoglo and colleagues, the combination of two drugs, including dimethomidine-propofol and doxedmotomidine-auto anti and doxedmotidine-thiopental, were used, while in our study, doxmodetomidine and propofol were compared with each other alone. However, in both studies, patients with emergency intubation candidates were examined, and only the lack of changes in SpO_2 between the studied groups was similar in the results of our study and Blokoglo was similar. In a study by Chang et al., in 2014 on children

with patents Cardiac Heart Surgery Applicant. This study aimed to compare the effects of dexmeditoimidine and propofol on hemodynamic responses and SpO_2 changes induced by intubation. The result of this study was that the hemodynamic changes induced by intubation in the dimethomidine group were lower than the propofol group (P = 0.01) In this study, 114 children were randomly divided into two groups of propofol and dexmedetomidine. The results of the comparison of the two groups were that changes in blood pressure and heart rate in the propofol group after intubation and after sternotomy was more than the group of dexmedotomidine (P < 0.01). The final result of this study indicated that dexmedmotomidine had a better effect on hemodynamic control in children undergoing cardiac surgery than Propofol [22]. The results of this study were completely consistent with our study. In our study, the hemodynamic changes induced by intubation in the dimethomidine group were lower than the propofol group. In both studies, there was no significant difference in SpO_2 changes. It should be noted that despite our study on adult subjects who were candidates for emergency intubation and the study of Cheng et al., On children who were candidates for cardiac surgery, the final results were almost consistent. Another study by Karimian et al., in 2006 aimed at comparing the effect of propofol and ketamine on hemodynamic changes in patients during intubation and induction of anaesthesia. The final result was that there was a significant difference between hemodynamic changes after intubation and induction of anaesthesia between the two groups could not be seen. Hemodynamic stability was found to be greater in the ketamine group than Propofol [23]. In simple terms, the use of ketamine has led to less hemodynamic changes in patients. Approximately the results of this study were consistent with our study. In our study, hemodynamic changes induced by intubation in the propofol group were more than the group of dexmedotomidine. By comparing the results of our study and the above studies, we can say that the use of Dexmedmotidine, as a sedative drug, improves hemodynamic stability during intubation of patients. Dexmedetomidine is a high-quality selective agonist that is used as an adjuvant in general anaesthesia and contributes to the sustained hemodynamic status of patients with central sympathetic effects [12] [13] [15]. In addition to anesthetic and sedative effects, the anti-Has a pain and reduces the need for opioids and reduces stress responses and releases catecholamines [13] [15] [16]. These beneficial effects of dexmedmotidine have led to its use as a high hemodynamic stability stabilizer when Intubation of patients. For intubation of patients, dexmedmotvydine as an agonist has been shown to lead to appropriate hemodynamic stability in patients. Dexmedetomidine is in fact an agonist with rapid intravenous effects that can, in addition to anti-anxiety and sedative effects, reduce the responses resulting from the release of catecholamines. One of the important issues during the intubation of patients, both in the operating room and in the emergency room, is the creation of an optimal condition with hemodynamic stability, which seems to lead to optimal conditions for patients during intubation in view of the use of doxedemotomidine [19] [18]. Propofol also has fast and fairly fast and fast-moving effects compared to other anesthetic drugs and also has the least effect on the CNS, and by inhibiting hypertension and heart rate, it reduces responses from stimulation of intubation [22]. However, in our study, the hemodynamic changes induced by intubation in the propofol group were greater than that of the dimethomidine group. Therefore, the hemodynamic stability of patients in the Dexmedmotidine group was higher.

In conclusion, the results of this study showed that comparison of the MAP and systolic and diastolic blood pressure and heart rate in patients with emergency intubation candidates in the group of dexmedmotomidine was lower than that of propofol, but changes in SpO_2 in the two groups did not have a significant difference.

Ethics approval and consent to participate

Ethics approval for this study was obtained from the Shahid Beheshti University of Medical Sciences Ethics Board. Written informed consent was obtained from each patient included in the study,

References

1. Kovac A. Controlling the hemodynamic response to laryngoscopy and endotracheal intubation. J Clin Anesth. 1996; 8(8):63-79. https://doi.org/10.1016/0952-8180(95)00147-6

2. Crawford D, Fell D, Achola K, Smith G. Effect of alfentanil on the presser and catecholamine responses to tracheal intubation. Br J Anaesth. 1987; 59(6):707-12. https://doi.org/10.1093/bja/59.6.707 PMid:3111508

3. Stoelting R. Attenuation of blood pressure response to laryngoscopy and tracheal intubation with sodium nitropruside. Anesth Analg. 1979; 58(2):116-9. https://doi.org/10.1213/00000539-197903000-00011 PMid:571234

4. Vucevic M, Purdy G, Ellis F. Esmolol hydrochloride for the management of the cardiovascular stress response to laryngoscopy and tracheal intubation. Br J Anaesth. 1992; 68(5):529-30. https://doi.org/10.1093/bja/68.5.529 PMid:1642945

5. Curran J, Crowley M, O'Sullivan G. Droperidol and endotracheal intubation. Anaesthesia. 1980; 35(3):290-4. https://doi.org/10.1111/j.1365-2044.1980.tb05099.x PMid:7396141

6. Taittonen M, Kirvela O. Cardiovascular and metabolic response to clonidine and midazolam premedication. Eur J Anaesthesia. 1997; 14(2):190-6.

https://doi.org/10.1097/00003643-199703000-00012

7. Miller R, Reves J, Glass P, Lubarsky D, McEvoy M. Intravenous non-opioid anaesthetics. Miller's Anaesthesia. Philadelphia: Elsevier Churchill Livingstone, 2015.

8. Saricaoglu F, Uzun S, Arun O, Arun F, Aypar U. A clinical comparison of etomidate-lipuro, propofol and admixture at induction. Saudi J Anaesth. 2011; 5:62-6. https://doi.org/10.4103/1658-354X.76509 PMid:21655019 PMCid:PMC3101756

9. Weisenberg M, Sessler D, Tavdi M, Gleb M, Ezri T, Dalton J, et al. Dose-dependent hemodynamic effects of propofol induction following brotizolam premedication in hypertensive patients taking angiotensin-converting enzyme inhibitors. J Clin Anesth. 2010; 22:190-5. https://doi.org/10.1016/j.jclinane.2009.07.008 PMid:20400005

10. Reagh O, Torres H, Rodríguez N, Gatica S. Alpha-2B adrenergic receptor mediated hemodynamic profile of etomidate. R Health Sci J. 2010; 29:91-5.

11. Sarkar M, Laussen P, Zurakowski D, Shukla A, Kussman B, Odegard K. Hemodynamic responses to etomidate on induction of anesthesia in pediatric patients. Anesth Analg. 2005; 101:645-50. https://doi.org/10.1213/01.ane.0000166764.99863.b4 PMid:16115968

12. Savola J, Ruskoaho H, Puurunen J, Salonen J, Kärki N. Evidence for medetomidine as a selective and potent agonist at alpha 2-adrenoreceptors. J Auton Pharmacol. 1986; 6(3):275-84. https://doi.org/10.1111/j.1474-8673.1986.tb00654.x PMid:2880852

13. Ebert T, Hall J, Barney J, Uhrich T, Colinco M. The effects of increasing plasma concentrations of dexmedetomidine in humans. Anesthesiology. 2000; 93(6):382-94. https://doi.org/10.1097/00000542-200008000-00016 PMid:10910487

14. Gurbet A, Basagan-Mogol E, Turker G, Ugun F, Kaya F, Ozcan B. Intraoperative infusion of dexmedetomidine reduces perioperativeanalgesic requirements. Can J Anaesth. 2006; 53(5):646-52. https://doi.org/10.1007/BF03021622 PMid:16803911

15. Hall J, Uhrich T, Barney J, Arain S, Ebert T. Sedative, amnestic, and analgesic properties of small-dose dexmedetomidine infusions. Anesth Analg. 2000; 90(5):699-705. https://doi.org/10.1097/00000539-200003000-00035 PMid:10702460

16. Blaudszun G, Lysakowski C, Elia N, Tramer M. Effect of perioperative systemic alpha2 agonists on postoperative morphine consumption and pain intensity: systematic review and meta-analysis of randomized controlled trials. Anesthesiology. 2012; 116(4):1312-22. https://doi.org/10.1097/ALN.0b013e31825681cb PMid:22546966

17. Bekker A, Haile M, Kline R, Didehvar S, Babu R, Martiniuk F, et al. The effect of intraoperative infusion of dexmedetomidine on the quality of recovery after major spinal surgery. J Neurosurg Anesthesiology. 2013; 25(1):16-24. https://doi.org/10.1097/ANA.0b013e31826318af PMid:22824921 PMCid:PMC3557800

18. Sivilotti M, Ducharme J. Randomized double blind study on sedative and hemodynamics during rapid sequence intubation in the emergency department: The Shred study. Ann Emerg Mwd. 1998; 31(3):313-24. https://doi.org/10.1016/S0196-0644(98)70341-5

19. Yazdi B, Khalili M, Talebi H, Fotovat A, Nikaeen A. Effect of adding ketamine to propofol infusion on hemodynamics and recovery time of patients under cataract surgery. Anesthesiology and Pain. 2011; 1(4).

20. Chalam K. A comparative study of intravenous dexmedetomidine-versus propofol-based sedation for awake fiberoptic intubation along with airway blocks in cervical discectomy patients. Karnataka Anaesthesia Journal. 2015; 1(1):21-7. https://doi.org/10.4103/2394-6954.149716

21. Bollucuoglu K, Hanci V, Yurtlu S, Okyay D, Ayoglu H, Turan I. Comparison of propofol-dexmedetomidine, tiopental-dexmedetomidine and etomidate-dexmedetomidine combinations' effects on the tracheal intubation conditions without using muscle relaxants. Bratisl Lek Listy. 2013; 114(9):514-8. https://doi.org/10.4149/BLL_2013_107

22. Cheng S, Hu d, Hei G. Comparision Dexmedetomidin and propofol in hyeamodynamic response in children with cardiovascular disease in heart surgery. Anesthesiology. 2014.

23. Karimian M, Emadi S, Nasiri E, Farzin D. Comparision of the effects of different doses of ketamine propofol on heamodynamic chandes of the patients during induction of anesthesia J Mazandaran UnivMed Sci. 2006; 16(54):7-13.

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Clinical Science

Therapeutic Effect of Adding Magnesium Sulfate in Treatment of Organophosphorus Poisoning

Fatemeh Jamshidi[1], Arash Yazdanbakhsh[1], Mohammad Jamalian[2], Peyman Khademhosseini[3], Koroosh Ahmadi[3], Alireza Sistani[1], Abolfazl Jokar[1*]

[1]Department of Emergency Medicine, Arak University of Medical Sciences, Arak, Iran; [2]Arak University of Medical Sciences, Arak, Iran; [3]Department of Emergency Medicine, Karaj University of Medical Sciences, Alborz, Iran

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***Correspondence:** Abolfazl Jokar. Department of Emergency Medicine, Arak University of Medical Sciences, Arak, Iran. E-mail: Dr.a.jokar@gmail.com

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Abstract

BACKGROUND: In recent years, the prevalence of poisoning has increased dramatically due to population growth and access to drugs and toxins. Today poisoning is one of the important reasons for visiting hospitals.

AIM: The present study aimed to investigate the effect of magnesium sulfate on organophosphorous toxicity.

METHODS: Patients who had inclusion criteria in the study were randomly assigned to one of two groups (control group or case group) by an emergency medicine specialist. Patients' data including age, sex, ECG, vital signs, arterial oxygen saturation were recorded for patients. Patients in the case group (40 subjects) received 2 mg magnesium sulfate 50%, while the control group (40 subjects) received 100 cc normal saline (as placebo) as an intravenous infusion

RESULTS: The distribution of gender in the two groups of patients was the same. Also, the mean age, Stature and weight of patients were similar in both groups. In the group receiving magnesium sulfate, diastolic blood pressure was lower when compared with another group, at 0 and 2 hours after intervention. Moreover, the mean of systolic blood pressure in both groups was determined to be the same at all hours. Furthermore, the heart rate in the group receiving sulfate was lower as compared to the control group for 8 hours, 16 and 24 hours after intervention.

CONCLUSION: The use of magnesium sulfate in organophosphate poisoning reduces therapeutic costs an average hospital length of stay and mortality compared to those who did not receive magnesium sulfate.

Introduction

In recent years, the prevalence of poisoning has increased dramatically due to the growth of societies and the ease of access to drugs and toxins [1]. In Iran, over 50 various pesticide combinations are utilised for agriculture, and today in Iran there are over 40 organophosphorus chemicals (OPs), with acute and subacute toxicity, as well as OPs, are applied in agronomy, homes, gardens and veterinary practice. [2]. Usually, the severity of poisoning in adolescents and adults is often acute, due to excessive consumption of oral medications or the misuse of some medications, environmental, industrial, and available agricultural products deliberately or accidentally [3] [4].

In general, the incidence of poisoning is quite different in each region and each country due to the geographical distribution of each particular region and the type of toxic substance [5] [6]. Many of the poisonings are drug-related, and the rest are classified as non-drug poisoning. Generally, common causes of poisoning include drugs, hydrocarbons, organophosphorus pesticides (OPPs), natural anticholinergic or chemical compounds, rodenticide, opiate, carbon monoxide, alcohol, fungi, insect and animal bites, *acid and basic material*s [5] [7]. Pesticides, especially organophosphorus, are distributed in many areas, to fight pest infestations. Because of their ease of access, poisoning from them has become more prevalent. Organophosphate

poisoning is one of the main clinical problems in the world, especially in developing countries, where is associated with high mortality [5] [8] [9]. About 28.4% of the agricultural pesticides used in Iran are organophosphorus [10]. Insecticides are organophosphorus compounds that lead to toxicity in humans by inhibiting the acetylcholinesterase enzyme [11]. As other studies indicated, the mortality rate from organophosphate poisoning has been reported by 3 to 25% [12]. The most common cause of death in this poisoning is respiratory failure due to respiratory depression, resulting from respiratory muscle weakness, central nervous system suppression, bronchus, bronchospasm and bradycardia [13]. Although poisoning and contamination with this toxin occur in all countries, in third world countries due to the low level of industrial and health care, the annual incidence of organophosphate poisoning and mortality is higher [14]. In Iran, organophosphate is widely used as an insecticide poison in the agricultural industry. According to a study conducted in Isfahan, this substance was the fourth most common poisoning and the second leading cause of death in patients referring to the poisoning department of the Noor Medical Center [15]. Magnesium sulfate is an inorganic salt containing magnesium, sulfur and oxygen, with the formula $MgSO_4$. The mechanism of magnesium effect is not well known but affects the Na + K + ATPase pump in sodium, potassium and calcium channels. It also reduces the release of acetylcholine at the site of the nerve-muscle.

The drug is used for various purposes, such as eliminating magnesium deficiency, helping to treat lethal arrhythmias called torsade, and preventing *eclamptic seizure*, as well as treating severe asthma and preventing premature uterine contractions during pregnancy. This drug prevents seizures or control of these attacks by blocking neuromuscular transmission [16]. Poisoning with this drug is not common, but excessive use of this drug can cause flushing and sweating, low blood pressure and reduced tendon reflexes. Atropine and oximes are traditionally used in the management of poisoning, but their efficacy is a subject of debate [17] [18] [19] [20] [21]. Animal findings indicate the low efficacy of pralidoxime in organophosphate poisoning [22] [23]. On the other hand, evidence suggests that it does not affect human poisoning [17] [19] [24].

Therefore, any pharmacotherapeutic agent that contributes to preventing or improving the toxicity of OPPs can also be helpful in reducing the cost of treatment and the length of hospitalisation. Animal experiments and uncontrolled human experiments demonstrate the effect of $MgSO_4$ [25] and clonidine for reducing organophosphate toxicity. Given the evidence mentioned above, the present study was conducted to investigate the effect of magnesium sulfate on organophosphate toxicity.

Material and Methods

Ethical approval and patient consents

All procedures performed in studies involving human participants were by the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Ethics Committee of Arak University of Medical Sciences. Patients who contacted us indicated their consent by signing a written consent form.

Study design and patient populations

This double-blind, randomised clinical trial was conducted on patients referred to the emergency department of Vali-e-Asr Hospital in Arak, Iran with symptoms of organophosphate poisoning. The diagnosis of this poisoning is based on a history of contact with these agents and specific clinical signs of poisoning, which are presented as follows: Defecation/Diarrhea, Urination, Muscle Weakness/ Miosis, Bradycardia/ Bronchorrhea/ Bronchospasm, Emesis, Tears Lacrimation, Salivation, which is called DUMBLE.

Patients in the case group (40 patients) received 2 g of magnesium sulfate 50% (4 cc) for intravenous infusion in a total volume of 100 cc, in half an hour. The same amount of drug (2 g) was successively injected 3 times every 2 hours. The drug was prepared by a nurse and under the supervision of a specialist in emergency medicine and was provided to the *corresponding person* who was not aware of the type of medication.

In the control group, 100 cc normal saline (as placebo) injected intravenously in half an hour for patients in the control group (40 patients). The same amount of drug (100 cc normal saline) was successively injected 3 times for half an hour. It should be noted that all patients in each of the two groups received the standard medicine, suggested in the reference books for organophosphate poisoning.

Standard treatment consists of gastric lavage, serum administration, oxygen intake, pulmonary and oral secretions suction, cardiopulmonary and respiratory monitoring, charcoal administration (1 g/kg), and washing of infected skin with water and soap, and intravenous injection of 0.5-5 mg atropine. The dosage of *Pralidoxime* is 20-40 mg/kg, which is administered intravenously throughout 10 to 5 minutes. Pralidoxime can be repeated every 6 to 24 hours, if necessary.

Keeping the airway open, oxygen was given and *fixation of hypertension* during treatment. Other factors were also evaluated, including electrolytes, fasting blood sugar, liver and kidney function tests,

arterial blood gases, pupil size, tendon reflexes, fasciculations intensity, respiratory crackles and *oral secretions*, and in the case of tracheal intubation. Also, if intubation was done, the secretion of the chip was checked, and the levels of atropine and *pralidoxime* were also recorded.

All data were analysed by SPSS software v.19. The variables were applied to measure the mean, standard deviation, standard error, percentage of frequency. Covariance analysis, Chi-square and Independent T-test or its nonparametric equation were also used to compare the variables.

The inclusion criteria included: 1. Age between 18-65 years; 2. Patients with acute OPPs toxicity who have not received advanced medical care at the other medical centre and less than two hours after the time of their poisoning; 3. Filling out the informed consent form of the patient.

Exclusionary *criteria* include 1. Unwilling to participate in the study; 2. The *concomitant use* of *other drugs* as a coincidence or suicide attempt; 3. History of severe complications or sensitisation due to magnesium sulfate; 4. History of the known cardiac block, *cardiovascular injury*, myocardial *injury* due to previous MI, severe renal failure, hepatitis, Addison's disease.

The sample size was calculated based on α = 0.05, and *generalising* the *prevalence* to a country (0.062), [26]. Finally, 40 subjects were assigned to each group, where the total sample size was estimated as 80 based on the formula below.

$$\alpha = 0.05$$
$$p_1 = 0.062$$
$$p2 = 0.5$$
$$\beta = 0.2$$

$$n_1 = n_2 = \frac{2(Z_{1-\alpha/2} + Z_{1-\beta})^2 \left[P_1(1-P_1) + P_2(1-P_2)\right]}{(P_1 - P_2)^2} = 4$$

All data were analysed by SPSS software v.19. The variables were applied to measure the mean, standard deviation, standard error, percentage of frequency. Covariance analysis, Chi-square and Independent T-test or its nonparametric equation were also used to compare the variables.

Results

This double-blind, randomised clinical trial was conducted on patients with organophosphate poisoning symptoms. Therefore, 90 patients were enrolled in the study and 10 were excluded from the study, of which 3 patients died before 24 hours, and 3 subjects used carbamate insecticide that was discharged from the hospital after 18 hours of admission and a relative improvement. Furthermore, one case of advanced liver cancer was excluded.

With regard to gender, the case group consisted of 25 (62.5%) males and 15 (37.5%) women, while the control group consisted of 22 (55%) males and 18 (45%), there was no statistically significant difference between two groups in terms of gender and both groups were poisoned in the same ratio (P = 0.391).

The mean and standard deviation of age in control and case groups were determined to be 35.90 ± 10.53 and 29.90 ± 8.87 years, respectively, where there was no statistically significant difference between the two groups regarding age and both groups were matched regarding mean age (P = 0.059).

The mean and standard deviation of weight in groups with or *without* sulfate were estimated as 71.4 ± 11.43 kg and 72.90 ± 11.19 kg, respectively. There was no statistically significant difference between the two groups regarding weight (P = 0.677).

Furthermore, mean and standard deviation of stature in groups with or *without* sulfate were determined to be166.30 ± 11.07 and 168.60 ± 11.05 cm, respectively, no significant difference was found regarding stature in the two groups (P = 0.515).

The mean systolic blood pressure in both groups between *0* to 24 hours after the intervention was not statistically significant (Table 1).

Table 1: Mean systolic blood pressure in both groups

	Group	Mean	Standard Deviation	P value
SBP Zero hour	Without sulfate	135	13.95	0.615
	With sulfate	133	10.80	
SBP 2 hours later	Without sulfate	129	9.67	0.689
	With sulfate	130/50	13.56	
SBP 4 hours later	Without sulfate	129	9.67	0.657
	With sulfate	130/50	11.45	
SBP 6 hours later	Without sulfate	129/30	9.47	0.570
	With sulfate	131/25	11.90	
SBP 8 hours later	Without sulfate	133	9.23	0.885
	With sulfate	133/50	12.25	
SBP 16 hours later	Without sulfate	130/05	9.17	0.264
	With sulfate	133/95	12.32	
SBP 24 hours later	Without sulfate	132	9.51	0.210
	With sulfate	136/55	12.80	

The mean and standard deviation of diastolic blood pressure 0 and 2 hours after intervention in the two groups showed a statistically significant difference, indicating that the magnesium sulfate group had higher blood pressure in these time zones than the group without magnesium sulfate (P = 0.004; P = 0.004). However, our results didn't show (Table 2) significant statistical difference for *the remaining hours* (Table 2).

Table 2: Mean diastolic blood pressure in both groups

	Group	Mean	Standard Deviation	P value
DBP Zero hour	Without sulfate	79.75	7.77	0.004
	With sulfate	72.90	6.38	
DBP 2 hours later	Without sulfate	77.95	7.18	0.004
	With sulfate	71.65	5.81	
DBP 4 hours later	Without sulfate	77.30	7.56	0.769
	With sulfate	76.60	7.39	
DBP 6 hours later	Without sulfate	77.30	7.58	0.444
	With sulfate	75.55	6.70	
DBP 8 hours later	Without sulfate	75.80	6.86	0.413
	With sulfate	77.60	6.88	
DBP 16 hours later	Without sulfate	74.40	7.09	1.000
	With sulfate	75.40	7.09	
DBP 24 hours later	Without sulfate	74.60	7.02	0.321
	With sulfate	76.90	7.44	

As indicated in Table 3, the mean and standard deviation of heart rate 8, 16, and 24 hours after intervention in the two groups showed a statistically significant difference, indicating that at these times, patients receiving magnesium sulfate had a lower heart rate per minutes (P = 0.028; P = 0.001; P = 0.017).

Table 3: Average heart rate (Pr) in both groups

Group		Mean	Standard Deviation	P value
Pr Zero hour	Without sulfate	57.90	5.37	0.139
	With sulfate	55.70	3.65	
Pr 2 hours later	Without sulfate	107.15	11.37	0.757
	With sulfate	108.20	9.91	
Pr 4 hours later	Without sulfate	105.85	10.00	0.252
	With sulfate	109.55	10.10	
Pr 6 hours later	Without sulfate	104.90	10.32	0.608
	With sulfate	106.50	9.21	
Pr 8 hours later	Without sulfate	104.55	9.43	0.028
	With sulfate	98.90	5.75	
Pr 16 hours later	Without sulfate	101.00	6.27	0.001
	With sulfate	92.25	3.94	
Pr 24 hours later	Without sulfate	95.20	6.27	0/017
	With sulfate	90.85	4.67	

There was no significant difference between the two groups in the number of breaths per minute at 0, 2, 4, 6, 8, 16, 24 hours after intervention. Moreover, No significant difference was found in the mean of arterial oxygen in the two groups at 0, 2, 4, 6, 8, 16, 24 hours after intervention.

Distribution of intubation frequency at 0, 2, 4, 6, 8, 16, 24 hours after intervention showed no statistically significant difference between the two groups regarding the need for intubation (Figure 1).

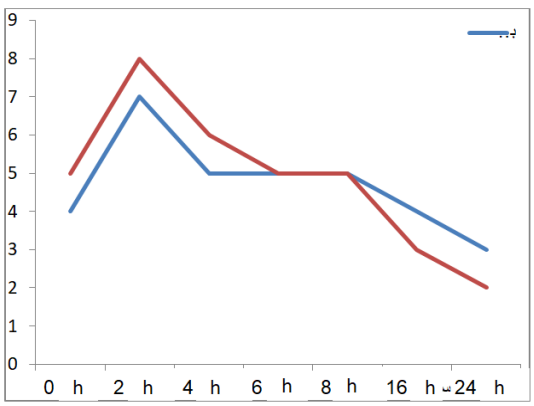

Figure 1: Frequency distribution of patients who were intubated at 0, 2, 4, 6, 8, 16, and 24 hours, based on the group

The frequency of pulmonary secretions at 0, 2, 4, 6, 8, 16, 24 hours after the intervention was not significantly different between the two groups and the lung secretions in both groups were approximately equal to one.

Moreover, the mean and standard deviation of admission hours showed that the use of magnesium sulfate in organophosphate poisoning reduced the number of admission hours (P = 0.006), (Table 4).

Table 4: Average hospitalization hours in both groups

Group		Mean	Standard Deviation	P value
Hospitali-zation	Without sulfate	58.05	12.17	0.006

Furthermore, the mean and standard deviation of atropine did not show a significant difference in the mean of atropine consumption (P = 67.9), (Table 5).

Table 5: Average atropine consumed in patients in both groups

	Group	Mean(mg)	Standard Deviation	P value
atropine	Without sulfate	1070	10	0.679
	With sulfate	1088	15	

Additionally, the mean and standard deviation of consumed *Pralidoxime* exhibited no significant difference (P = 0.232), (Table 6).

Table 6: The mean of consumed *Pralidoxime* at the end of 24 hours in patients in both groups

	Group	Mean(g)	Standard Deviation	P value
Pralidoxime	Without sulfate	344	26	0.232
	With sulfate	352	22	

On the other hand, the distribution of pupil diameter in both groups at 0, 2, 4, 6, 8, 16, 24 hours after intervention suggested no statistically significant difference (Table 7).

Table 7: Distribution of pupil diameter in both groups

P value	Group		Pupil diameter	
	With sulfate	Without sulfate		
-	20	20	Lower 3 mm	Zero hour
	0	0	Between 3 and 6 mm	
	0	0	Up to 6 mm	
0.824	1	2	Lower 3 mm	2 hours later
	9	10	Between 3 and 6 mm	
	9	9	Up to 6 mm	
1.000	0	0	Lower 3 mm	4 hours later
	3	4	Between 3 and 6 mm	
	17	16	Up to 6 mm	
1.000	0	0	Lower 3 mm	6 hours later
	3	4	Between 3 and 6 mm	
	17	16	Up to 6 mm	
0.301	0	0	Lower 3 mm	8 hours later
	8	4	Between 3 and 6 mm	
	12	16	Up to 6 mm	
0.748	0	0	Lower 3 mm	16 hours later
	11	13	Between 3 and 6 mm	
	9	7	Up to 6 mm	
0.501	0	0	Lower 3 mm	4 hours later
	12	15	Between 3 and 6 mm	
	8	5	Up to 6 mm	

Discussion

This study aimed to investigate the effect of magnesium sulfate on organophosphate poisoning. Organophosphate poisoning is one of the main clinical problems in the world with thousands of victims per year [27]. By inhibiting acetylcholinesterase activity, these compounds cause acetylcholine to accumulate in some brain synapses and neuromuscular synapses. Organophosphate poisoning is defined by various disorders such as the four clinical syndromes, *cholinergic crisis*, interstitial syndrome, organophosphate-induced *delayed polyneuropathy* (OPIDP), and organophosphate-induced chronic neuromuscular disorders. Each of these syndromes has its symptoms and symptoms [28]. Patients who receive first *auxiliary treatment* and emergency medical treatment have a better chance of recovery. However, the presence of arrhythmia and respiratory failure associated with poor prognosis. Ultimately, early diagnosis and proper treatment of complications can potentially reduce mortality.

Based on results presented in this study, the frequency of gender distribution was similar in both groups of patients and also the mean age, stature and weight of patients in both groups were determined to be similar. In the group receiving magnesium sulfate, diastolic blood pressure was found to be lower 0 and 2 hours after intervention as competed to patients who did not receive sulfate. The mean of systolic blood pressure in both groups was the same at all hours. The heart rate per minute in 8, 16 and 24 hours after the intervention was lower in the magnesium sulfate group than patients *without receiving* magnesium sulfate. Also, the number of hospital days in the group receiving magnesium sulfate was lower than that of other patients.

$MgSO_4$ inhibits acetylcholine release in the central nervous system, peripheral sympathetic and parasympathetic synapses. This prevents calcium channels in presynaptic nerve terminals, which release acetylcholine and increases the hydrolysis of some pesticides. It reduces arrhythmias associated with organophosphorus compounds and atropine, in the central nervous system decreases overstimulation by organophosphorus compounds, acting on the N-methyl-D-aspartate receptor, and reverses neuromuscular faint in the peripheral nervous system [29] [30] [31].

In the study of Pajoumand et al., The use of $MgSO_4$ at a dose of 4 g/day was considered useful in the treatment of acute human organophosphate toxicity, leading to a decrease in hospitalisation days and mortality rates that were consistent with the results of our study [32]. Our study also revealed that the number of hospital days in patients receiving magnesium sulfate was higher than those who did not receive magnesium sulfate, where reduces the cost of treatment. Basher in 2013 investigated the effect of magnesium sulfate on the toxicity of acute organophosphorus pesticides, and no adverse effects of magnesium were reported. In our study, no adverse effects were also found in patients receiving magnesium sulfate [33].

Recent advances in the treatment of organophosphate pesticide poisoning have shown that the alkalization of blood with sodium bicarbonate and magnesium sulfate can be *promising auxiliary treatment* [34], furthermore, Ahmed et al., in 2010, investigated the role of fresh plasma and magnesium sulfate in the treatment of acute toxicity of arganophosphamide insecticides, and showed that the addition of magnesium sulfate and fresh plasma to conventional treatments in organophosphate poisoning reduced the rate of hospitalization and death, where the results of this study were consistent with our findings [32].

In conclusion, the results of our study showed that the use of magnesium sulfate in organophosphate poisoning reduces therapeutic costs and the number of hospitalisation days and mortality compared to those who did not receive magnesium sulfate. Also, magnesium sulfate is also used to control tachycardia, ventricular arrhythmias, muscle fasciculations, where is therefore preferred to traditional therapies.

References

1. Moghaddamnia AA. Survey of acute suicidal poisoning in the west of Mazandaran province during the years 1994-97. J Mazandaran Univ Medical Sciences. 1999; 9(22-23):18-25.

2. Abdollahi M, Jalali N, Sabzevari O, Hoseini R, Ghanea T. A retrospective study of poisoning in Tehran. J Toxicol Clin Toxicol. 1997; 35:387-93. https://doi.org/10.3109/15563659709043371 PMid:9204099

3. Marx J, Walls R, Hockberger R. Rosen's Emergency Medicine-Concepts and Clinical Practice E-Book. Elsevier Health Sciences, 2013.

4. Paudyal BP. Poisoning: pattern and profile of admitted cases in a hospital in central Nepal. J Nepal Med Assoc. 2005; 44(159):6-92.

5. Moghadamnia AA, Abdollahi M. An epidemiological study of poisoning in northern Islamic Republic of Iran. East Mediterr Health J. 2002;8(1):88-94. PMid:15330564

6. Yaraghi A, Izadi Mood N, Gheshlaghi F, Rezvan M, Pazooki S. Evaluation of rodenticide poisoning distribution based on demographic characteristics, poisons, causes of intoxication, duration of hospitalization and mortality rate. Iranian J Toxicol. 2007; 2(1):100-4.

7. Ghorashi Z, Sultani Ahari H. A Study of the acute poisoning in patients admitted to Tabriz pediatrics medical center. J Ardabil Univ Med Sci Health Serv. 2003; 3(9):59-64.

8. Kanchan T, Menezes RG. Suicidal poisoning in Southern India: gender differences. J Forensic Leg Med. 2008; 15(1):7-14. https://doi.org/10.1016/j.jflm.2007.05.006 PMid:18096509

9. Rahimi R, Nikfar S, Abdollahi M. Increased morbidity and mortality in acute human organophosphate- poisoned patients

treated by oximes: a meta- analysis of clinical trials. Hum Exp Toxicol. 2006; 25(3):157-62. https://doi.org/10.1191/0960327106ht602oa PMid:16634335

10. Dehghani R, Moosavi SG, Esalmi H, Mohammadi M,Jalali Z,Zamini N.Surveying of Pesticides Commonly on the Markets of Iran in 2009 .Journal of Environmental Protection. 2011; 2:1113-1117. https://doi.org/10.4236/jep.2011.28129

11. Katz K, Brooks D. Toxicity organophosphate. Available from: http://emedicine. Medscape. com/ article/ 167726-overview.

12. Cynthia K. Organophosphates and carbamate. Ford M, Delaney K, Ling L, Erickson T. Clinical toxicology, 2001: 819-29.

13. Verhulst L, Waggie Z, Reynold L, Hatherill M, Argent A. presentation and outcome of sever anticholinesterase insecticide poisoning. Archives of Disease in childhood. 2002; 86:352-55. https://doi.org/10.1136/adc.86.5.352 PMid:11970930 PMCid:PMC1751109

14. Grenvik A, Ayzes SM, Hoebrook PR, Shoemaker WC. Text book of critical care. 4th ed. Philadelphia, Pennsylvania: W.B Saunders company, 2000: 2074-5

15. Sharafi E. A survey in death due to poisoning in poisoning emergency dep. In Noor hospital in isfahan 1378 – 1380. Doctora Thesis, 1382, Isfahan university of medical sciences [Persian].

16. Culture Iranian generic drugs, doctor heshmati, 2008.

17. Abdollahi M, Jafari A, Jalali N, Balai MM, Kebriaeeza-deh A, Nikfar S. A new approach to the efficacy of oximes in the management of acute organophosphate poisoning. Irn JMed Sci. 1995; 20:105-109.

18. Cherian AM, Peter JV, Samuel J, Jaydevan R, Peter S,Joel S et al. Effectiveness of pralidoxime in thetreatment of organophosphorus poisoning: a rando-mized, double blind placebo controlled clinical trial. J Assoc Physicians India. 1997; 45:22-24.

19. De Silva HJ, Wijewickrema R, Senanayake N. Doespralidoxime affect outcome of management in acute organophosphorus poisoning? Lancet. 1992; 339:1136-38. https://doi.org/10.1016/0140-6736(92)90733-J

20. Sivagnanam S. Potential therapeutic agents in the management of organophosphorus poisoning. Crit Care. 2002; 6:260-61. https://doi.org/10.1186/cc1500 PMid:12133189 PMCid:PMC137451

21. Sungur M, Guven M. Intensive care management of organophosphate insencticide poisoning. Crit Care. 2001; 5:15-211. https://doi.org/10.1186/cc1025

22. Rossic J. Partial antagonism by cholinesterase reacti-vators of the effects of organophosphate compounds on shuttle-box avoidance. Arch Int Pharmacodyn. 1970; 183:139-47.

23. Sanderson DM. Treatment of poisoning by anticholi-nesterase insecticides in the rat. J Pharm Pharmacol. 1961; 13:435-39. https://doi.org/10.1111/j.2042-7158.1961.tb11849.x PMid:13746163

24. Du Toit PW, Muller FO, Nan Tonder MW. Experience with intensive care management of organophos-phate insecticide poisoning. SA Med Tydskrif. 1981; 60:227-29.

25. Buccafusco JJ, Aronstam RS. Clonidine protectionfrom the toxicity of soman, an organophosphate acetyl cholinesterase inhibitor, in the mouse. J Phar-macol Exp Ther. 1986; 239:43-47. PMid:3761196

26. Shadnia Sh, Esmaily H, Sasanian Gh, Pajoumand A, Hassanian-Moghaddam H, Abdollahi M. Pattern of acute poisoning in Tehran-Iran in 2003. Human & Experimental Toxicology. 2007; 26:753–756. https://doi.org/10.1177/0960327107083017 PMid:17984147

27. Sokołowski R, Płusa T. Today's threat of use of organophosphorus compounds. Pol Merkur Lekarski. 2015; 39(231):176-80.

28. Costa LG. Organophosphorus Compounds at 80: Some Old and New Issues. Toxicol Sci. 2018; 162(1):24-35. https://doi.org/10.1093/toxsci/kfx266 PMid:29228398

29. Naguib M, Lien CA, Meistelman C. Pharmacology of neuromuscular blocking drugs. In: Miller RD, Eriksson LI, Cohen NH, Fleisher LA, Wiener-Kronish JP, Young WL, editors. Millers? Anesthesia. 8th ed. Philadelphia, PA: Elsevier Churchill Livingstone, 2015. p. 982.

30. Eddleston M, Chowdhury FR. Pharmacological treatment of organophosphorus insecticide poisoning: The old and the (possible) new. Br J Clin Pharmacol. 2016; 81:462–70. https://doi.org/10.1111/bcp.12784 PMid:26366467 PMCid:PMC4767211

31. Vijayakumar HN, Kannan S, Tejasvi C, Duggappa DR, Veeranna Gowda KM, Nethra SS.Study of Effect of Magnesium Sulphate in Management of Acute Organophosphorous Pesticide Poisoning.Anesth Essays Res. 2017; 11(1):192-196. https://doi.org/10.4103/0259-1162.194585 PMid:28298783 PMCid:PMC5341676

32. Pajoumand A, Shadnia S, Rezaie A, Abdi M, Abdollahi M. Benefits of magnesium sulfate in the management of acute human poisoning by organophosphorus insecticides. Hum Exp Toxicol. 2004; 23(12):565-9. https://doi.org/10.1191/0960327104ht489oa PMid:15688984

33. Basher A, Rahman SH, Ghose A, Arif SM, Faiz MA, Dawson AH.Phase II study of magnesium sulfate in acute organophosphate pesticide poisoning.Clin Toxicol (Phila). 2013; 51(1):35-40. https://doi.org/10.3109/15563650.2012.757318 PMid:23311540

34. Balali-Mood M, Balali-Mood K. Neurotoxic disorders of organophosphorus compounds and their managements. Archives of Iranian Medicine. 2008; 11(1):65-89. PMid:18154426

35. Syed M Ahmed, Bikramjit Das, Abu Nadeem, and Rajiv K Samal. Survival pattern in patients with acute organophosphate poisoning on mechanical ventilation: A retrospective intensive care unit-based study in a tertiary care teaching hospital. Indian J Anaesth. 2014; 58(1):11–17. https://doi.org/10.4103/0019-5049.126780 PMid:24700893 PMCid:PMC3968644

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Clinical Science

The Estimation of Survival and Associated Factors in Self-Immolation Attempters in Ilam Province of Iran (2011-2015)

Jafar Bazyar[1], Katayoun Jahangiri[2], Hamid Safarpour[2*], Meysam Safi Keykaleh[2], Saeideh Varasteh[3], Leila Malekyan[4], Ehsan Mohammadi[5]

[1]Health in Emergency and Disaster Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; [2]Department of Health in Disasters and Emergencies, School of Public Health and Safety, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [3]Department of Nursing, School of Nursing and Midwifery, Zahedan University of Medical Sciences, Zahedan, Iran; [4]Department of Nursing, School of Nursing and Midwifery, Bam University of Medical Sciences, Bam, Iran; [5]Department of Nursing, School of Nursing and Midwifery, Ilam University of Medical Sciences, Ilam, Iran

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***Correspondence:** Hamid Safarpour. Department of Health in Disasters and Emergencies, School of Public Health and Safety, Shahid Beheshti University of Medical Sciences, Tehran, Iran. E-mail: h.safarpour@sbmu.ac.ir

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Abstract

BACKGROUND: Self-immolation is the most common method of suicide in Ilam province.

AIM: This study aimed to estimate the survival rate in self-immolation attempters in Ilam and identify the associated factors.

METHODS: A descriptive-analytic study was conducted based on data collected at Taleghani Hospital in Ilam, Iran. All persons passed away due to self-immolation or those hospitalised in the centre of the self-burned patients located in Ilam during 2011 to 2015 were assessed. Survival rate was calculated based on Kaplan-Meier. To compare the survival rate between groups, Univariate Log Rank and for multivariate analysis, the Cox test of STATA12 software was used.

RESULTS: During 2011 to 2015, 236 persons including 168 females and 69 males committed self-immolation. The mean and median of survival time in attempters were 32.2 ± 4.7 and 3 ± 0.33 days, respectively. In Univariate Log-Rank test, the following variables including age, sex, burn degree, Total Body Surface Area (TBSA), and burns in neck and head and lower limbs had a significantly meaningful relation with survival, while in multivariate Cox Regression test only two variables including Total Body Surface Area and age remained in the model.

CONCLUSION: The mean and median survival rate in self-attempters are very low. Quickly hospitalisation without waste of time should be considered. Providing prompt treatments and compensating dehydration in early hours especially within the first 24 hours of self-immolation are very vital. Younger persons and those with lower burn surface have more chance for longer survival and recuperation.

Introduction

Suicide is a major and one of the most important public health problems with an incidence which varies worldwide [1]. Suicide is an act where a person consciously attempts to end his/her life. The increasing rate of suicides at the beginning of the third millennium is so alarming that the World Health Organization and the International Association for Suicide Prevention have designated the tenth day of September as World Suicide Prevention Day [2]. Suicide is an indicator of a society mental health [1].

Suicides have many socio-demographic and psychopathological risk factors. Some studies showed the financial hardship, intimate relationship break-ups, and personal history of suicide attempts was risk factors for self-immolation [3]. Palacio et al., [4] in their study showed that those who reported adverse life-events in the last six months, and those who had a family history of suicide, had a higher risk of suicide. In another study, Zhang and colleagues [5] reported that hopelessness, negative life events, and family history of suicide were risk factors of attempted

suicide. Also Zhao et al. showed the work and study problems, marriage frustration, family conflict and fanaticism, somatic disease, and history of mental disorders were all significantly associated with suicide attempts [6]. Committing suicide has had an increasing rate in recent years which not only has caused tension and concern among communities to rise but also has affected the whole world [7]. The methods of choice for suicide attempt vary across countries and sometimes regions and have a significant cultural and ethnic influence [8]. The rate of suspected death due to suicide in Iran has been reported 4.7 cases per hundred thousand people [9]. One of the violent and dramatic ways of suicide is self-immolation [10]. Self-immolation, the purposely act of self-inflicting burns, is among the most lethal means of attempting suicide [11]. It is expected that self- harm caused by self-immolation, is the most damaging method of self-destruction [12] [13]. In addition to the high probability of mortality, due to self-immolation, even if the victims survive, mutilation and cosmetic changes can lead to pain and discomfort of the person and family [14]. The high rate of mortality (70-90%) and morbidity caused by self-immolation is considerable in Middle East countries in which self-immolation is a common method for suicide [15]. Self-immolation in Iran is a common method of suicide among young adults, who were mostly women, well-educated and mentally healthy [10]. In Iran, from 1.39 to 40 per cent of the suicides are due to self-immolation [16]. Evaluation of absolute and relative frequencies of suicide cases based on sex and the method of suicide in Ilam province shows that in both sexes, self-immolation with 71% has been the most common suicide method [16], [17]. The Khankeh et al., the study showed that there were five main motives for attempting self-immolation: cultural context, mental health problems, family conflicts, self-immolation as a threat, and distinct characteristics of the suicidal method [11]. The main concern of the government is to prevent self- harm through different kinds of suicide. The next phenomenon faced by the government after prevention is treatment because when people commit suicide, saving their life is a priority. Therefore this study aimed to estimate the survival rates and the associated factors in self-immolation attempters in Ilam province during 2011 to 2015.

Material and Method

This was a descriptive-analytic study based on data collected at Taleghani Hospital in Ilam, Iran. The medical records of all patients admitted to Taleghani hospital from March 2011 to February 2015 were reviewed. Taleghani hospital is the only burn centre in the province of Ilam and was unlikely due to the urgent reception of patients outside the province, according to the reports of the Emergency operation centre (EOC), it was unlikely that the transfer would be rapid and admission to other medical centres outside the province of Ilam. A total of 236 patients who had the experience of self-immolation participated in the study.

Based on the purpose of the study, the required variables such as demographic variables were obtained through the medical records of the patients. The researcher introduced her and the study purpose, and then explained ethical considerations such as secrecy of data and permission to leave the study at any time they want. Oral consent was obtained for all participants. This research was reviewed and approved by the Ethics Committee of Ilam University of Medical Sciences. The patient who had data of medical records of self-immolation attempting in the hospital and forensic medicine were included to study. If the patient was not referred to the hospital and self-immolation is reported by relatives or other persons, his profile was recorded in the judiciary and forensic medicine. Therefore, to investigate all cases of self-immolation and to increase the accuracy, after removing duplicates, the data of medical records were used in forensic medicine. The patients who were readmitted to the hospital due to self-immolation side-effects such as a scar or for plastic surgery were excluded.

Three established and commonly used estimation methods for the burn surface area are Rule of Palm, Rule of Nines, and Lund-Browder Chart [18]. The estimation of patients' burn surface area was extracted out of the charts available in their records which were calculated according to Lund and Browder's method [19]. All the self- immolators were followed until death or recuperation. The ultimate fate of people sent to burn centres outside the province was also followed through those medical centres. The rate of survival was calculated using the Kaplan method. In the univariate analysis of survival of the groups, Log-Rank test was used. In the multivariate analysis of survival, the factors which were significantly less than 2.0 in the Log-Rank method were analysed within the Cox model using the "forward" method. Then the most important factors associated with survival were identified. All analyses were carried out by the help of STATA12 software by taking the significant level of $P < 0.05$ into account.

Results

From 2011 to 2015, 236 patients including 168 (71.2%) females and 68 (28.8%) males had attempted self-immolation in Ilam province. The mean age of participants was 18.84 ± 34.85, ranging from 11 to 90 years. The mean of total Total Body Surface Area was 80%, ranging from 20 to 100 per cent of

burning so that the levels of burn-in women and men were 83 and 73 per cent, respectively. There was a statistically significant difference between the level of burn and gender (P = 0.002). More than 65 per cent of suicide attempters had burns over 80 per cent. Considering the future therapy of patients and the patients dispatched to the outside of the province, we figured out that 81.4 and 18.6 per cent of the patients were respectively died and had partial or complete remission. A total of 43 patients (18.2%) were dispatched to burn centres outside the province.

Table 1: Rates of suicide attempters' survival regarding the forthcoming days after the self-immolation

Days after the self-immolation	Death frequency	Improvement frequency	Survival rate based on per cent
Less than a day	60	0	74.57
1	84	1	64.4
7	160	5	32.2
14	174	20	26.2
22	179	24	24.1
28	181	26	23.3
35	183	28	22.45
90	186	31	21.1

The mean and median of survival in suicide attempters in the present study were 32.2 ± 4.7 and 3 ± 33 respectively. Kaplan-Meier graph shows the survival rate (Table 1 and Figure 1).

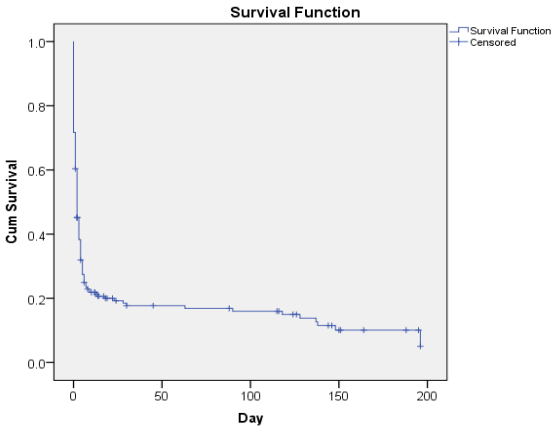

Figure 1: Overall survival rates of suicide attempters regarding the day, based on Kaplan-Meier

Based on this chart 25.43% of the victims died in the early hours after the self-immolation, having a survival of fewer than 24 hours. Only 32.2 % of the victims died 7 days after the self- immolation, that is to say, the total rate of mortality after a week amongst the self- immolators in Ilam province were equal to 67.8 %. The three- month survival rate was also 21.1 % (Table 2).

The association of all variables with survival was specified using the Log-Rank test. There was a significant relationship between genders, age, per cent of burn, burn degree and survival rate, accordingly. People who had suffered burn in the head, neck, limbs and reproductive organs had a lower rate of survival.

Table 2: Univariate analysis of factors affecting the survival rate of self-immolation attempters in Ilam province during 2011 to 2015

Variable	Subgroup	Frequency	Death (per cent)	Improvement (per cent)	Log-rank test
Gender	Male	68	(67.4)46	(32.4)22	0.024*
	Female	168	(86.9)146	(13.1)22	
Age	10- 30 years old	141	(79.1)113	(19.9)28	0.000*
	31- 60 years old	67	(79.6)52	(22.4)15	
	Over 61 years old	28	(96.4)27	(3.6)1	
Education	Illiterate	61	(83.6)51	(16.4)10	0.64
	Primary school	46	(82.6)38	(17.4)8	
	Secondary school	41	(78)32	(22)9	
	High school	29	(82.8)24	(17.2)5	
	Diploma	46	(76.1)35	(23.9)11	
	University	13	(92.3)12	(7.7)1	
Employment	Employee and retired	5	(80)4	(20)1	0.833
	Student	27	(85.2)23	(14.8)4	
	Worker	7	(85.7)6	(14.3)1	
	Householder, unemployed	175	(81.7)143	(18.3)32	
	Self- employed	22	(72.7)16	(27.3)6	
Marital status	Married	130	(81.5)106	(18.5)24	0.070
	Single	98	(79.6)78	(20.4)20	
	Other (widowed, divorced)	8	(100)8	(0)0	
Residency status	City	161	(79.5)128	(20.5)33	0.677
	Rural area	75	(85.3)64	(14.7)11	
Per cent of burn	Less than 30%	11	(10)1	(90) 10	0.000*
	31- 70 %	55	(40)22	(60) 33	
	Over 71%	169	(99.4)168	(.6)1	
Ignition material Used	Oil	228	(81.6)186	(18.4)42	0.922
	Benzene	6	(66.7)4	(33.3)2	
	Gasoline	2	(100)2	(0)0	
Burn degree	First- degree	0	(0)0	(0)0	*0.000
	Second- degree	8	(2.5)1	(87.5)7	
	Third- degree	1	(100)1	(0) 0	
	First and second degree	12	(0)0	(100)12	
	Second and third degree	144	(93.7)135	(6.3)9	
	First, second and third degree	71	(77.5)55	(22.5)16	
Season	Spring	67	(77.7)52	(22.3)15	0.472
	Summer	60	(78)49	(22)11	
	Fall	50	(78)39	(22)11	
	Winter	59	(79.7)46	(20.3)12	
Head, neck burn	With	198	(94.4)187	(5.6)11	*0.000
	Without	38	(13.2)5	(86.8)33	
Body burn	With	227	(81.9)186	(18.1)41	0.288
	Without	9	(66.7)6	(33.3)3	
Upper parts burn	With	183	(80.3)147	(19.7)36	0.066
	Without	53	(84.9)45	(15.1)8	
Lower parts (reproductive organs) burn	With	195	(95.9)187	(4.1)8	*0.000
	Without	41	(2.2)5	(87.8)36	
Addiction	With	15	(73.3) 11	(26.7)4	0.666
	Without	221	(81.9)181	(18.1)40	

There was not a significant correlation between education level, employment status, marital status, ignition material used, residency status, time of the year and addiction with survival (Table 3).

Table 3: Multivariate analysis of factors affecting the survival of self-immolation attempters in Ilam province during 2011 to 2015

Variable	Sub- group		CI95%HR
Burn percentage	Below 30%		----
	31- 70%	3.94	0.531-29.32
	Over 71%	26.21	3.661-187.699
Age	10- 30 years	1	----
	31- 6- years	1.3	0.934-3.368
	Over 61 years	2.2	1.435-3.367

*Hazard Rate.

Men had a higher rate of survival in comparison with women, and this correlation was statistically significant (P = 0.024). The variables having P < 0.2 in univariate analysis using the Log-Rank was analysed using multivariate Cox analysis method. Multivariate analysis showed that only two variables, namely, Total Body Surface Area and age could remain in the Cox Regression model and the rest of the other variables were excluded from the model.

Total Body Surface Area was introduced as the strongest factor associated with survival.

Discussion

The mean and median of suicide attempters in the present study were, respectively, 32.2 ± 4.7 and 3 ± 33. In the study conducted by Najafi et al., the mean and median of the suicide attempters was respectively, 11 ± 2 and 33 ± 2.6 in Kermanshah [20]. There was a significant difference in survival rate of both genders. It was in contrast with them. Moradinazar et al., study which reported that no significant difference in survival rate of both genders was found [21]. In our study 25.43% of the victims died in the early hours after self- immolation having survival rate lower than 24 hours, which was consistent with the results of the of Moradinazar et al., study which showed that the highest mortality rate of self-immolation was in the first 24 hours after accident [21]. Only 32.2 % of the victims died after 7 days after self-immolation, while in Najafi's study 11% and nearly 70% of the victims have respectively, the survival rates of lower than 1 and 7 days [20]. It can be said that 24 and 7 days survival of the self-immolation attempters in Kermanshah province was rather two times more than our study's.

In the present study, through using univariate analysis by the help of the Log Rank, it was revealed that there is a significant relationship between, age, sex, burn degree, Total Body Surface Area, head, neck, limbs and genitals' burn with survival rate. In Najafi's study, in univariate analysis, factors such as age, mental disorder, drug addiction and burn percentage were significant [20]. In our study, in contrast with Najafi's, gender was significant, while drug addiction was not so. In Dastgiri's study as far as univariate analysis is taken into consideration, gender and age were not significant [22]. In our study, the victims suffering head, neck and limb's burn had a lower rate of survival which is inconsistent with the study done by Dastgiri et al., [22].

In the present study, only Total Body Surface Area and age variables could remain in the multivariate analysis and other variables were excluded out of the model. The findings of the study were well correlated with Najafi's in Kermanshah [20]. In this study, like Najafi's study, the percentage of the burn was the most powerful detected factor in the survival. Also in Moradinazar et al. study the strongest risk factor affecting the survival of self-immolation attempters was Total Body Surface Area [21]. In the present study, the risk factor in those participants with more than 71% of the burn was 26.2 times more than those with less than 30% of burn. These results are consists with Moradinazar that demonstrated the risk of those with burns percentage higher than 70% was 17 times more than those with burns percentage lower than 30%. Also, this rate was 17.3% in Najafi's study [20].

In the analysis of age as the second associated factor in survival, it was found that the risk factor for people ranging from 31 to 60 years was 1.3 times and in people over 61 years, 2.2 times more than those under the age of 30, and in Najafi's study, these rates were, 1 and 3.08, respectively [20]. Also, the results of Moradinazar et al. study showed the ratio of fatal self-immolations up to the age of 45 was constant. But, after the age of 45, the ratio of fatal suicides increases [21]. It is believed that with ageing, the human skin, major changes such as perforation of dermal vessels, reducing subcutaneous fat and atrophy of the skin-dependent structures, which means that the immune system's ability to fight secondary infections is will be reduced.

In conclusion, the mean and median of self-immolation attempters' survival rate is very low, and the mortality rate is by contrast high. Although preventive measures to avoid the phenomenon of suicide is much more important than medical intervention, self-immolation attempters with a lower degree of burn and age have more chances to survive and even improve. It seems that even in case of severe burns, the younger victims have a chance of survival. So, quickly hospitalisation without waste of time should be considered. Urgent medical treatments in the first hours, especially in the early 24 hours after self- immolation, as well as compensation for body's fluid and electrolytes, can be very determinative in survival rate. Activating the emergency medical system and doing medical treatments at the place of self- immolation towards the first burning centre can help revival and keep body fluid.

Our results regarding the survival of people committing self-immolation, due to the limitations of the studies in this field, were compared with a few other studies. It is recommended to conduct further studies with sufficient sample size. Such factors as the risk of nosocomial infection and the rate of inhaled damages will certainly have an impact on survival. In the present study, due to lack of access to this information, we were unable to estimate the impact of these factors. Considering the impact of these issues in future researches is also suggested. Many patients due to lack equipped burn centre in the province are being sent to distant provinces. Perhaps, establishing Burn Centers in Ilam or the west of the country, having the high incidence of suicide, can be helpful.

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References

1. Kanchan T, Menon A, Menezes RG. Methods of choice in completed suicides: gender differences and review of literature. Journal of forensic sciences. 2009; 54(4):938-42. https://doi.org/10.1111/j.1556-4029.2009.01054.x PMid:19486437

2. WHO. Mental health: suicide prevention 2014 [accessed Nov 5, 2014]. Available from: http://www.who.int/mental_health/suicide-prevention/en/

3. Ahmadi A, Schwebel DC, Bazargan-Hejazi S, Taliee K, Karim H, Mohammadi R. Self-immolation and its adverse life-events risk factors: results from an Iranian population. Journal of injury and violence research. 2015; 7(1):13. PMid:25618437 PMCid:PMC4288291

4. Palacio C, García J, Diago J, Zapata C, Lopez G, Ortiz J, et al. Identification of suicide risk factors in Medellin, Colombia: a case-control study of psychological autopsy in a developing country. Archives of Suicide Research. 2007; 11(3):297-308. https://doi.org/10.1080/13811110600894223 PMid:17558615

5. Zhang Y, Yuan G, Li G-L, Yao J, Cheng Z, Chu X, et al. A case-control study on the risk factors for attempted suicide in patients with major depression. Zhonghua liu xing bing xue za zhi= Zhonghua liuxingbingxue zazhi. 2007; 28(2):131-5.

6. Zhao C-j, Dang X-b, Su X-l, Bai J, Ma L-y. Epidemiology of Suicide and Associated Socio-Demographic Factors in Emergency Department Patients in 7 General Hospitals in Northwestern China. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2015; 21:2743-9. https://doi.org/10.12659/MSM.894819 PMid:26369363 PMCid:PMC4576919

7. Murray B, Wright K. Integration of a suicide risk assessment and intervention approach: the perspective of youth. Journal of Psychiatric and Mental Health Nursing. 2006; 13(2):157-64. https://doi.org/10.1111/j.1365-2850.2006.00929.x PMid:16608470

8. Mehrpour O, Javadinia SA, Malic C, Dastgiri S, Ahmadi A. A survey of characteristics of self-immolation in the east of Iran. Acta Medica Iranica. 2012; 50(5):328-34. PMid:22837086

9. Shojaee A SH, Moradi S, Alaoddini F, Khademi A. Investigate suspicious death rate of suicide referral to Legal Medicine of 2009 winter to 2010 autumn comparison with the global rates. Journal of Forensic Medicine. 2012; 18:7-15.

10. Parvareh M, Hajizadeh M, Rezaei S, Nouri B, Moradi G, Nasab NE. Epidemiology and socio-demographic risk factors of self-immolation: A systematic review and meta-analysis. Burns. 2017. PMid:29032973

11. Khankeh HR, Hosseini SA, Rezaie L, Shakeri J, Schwebel DC. A model to explain suicide by self-immolation among Iranian women: a grounded theory study. Burns. 2015; 41(7):1562-71. https://doi.org/10.1016/j.burns.2015.03.015 PMid:25958251

12. Malic C, Karoo R, Austin O, Phipps A. Burns inflicted by self or by others—an 11 year snapshot. Burns. 2007; 33(1):92-7. https://doi.org/10.1016/j.burns.2006.04.008 PMid:17071003

13. Thombs BD, Bresnick MG, Magyar-Russell G. Who attempts suicide by burning? An analysis of age patterns of mortality by self-inflicted burning in the United States. General hospital psychiatry. 2007; 29(3):244-50. https://doi.org/10.1016/j.genhosppsych.2007.01.012 PMid:17484942

14. Massoudhamidi A, Massoudhamidi F. The relationship between suicide and demographic characteristics of patients admitted to the Burn Center. Faculty Nurs Midwife. 2005; 5:12-7.

15. Rezaie L, Hosseini SA, Rassafiani M, Najafi F, Shakeri J, Khankeh HR. Why self-immolation? A qualitative exploration of the motives for attempting suicide by self-immolation. Burns. 2014; 40(2):319-27. https://doi.org/10.1016/j.burns.2013.06.016 PMid:23891233

16. Suhrabi Z, Delpisheh A, Taghinejad H. Tragedy of women's self-immolation in Iran and developing communities: a review. International journal of burns and trauma. 2012; 2(2):93. PMid:23071907 PMCid:PMC3462521

17. REZAEIAN M, SHARIFIRAD GR. Seasonal Pattern of Suicide And Attempted Suicide In Dam Province During 1995-2002. 2008.

18. Giretzlehner M, Dirnberger J, Owen R, Haller H, Lumenta D, Kamolz L-P. The determination of total burn surface area: how much difference? Burns. 2013; 39(6):1107-13. https://doi.org/10.1016/j.burns.2013.01.021 PMid:23566430

19. Lund CC. The estimation of areas of burns. Surg Gynecol Obste. 1944; 79:352-8.

20. Najafi F, Ahmadijouybari T, Moradinazar M, Ataie M, Hatami M, Almasi A. The survival rate of self-immolators in Kermanshah Province 2010-2011. Journal of Kermanshah University of Medical Sciences. 2013; 17(9):563-71.

21. Moradinazar M, Amini S, Baneshi M, Najafi F, Abbasi N, Ataee M. Survival probability in self immolation attempters: a prospective observational cohort study. Ulus Travma Acil Cerrahi Derg. 2016; 22(1):23-8. PMid:27135074

22. Dastgiri S, Kalankesh LR, Pourafkary N, Vahidi RG, Mahmoodzadeh F. Incidence, survival pattern and prognosis of self-immolation: a case study in Iran. Journal of Public Health. 2006; 14(1):2-6. https://doi.org/10.1007/s10389-005-0001-9

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Clinical Science

The Comparison of Simple Anthropometric and Biochemical Parameters for Predicting Liver Steatosis in Obese Balinese Young Women

I Wayan Weta[1], Tjok Gde Bagus Mahadewa[2*], Wayan Putu Sutirtayasa[3], AAN Subawa[3], Firman P. Sitanggang[4], I Putu Eka Widyadharma[5]

[1]Department of Public Health, Preventive Medicine, and Clinical Nutrition, Faculty of Medicine, Udayana University, Bali, Indonesia; [2]Department of Neurosurgery, Faculty of Medicine, Udayana University, Bali, Indonesia; [3]Department of Clinical Pathology, Faculty of Medicine, Udayana University, Bali, Indonesia; [4]Department of Radiology, Faculty of Medicine, Udayana University, Bali, Indonesia; [5]Department of Neurology, Faculty of Medicine, Udayana University, Sanglah General Hospital, Bali, Indonesia

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***Correspondence:** Tjok Gde Bagus Mahadewa. Department of Neurosurgery, Faculty of Medicine, Udayana University, Bali, Indonesia. E-mail: tjokmahadewa@unud.ac.id

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Abstract

BACKGROUND: The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing globally. Early identification of liver steatosis (LS) status is critical to prevent the development of NAFLD into non-alcoholic steatohepatitis (NASH) fibrosis.

AIM: This study aimed at exploring the validity of simple anthropometric and biochemical parameters to predict LS in young obese women.

MATERIALS AND METHODS: This is a cross-sectional study involving 132 young obese women. We collected the data of measured waist circumference (WC), body mass index (BMI), serum triglyceride (TG), and gamma-glutamyltransferase (GGT). The lipid accumulation product (LAP) was designed from TG and WC variables. Fatty liver index (FLI) was calculated from TG, BMI, WC, and GGT variables. LS status was measured using ultrasonography assay. Statistical significance was set at $p < 0.05$.

RESULTS: A positive correlation was found between BMI, WC, TG, GGT, LAP, FLI, and LS ($p = 0.001$). We found that BMI is a better predictor for LS to WC. Our multiple linear regression analysis revealed that BMI, GGT,and TG could predict 41.4% of LS. The validity (specificity, sensitivity, and odds ratio) of simple body fat parameters in predicting LS were as follows: BMI ≥ 30 kg/m^2 (69.6%, 74.4%, and 6.21), WC ≥ 90 cm (67.4%, 70.0%, and 4.28), TG ≥ 100 mg/dL (70.6%, 70.0%, and 5.62) and GGT ≥ 20 µg/L (69.6%, 77.5%, and 7.87), as well as LAP ≥ 30 (82.6%, 70.0%, and 11.1), and FLI ≥ 2.5 (79.3%, 72.5%, and 10.1), significantly.

CONCLUSION: Simple anthropometric and biochemical parameters (BMI, WC, and TG, GGT), are appropriately predicting LS as well as LAP, and FLI among obese Balinese young women.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is related to metabolic syndrome, while non-alcoholic steatohepatitis (NASH) can develop cirrhosis and liver failure. As the prevalence of NAFLD increases, it arises as a public health problem. It is predicted that within one decade the prevalence of NAFLD will be twice as much. In the western population, its prevalence ranges from 15 to 35%, with 10% of these will progress to NASH.

The prevalence of NAFLD is 58% in overweight people and 98% in non-diabetic, obese population [1]. Within 10 years, NAFLD can progress from liver steatosis (LS) to NASH (47%), and to advanced NASH fibrosis and cirrhosis (20-50%) [2]. The prevalence of NAFLD in Asia is 15-30%, and this figure rises to 50% in those with metabolic syndrome [3] [4].

LS is initiated by the increased of visceral fat mass in an individual with obese. Measuring visceral fat mass is essential for identifying the risk to LS. While computer tomographic (CT) scan is still the gold

standard to measure visceral fat, some specific body fat parameters which include waist-to-height ratio (WtHR), conicity index (c-index), visceral adiposity index (VAI), and lipid accumulation product (LAP) can also be used to predict visceral fatness [5]. Bedogni et al., developed the fatty liver index (FLI), that is calculated from the waist circumference (WC), body mass index (BMI), gamma-glutamyltransferase (GGT), and serum triglyceride (TG) [6].

A biopsy of the liver is the gold standard in diagnosing NAFLD, especially in NASH. However, other methods are available as a less invasive option, such as imaging. Ultrasonography (US) is one tool that is generally used for steatosis screening, despite its lack of sensitivity [7]. It is a cost-effective and well-established as an imaging technique in diagnosing LS. Its downside is an operator-dependent diagnosis tool, rather qualitative than quantitative, and lacking accuracy in detecting mild hepatic steatosis [8].

In the early stage, NAFLD presents with asymptomatic LS which can progress further to NASH. However, in many countries, there is no regular screening program for detecting asymptomatic LS. Identification of early and asymptomatic LS is critical because of its progression to NASH, advanced fibrosis, and cirrhosis can be prevented. Simple body fat parameters and biochemical measurements could be used to predict asymptomatic LS, while the progression of asymptomatic LS to the late stage of NAFLD, NASH, advanced fibrosis, and cirrhosis can be achieved through managing lifestyle, body weight, diet, and exercise.

Material and Methods

This was a cross-sectional study involving 132 young, obese (aged 18-25 years, BMI > 25 kg/m^2) women in Bali, Indonesia. Data were collected from April to September 2013. A written informed consent was obtained before commencing the study, and the participants' anonymity was maintained throughout. The study protocol was approved by the Committee of Ethical Research of Udayana University/Sanglah General Hospital.

Anthropometric and biochemical variables were assessed. All anthropometric variables were measured twice, and for the final analyses mean values were used. Body weight (BW) was measured by a digital scale (Omron HBF-362, Japan) and presented in kilogram (kg). Body height (BH) was measured using a stature meter (General Care 26SM) and presented in centimetre (cm). WC was measured using a non-elastic, flexible tape at the middle level of the abdomen. Both TG and GGT were analysed by a colourimetric method (Cobas 6000, Roche Diagnostics, Germany). LAP for women was calculated as [WC (cm) −(5,9,10,11) Finally, FLI was calculated as (e0.953* loge(TG) +0.139*(BMI) +0.718*loge(GGT) +0.053*WC -15.745)/ (1- e0.953* loge(TG) +0.139*(BMI) +0.718*loge(GGT) +0.053*(WC) -15.745) * 100. No adjustment of any kind was made in this study regarding any calculations of the anthropometry [5] [6] [9] [10] [11].

Liver steatosis (LS) was assessed using ultrasonography (US) imaging (Logiq 500, GE, Solingen, Germany), setting at 3-5 MH curve linear frequencies. The imaging was focused on the right subcostal-longitudinal and transversal axis line of the subject. The results were interpreted independently by three radiologists, and the final interpretation was concluded by the majority decision.

LS criteria were described as: (1) normal liver, absence of steatosis and other liver disorder, (2) mild steatosis, marked by the appearance of liver parenchymal or hepatorenal echo contrast, a little bit brighter without disorder of intrahepatic vascular, (3) moderate steatosis, marked by liver parenchymal or hepatorenal appearance brighter in most area without intrahepatic vascular disorder, and (4) severe steatosis, marked by a diffuse and brighter liver appearance with blunting intrahepatic vascular [12] [13].

Statistical analyses were performed using Stata 12.1 (Stata Corp, College Station, TX, USA). The normal distribution of continuous data is presented as a mean ± standard error of the mean (SE). We employed a Pearson correlation test for continues data to assess the relationship between anthropometrical, biochemical, and LS, followed by multiple linear regression. One way ANOVA test was used to compare mean values of risk variables by the stage of LS. For ordinal variables, we employed non-parametric analyses which include calculation of odds ratio (OR), specificity, and sensitivity value to predict liver steatosis. Statistical significance was set at $p < 0.05$ (95% CI).

Results

This study enrolled 132 obese young women. Most of them were Balinese (94.7%), and a university student (90.9%). Table 1 depicts the demographic characteristics and descriptive data of the study participants. Average of BMI and WC were 30.4 ± 0.43 kg/m^2 and 90.4 ± 1.06 cm respectively. Most subjects (87.1%) had abdominal obesity (WC ≥ 80 cm). Mean TG and GGT serum concentrations were 104.6 ± 4.31 mg/dL and 24.4 ± 1.47 µg/L respectively, which were above the normal level. LS was found in 30.3% subjects, varied from mild (13.6%), moderate (6.8%), and severe (9.8%).

Table 1: Characteristics description of subjects (n = 132)

Parameters	Mean ± SE	N (%)
Age (year)	20.7 ± 0.20	
Height (cm)	158.2 ± 0.74	
Weight (kg)	76.7 ± 2.00	
BMI (kg/m^2)	30.4 ± 0.43	
25.0-29.9		70 (53.0)
30.0-39.9		54 (40.9)
≥ 40		8 (6.1)
WC (cm)	90.4 ± 1.06	
< 80		17 (12.9)
≥ 80		115 (87.1)
TG (mg/dL)	104.6 ± 4.31	
GGT (µg/L)	24.4 ± 1.47	
Liver Steatosis		40 (30.3)
Mild		18 (13.6)
Moderate		9 (6.8)
Severe		13 (9.8)

Presented in mean ± SE (standard error of mean) for continous data; N(%) for catagorical data. BMI = body mass index, WC = waist circumference; TG = trygliceride, GGT = gamma-glutamyltransferase.

Table 2 shows the association between all anthropometric variables, biochemical parameters, body fatness, and LS status. We found a strong positive correlation (p-value < 0.001) between anthropometric variables, biochemical parameters (TG, GGT), body fatness, and LS status.

Table 2: Correlation matrix of body mass index (BMI), waist circumference (WC), Waist to Height Ratio (WHtR), Lipid accumulation product (LAP), fatty liver index (FLI), plasma Triglyceride (TG), plasma gamma-GT (GGT) and liver steatosis (LS)

Parameters	BMI	WC	CI	WHtR	TG	GGT	LAP	FLI	LS
BMI	1								
WC	0.867*	1							
CI	0.462*	0.826*	1						
WHtR	0.854*	0.960*	0.823*	1					
TG	0.460*	0.467*	0.361*	0.494*	1				
GGT	0.470*	0.489*	0.319*	0.466*	0.379*	1			
LAP	0.746*	0.797*	0.624*	0.804*	0.861*	0.494*	1		
FLI	0.860*	0.787*	0.446*	0.756*	0.465*	0.529*	0.776*	1	
LS	0.588*	0.588*	0.417*	0.607*	0.449*	0.494*	0.592*	0.567*	1

Presented in Pearson's correlation coefficient. *p < 0.001. LS 1 = none, 2 = mild, 3 = moderate, 4 = severe.

Our multiple linear regression suggested that BMI, GGT, and TG could predict 41.4% of liver steatosis, as shown in Table 3. We found a weak association between WC and LS in comparison to BMI, TG, and GGT. Therefore we removed the WC variable from the final model.

Table 3: Regression of body mass index (BMI), plasma gamma-glutamylTransferase (GGT), and triglyceride (TG) to liver steatosis

Dependent	Independents	B	SE	Beta	*p*	R square
	(Constant)	-1.555	0.438		0.001	
Liver	BMI	0.080	0.017	0.395	< 0.001	0.414
Steatosis	GGT	0.014	0.005	0.242	0.003	
	TG	0.003	0.002	0.171	0.031	

The variation of LS value determined by the model 41.4%.

Analysed using linear regression, Independent variable enter; BMI, WC, TG and GGT.

Table 4 depicts the relationship between predictor variables and LS status. We found a significant association between all predictor variables and LS status. We found a consistent increase of association based on the stages of LS.

Table 4: Relationship of some determinants parameter to Liver Steatosis

Determinant	Liver Steatosis				
	None (92)	Mild (18)	Moderate (9)	Severe (13)	*p*
Body Mass Index (kg/m^2)	28.7 ± 0.33*†‡	32.0 ± 0.85*§	32.4 ± 1.22†‖	38.2 ± 2.28‡§‖	*0.002 †0.009 ‡< 0.001 §< 0.001 ‖0.001
Waist Circumference (cm)	86.4 ± 0.88*†‡	94.3 ± 2.39*§	95.0 ± 3.71†‖	110 ± 4.58‡§‖	*0.002 †0.014 ‡< 0.001 §< 0.001 ‖< 0.001
Conicity Index	1.18 ± 0.008*†‡	1.22 ± 0.020*§	1.23 ± 0.034†	1.29 ± 0.022‡§	*0.054 †0.057 ‡< 0.001 §0.013
Waist to Height Ratio	0.545 ± 0.005*†‡	0.594 ± 0.012*§	0.606 ± 0.025†‖	0.686 ± 0.026‡§‖	*0.001 †0.003 ‡< 0.001 §< 0.001 ‖< 0.001
Triglyceride (mg/dL)	89.7 ± 3.54*†‡	134 ± 12.3*	122 ± 21.2†	157 ± 20.0‡	*< 0.001 †0.037 ‡< 0.001
Gamma-Glutamyltransferase (µg/L)	19.5 ± 1.26*‡	30.8 ± 3.76*§	25.1 ± 4.80‖	48.7 ± 6.79‡§‖	*0.003 ‡< 0.001 §0.001 ‖< 0.001
Lipid Accumulation Product	29.6 ± 1.81*†‡	55.2 ± 5.81*§	53.1 ± 12.8†‖	98.3 ± 17.9‡§‖	*0.001 †0.017 ‡< 0.001 §< 0.001 ‖< 0.001
Fatty Liver Index	1.88 ± 0.38‡	5.63 ± 1.51§	6.13 ± 3.08‖	30.5 ± 8.65‡§‖	‡< 0.001 §< 0.001 ‖< 0.001

Presented in mean ± standard error of the mean (SE). Analysed using one-way ANOVA. The significance level (*p*-value) of; none vs mild steatosis*, none vs moderate steatosis†, none vs severe staetosis‡, mild vs severe steatosis§, and moderate vs severe steatosis‖.

Simple anthropometric parameters (BMI, WC, CI, and WHtR), biochemical indexes (TG, GGT), and complex body fatness indicators could predict liver steatosis significantly (Table 5).

Table 5: Specificity, Sensitivity and the OR to predict Liver Steatosis (US) of somebody fatness parameters

Parameters (cut off)	Specificity (None = 92) F (%)	Sensitivity (Steatosis = 40) F (%)	OR (95% CI)	*p*
BMI (≥ 30 kg/m^2)	64 (69.6)	29 (74.4)	6.21 (2.66-14.5)	< 0.001
WC (≥ 90 cm)	62 (67.4)	28 (70.0)	4.82 (2.16-10.8)	< 0.001
CI (≥ 1.2)	59 (64.1)	29 (74.4)	4.53 (1.99-10.3)	< 0.001
WHtR (≥ 0.55)	58 (63.0)	31 (77.5)	6.34 (2.59-15.5)	< 0.001
TG (≥ 100 mg/dL)	65 (70.6)	28 (70.0)	5.62 (2.50-12.6)	< 0.001
GGT (≥ 20 µg/L)	64 (69.6)	31 (77.5)	7.87 (3.31-18.7)	< 0.001
LAP (≥ 30)	76 (82.6)	28 (70.0)	11.1 (4.67-26.3)	< 0.001
FLI (≥ 2.5)	73 (79.3)	29 (74.4)	10.1 (4.29-23.9)	< 0.001

BMI = body mass index, WC = waist circumference, CI = conicity index, WHtR = waist to height ratio, TG = triglyceride, GGT = gamma glutamyltransferase, LAP = lipid accumulation product, FLI = fatty liver index, OR = odd ratio.

The validity indicators (specificity, sensitivity and the odds ratio (95%CI)) of body fat parameters which could predict LS were as follows: BMI ≥ 30 kg/m^2 (69.6%, 74.4% and 6.21(2.66-14.5)), WC ≥ 90 cm (67.4%, 70.0% and 4.28(2.16-10.8)), CI ≥1.2 (64.1%, 74.4%, and 4.53(1.99-10.3)), TG ≥ 100 mg/dL (70.6%, 70.0% and 5.62(2.50-12.6)), GGT ≥ 20 µg/L (69.6%, 77.5% and 7.87(3.31-18.7)), LAP ≥ 30 (82.6%, 70.0% and 11.1(4.67-26.3)), and FLI ≥ 2.5 (79.3%, 72.5% and 10.1(4.29-23.9)).

Discussion

NAFLD has emerged into a serious problem worldwide. LS is an early stage of NAFLD that naturally will progress to NASH (47%), of which as many as 25-50% will eventually progress to cirrhosis or fibrosis. Following the next ten years, as many as 7% will progress to hepatocellular carcinoma, contribute to 20% of liver-related death, and 50% will require a liver transplant [2]. It is important for healthcare providers to understand and identify this entity at an earlier stage and deliver the appropriate treatment.

The objective of treating NAFLD is to prevent fibrosis and improve steatosis. Current treatment relies on treating existing related entities such as obesity and insulin resistance. Weight loss, achieved through the lifestyle and behavioural interventions such as diet and exercise, is still the main strategy to prevent the progression of NAFLD [14]. Our previously randomised clinical trial [15] [16] found that three months restriction energy intake with a supplement of low n6:n3 (2:1) polyunsaturated fatty acid (PUFA) ratio, and weekly moderate exercise, decreased body fat parameters, improved cytokines levels, controlled fasting blood glucose, and reduced LS.

Body fatness, particularly visceral fat, is strongly associated with NAFLD. Oshakbayev et al., [17] reported NAFLD patients were found to have increased visceral fat rating, increased metabolic age by 9.6 years, and a basal metabolic rate of 209 kcal/day.

FLI, LAP, and VAI are body fatness indexes which can be used to predict cardiovascular, metabolic accurately, and liver-related diseases [6] [9] [10]. Both LAP and FLI can be utilised to recognise patients with hepatic steatosis [18]. FLI is a non-invasive diagnostic tool that is known for its strong agreement to SteatoTest and moderate agreement to abdominal ultrasound or hepatorenal imaging [19]. Du et al., [20] reported that VAI and LAP are sensitive in recognising the metabolic obese-normal weight (MONW) phenotype among Chinese adults.

Despite FLI's ability in recognising NAFLD, a simpler visceral fat assessment like waist circumference was found to have a similar performance [21]. Rinella et al., [22] investigated that there was a proven correlation between the overall grade of steatosis and BMI in liver donors. A population-based study by Stranges et al., [23] found that abdominal height was related to GGT and ALT levels, with women showed a stronger correlation.

Our study aims to identify predictors of NAFLD instead of defining the diagnosis of NAFLD. Understanding these predictors can guide the management of NAFLD especially in modifying risk factors associated with the development of NAFLD to NASH. Until recently, there is no definitive and specific pharmacologic regiment available for treating NAFLD. Simple anthropometric and biochemical predictors such as BMI, WC, TG, and GGT are readily available at all level of medical care services, especially at primary care. These predictors can be modified through behavioural interventions to prevent the progression of NAFLD to advanced NASH. Also, managing these predictors is also beneficial to reduce the risk for cardiovascular and metabolic-related diseases effectively.

In conclusion, there is a significant relationship between liver steatosis with all body fat parameters. These phenomena indicate that the simple single anthropometric (BMI, WC) and also the biochemical (TG, GGT) parameters are appropriate as hallmarks for predicting liver steatosis as well as the complex fatness parameters (such as FLI and LAP) among the obese Balinese young women.

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References

1. Schwenger KJ, Allard JP. Clinical approaches to non-alcoholic fatty liver disease. World Journal of Gastroenterology. 2014; 20(7):1712-1723. https://doi.org/10.3748/wjg.v20.i7.1712 PMid:24587650 PMCid:PMC3930971

2. More JB. Non-alcoholic fatty liver disease: the hepatic consequence of obesity and metabolic syndrome. Proc Nutr Soc. 2010; 69(2):211-20. https://doi.org/10.1017/S0029665110000030 PMid:20158939

3. Wong VW-S. Nonalcoholic fatty liver disease in Asia: A story of growth. Journal of Gastroenterology and Hepatology. 2012; 28(1):18-23. https://doi.org/10.1111/jgh.12011 PMid:23094755

4. Marengo A, Jouness RIK, Bugianesi E. Progression and Natural History of Nonalcoholic Fatty Liver Disease in Adults. Clinics in Liver Disease. 2016; 20(2):313-324. https://doi.org/10.1016/j.cld.2015.10.010 PMid:27063271

5. Roriz AKC, Passos LCS, de Oliveira CC, Eickemberg M, Moreira P de A, Sampaio LR. Evaluation of the Accuracy of Anthropometric Clinical Indicators of Visceral Fat in Adults and Elderly. PLoS ONE. 2014; 9(7):e103499. https://doi.org/10.1371/journal.pone.0103499 PMid:25078454 PMCid:PMC4117503

6. Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterology. 2006; 6(1). https://doi.org/10.1186/1471-230X-6-33 PMid:17081293 PMCid:PMC1636651

7. Castera L, Vilgrain V, Angulo P. Noninvasive Evaluation of NAFLD. Nature Reviews Gastroenterology & Hepatology. 2013; 10(11): 666–75. https://doi.org/10.1038/nrgastro.2013.175 PMid:24061203

8. Lee SS, Park SH. Radiologic evaluation of nonalcoholic fatty liver disease. World Journal of Gastroenterology. 2014; 20(23):7392-7402. https://doi.org/10.3748/wjg.v20.i23.7392 PMid:24966609 PMCid:PMC4064084

9. Bedogni G, Kahn HS, Bellentani S, Tiribelli C. A simple index of lipid overaccumulation is a good marker of liver steatosis. BMC Gastroenterology. 2010; 10(98). https://doi.org/10.1186/1471-230X-10-98

10. Chiang J-K, Koo M. Lipid accumulation product: a simple and accurate index for predicting metabolic syndrome in Taiwanese people aged 50 and over. BMC Cardiovascular Disorders. 2012; 12(1). https://doi.org/10.1186/1471-2261-12-78 PMid:23006530 PMCid:PMC3506496

11. Kahn HS. The "lipid accumulation product" performs better than the body mass index for recognizing cardiovascular risk: a population-based comparison. BMC Cardiovascular Disorders. 2005; 5:26. https://doi.org/10.1186/1471-2261-5-26 PMid:16150143 PMCid:PMC1236917

12. Hamaguchi M, Kojima T, Itoh Y, et al. The Severity of Ultrasonographic Findings in Nonalcoholic Fatty Liver Disease Reflects the Metabolic Syndrome and Visceral Fat Accumulation. The American Journal of Gastroenterology. 2007; 102(12):2708-2715. https://doi.org/10.1111/j.1572-0241.2007.01526.x PMid:17894848

13. Festi D, Schiumerini R, Marzi L, et al. Review article: the diagnosis of non-alcoholic fatty liver disease - availability and accuracy of noninvasive methods. Aliment Pharmacol Ther. 2013; 37(4):392-400. https://doi.org/10.1111/apt.12186 PMid:23278163

14. Wree A, Broderick L, Canbay A, Hoffman HM, Feldstein AE.From NAFLD to NASH to Cirrhosis - new insights into disease mechanisms. Nat RevGastroenterolHepatol. 2013; 10(11):627-636. https://doi.org/10.1038/nrgastro.2013.149

15. Weta IW, Mahadewa TGB, Sutirtayasa WP, Subawa AAN, Malik SG, Widyadharma IPE. Supplementation With 2:1 Ratio Of N-6:n-3 Polyunsaturated Fatty Acid Improves Liver Steatosis And Serum Cytokine Levels In Young Obese Balinese Women: A Randomized Clinical Trial. Asian Journal of Pharmaceutical and Clinical Research. 2017; 10(12):74. https://doi.org/10.22159/ajpcr.2017.v10i12.20851

16. Weta IW, Sutirtayasa WP, Subawa AAN, Malik SG. Supplementation 2000mg and 1000mg of linoleic acid and alfa linolenic acid delayed pre diabetic state in Balinese young obese women: A Randomised Clinical Trial. Bali Medical Journal. 2017; 6(3):55. https://doi.org/10.15562/bmj.v6i3.721

17. Oshakbayev K, Nersesov A, Izatullayev E, Kaybullayeva J, Nugmanova M, Ilyassova B. Correlation between body fat mass and nonalcoholic fatty liver disease. Medical and Health Science Journal. 2011; 6:60-67. https://doi.org/10.15208/mhsj.2010.109

18. Cuthbertson DJ, Weickert MO, Lythgoe D, et al. External validation of the fatty liver index and lipid accumulation product indices, using 1H-magnetic resonance spectroscopy, to identify hepatic steatosis in healthy controls and obese, insulin-resistant individuals. European Journal of Endocrinology. 2014; 171(5):561-569. https://doi.org/10.1530/EJE-14-0112 PMid:25298375

19. Zelber-Sagi S, Webb M, Assy N, et al. Comparison of fatty liver index with noninvasive methods for steatosis detection and quantification. World Journal of Gastroenterology. 2013; 19(1):57-64. https://doi.org/10.3748/wjg.v19.i1.57 PMid:23326163 PMCid:PMC3542754

20. Du T, Yu X, Zhang J, Sun X. Lipid accumulation product and visceral adiposity index are effective markers for identifying the metabolically obese normal-weight phenotype. Acta Diabetologica. 2015; 52(5):855-863. https://doi.org/10.1007/s00592-015-0715-2 PMid:25690647

21. Motamed N, Sohrabi M, Ajdarkosh H, et al. Fatty liver index vs waist circumference for predicting non-alcoholic fatty liver disease. World Journal of Gastroenterology. 2016; 22(10):3023-3030. https://doi.org/10.3748/wjg.v22.i10.3023 PMid:26973398 PMCid:PMC4779925

22. Rinella ME, Alonso E, Rao S, et al. Body mass index as a predictor of hepatic steatosis in living liver donors. Liver Transpl. 2001; 7(5): 409-414. https://doi.org/10.1053/jlts.2001.23787 PMid:11349260

23. Stranges S, Dorn JM, Muti P, et al. Body fat distribution, relative weight, and liver enzyme levels: apopulation-based study. Hepatology. 2004; 39(3):754-763. https://doi.org/10.1002/hep.20149 PMid:14999694

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Clinical Science

Association between Increased Matrix Metalloproteinase-9 (MMP-9) Levels with Hyperglycaemia Incidence in Acute Ischemic Stroke Patients

Ismail Setyopranoto[1*], Rusdy Ghazali Malueka[1], Andre Stefanus Panggabean[1], I Putu Eka Widyadharma[2], Ahmad Hamim Sadewa[3], Rusdi Lamsudin[1], Samekto Wibowo[1]

[1]Department of Neurology, Faculty of Medicine, Universitas Gadjah Mada and Dr Sardjito General Hospital, Yogyakarta, Indonesia; [2]Department of Neurology, Faculty of Medicine, Udayana University and Sanglah General Hospital, Bali, Indonesia; [3]Department of Biochemistry, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

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***Correspondence:** Ismail Setyopranoto. Department of Neurology, Faculty of Medicine, Universitas Gadjah Mada and Dr Sardjito General Hospital, Yogyakarta, Indonesia. E-mail: ismail.setyopranoto@ugm.ac.id

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Abstract

BACKGROUND: Hyperglycemia is common in acute stroke patients. Hyperglycemia can induce the production of reactive oxygen species, causing increased activity of matrix metalloproteinase-9 (MMP-9).

AIM: This study aimed to determine an association between the increased levels of MMP-9 and the incidence of hyperglycemia in acute ischemic stroke patients.

METHODS: This is a case-control study. Acute ischemic stroke patients admitted to the Stroke Unit of a reference hospital in Yogyakarta, Indonesia was divided into the hyperglycemic and non-hyperglycemic group. Demographic and clinical characteristics of each subject were recorded, and blood levels of MMP-9 were measured. Seventy-one patients were recruited, 40 subjects in the hyperglycemic group and 31 subjects in the non-hyperglycemic group.

RESULTS: The median levels of blood MMP-9 level in the hyperglycemic and non-hyperglycemic group were 974.37 and 748.48 ng/mL, respectively, and the difference was statistically not significant (95% CI, 191.24-2849.53; p = 0.07). When the calculated cut-off point of 600.99 ng/mL was used, the proportion of patients with higher MMP-9 levels was significantly more in the hyperglycemic group compared with the ones in the non-hyperglycemic group (82.5% and 54.8%, respectively; OR = 3.88; p = 0.011).

CONCLUSION: We concluded that the proportion of patients with MMP-9 level >600.99 ng/mL was significantly higher in acute ischemic stroke patients with hyperglycemia.

Introduction

Hyperglycemia, as defined by fasting blood glucose over 126 mg/dL (7.0 mmol/L) is common in acute ischemic stroke patients with the incidence rate of approximately 60% [1] [2]. Hyperglycemia in patients with acute stroke can be caused by some of the underlying mechanisms; one of which is the activation of the hypothalamic-pituitary-adrenal axis due to a direct impact of the brain ischemia [3]. This hyperglycemia condition is associated with cytotoxic injury of the brain and increased mortality and poor recovery for the patients [4].

The hyperglycemia may induce the production of reactive oxygen species (ROS), which resulted in the increased activity of matrix metalloproteinase-9 (MMP-9) [5]. Glucose intake is also known to increase levels of pro-inflammatory transcription factors, such as activator protein-1 (AP-1) and the early growth response-1 (Egr-1). AP-1 regulates the transcription of

MMP. Hence the expression of MMP-2 and MMP-9 are increased and rapidly regulated in stroke pathogenesis [6] [7]. It positively correlates with the severity of the stroke, and it increases the permeability of the blood-brain barrier [8] [9] [10]. Polymorphism in MMP-9 (e.g. MMP9 rs3918242) may induce the development of ischemic stroke and is found more frequent in diabetic type 2 patients, indicating the role of the MMP protein in pathological mechanism of stroke [11] [12].

There is evidence that shows the detrimental role of MMP-9, i.e., enlarging the region of brain injury following the focal ischemia. A recent study demonstrated that MMP-9 can directly trigger anoikis, e.g., the death of neurons due to the occurrence of signal interference between cells and their matrix [13]. Inhibition of MMP-9 either by minocycline or gene silencing in ischemic models shows a protective effect as indicated by decreased infarct size, indicating the involvement of MMP-9 in ischemic stroke pathology [14].

The purpose of this study was to determine the relationship between levels of MMP-9 with the incidence of hyperglycemia in patients with acute ischemic stroke.

Material and Methods

This was a case-control study approved by the Ethical Committee of the Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia. Blood samples were consecutively withdrawn from acute ischemic stroke patients who were treated during the first attack at the Stroke Unit in Dr Sardjito General Hospital, Yogyakarta, Indonesia. The inclusion criteria were age 50-70 years, and the maximum onset of stroke was 6 days evidenced by the presence of cerebral infarction in the head CT scan. Subjects were divided into 2 groups, i.e., the hyperglycemic group (fasting plasma blood glucose levels > 126 mg/dL) including patients with a history of diabetes mellitus before the stroke and the non-hyperglycemic group (fasting plasma blood glucose < 126 mg/dL). This study selected subjects with age over 50 years old because a previous study demonstrated that the relationship between MMP-9 with certain complications was also influenced by the age factor. Indeed, it was reported that MMP-9 genotypes were associated with extensive lesions of cardiac infarction in patients aged 53 years and over [15]. The exclusion criteria were patients with bleeding transformation or space-occupying lesion by other causes and patients who did not complete the study procedure.

The analyses of multiple comorbidities on stroke were performed on the recruited subjects, including their demographics, risk factors, blood pressure, blood chemistry, and clinical outcomes based on the Gadjah Mada Stroke Scale (GMSS). GMSS is a modified version of the National Institute of Health Stroke Scale (NIHSS). GMSS is designed to serve as a clinical measurement tool to evaluate and to monitor the neurologic status of stroke patients. The GMSS has been tested for its validity and reliability, in which it yields a Kappa coefficient between 0.85-1. The GMSS threshold is set at 23: score more than 23 indicates mild to moderate neurologic deficits, while the one less than 23 refers to severe neurologic deficits [16]. Plasma blood glucose levels were examined within the first 24 hours of care after fasting for at least 12 hours. The MMP-9 level was measured with the technique of quantitative sandwich enzyme-linked immunosorbent assay (ELISA) using a special kit (Bio-Rad Laboratories, Inc., Hercules, CA 94547, USA). A comparative analysis of MMP-9 levels was conducted between acute ischemic stroke subjects in the hyperglycemic and the non-hyperglycemic groups. To determine the cut-off point of MMP-9 levels, the receiver operating characteristic (ROC) curve was created. Sensitivity and specificity are two components used to measure the validity of a diagnostic test compared to the gold standard. ROC curve is a graph of the sensitivity (Y-axis) with 1-specificity (X-axis), aims to determine the cutoff point of the diagnostic test that is continuous or ordinal scale, and the ROC is an effective method for assessing the performance of a diagnostic test [17]. Spearman correlation analysis was used to analyse the correlation between MMP-9 level and stroke outcome. The Chi-square, t-test or Mann-Whitney analyses were performed for basic demographic characteristics with a chosen significance level of 0.05 or 95% confidence level ($p < 0.05$) by using the statistical program.

Results

Seventy-one patients with acute ischemic stroke were recruited, consisting of 40 hyperglycemic and 31 non-hyperglycemic subjects. The baseline characteristics between the two groups are shown in Table 1. The mean age of both groups was 60 and 58 years, in which the majority of subjects were males with a history of hypertension. As expected, the incidence of diabetes mellitus was higher in the group of hyperglycemia (32.5% vs 6.5%; $p = 0.011$). The systolic blood pressure was higher in hyperglycemia group compared to non-hyperglycemia group (155.90 ± 25.72 mmHg vs 143.13 ± 22.12 mmHg respectively;

p = 0.031). Other variables did not show a significant difference between the two groups.

Table 1: Baseline characteristics of the subjects

Variables	Hyperglycemia (n = 40)	Non-hyperglycemia (n=31)	Total	t/χ^2	p
Demography					
Age (years)	60.55 ± 8.64	57.90 ± 12.56	59.39 ± 10.53	1.051	0.297
Male (n; %)	22 (55.0%)	19 (61.3%)	41 (57.7%)	0.283	0.595
Female (n; %)	18 (45.0%)	12 (38.7%)	30 (42.3%)		
Therapeutic windows (hour)	28.13 ± 26.23	25.32 ± 27.02	26.90 ± 26.42	0.441	0.661
Risk Factor of stroke					
Hypertension (n; %)	29 (72.5%)	18 (58.1%)	47 (66.2%)	5.697	0.058
Diabetes Mellitus (n; %)	13 (32.5%)	2 (6.5%)	15 (21.1%)	8.970	0.011
Cardiac disease (n; %)	3 (7.5%)	3 (9.7%)	6 (8.5%)	0.367	0.832
Hypercholesterolemia (n; %)	9 (22.5%)	4 (12.9%)	13 (18.3%)	1.101	0.577
Smoking cigarette (n; %)	15(37.5%)	8 (25.8%)	23 (32.4%)	1.090	0.296
Blood pressure					
Systolic (mmHg)	155.90 ± 25.72	143.13 ± 22.12	150.32 ± 24.88	2.204	0.031
Diastolic (mmHg)	89.00 ± 13.05	85.52 ± 12.58	87.48 ± 12.87	1.133	0.261
Blood chemistry					
BUN (mg/dL)	18.77 ± 12.72	17.27 ± 11.15	18.11 ± 12.00	0.520	0.605
Creatinine (mg/dL)	1.07 ± 0.56	1.21 ± 1.35	1.13 ± 0.98	-0.592	0.556
Total cholesterol (mmol/L)	204.78 ± 52.01	188.19 ± 44.86	197.54 ± 49.38	1.413	0.162
LDL (mmol/L)	130.56 ± 47.34	117.45 ± 39.55	124.84 ± 44.29	1.242	0.218
Albumin (g/dL)	3.10 ± 0.63	3.20 ± 0.60	3.14 ± 0.62	-0.613	0.542
Stroke outcome					
Gadjah Mada Stroke Scale (GMSS)	25.25 ± 8.93	28.52 ± 8.14	26.68 ± 8.69	-1.588	0.117

BUN: blood urea nitrogen, LDL: low-density lipoprotein.

Figure 1 illustrates the distribution of MMP-9 levels between the two groups. The median levels of MMP-9 in the hyperglycemic group were higher than the ones in the non-hyperglycemic group (974.37 and 748.48 pg/mL, respectively). No outlier data were observed.

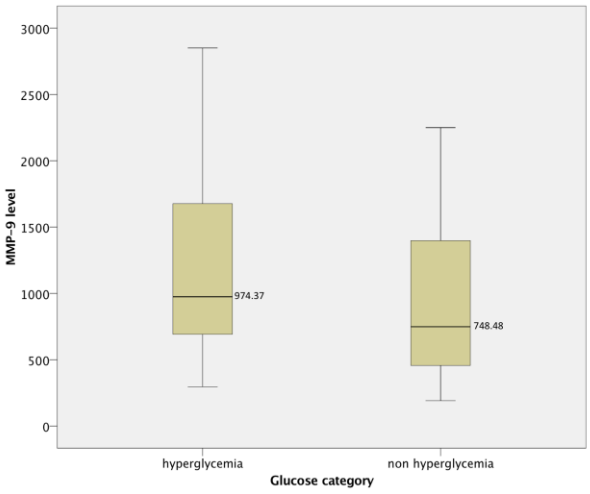

Figure 1: Boxplot distribution of MMP-9 levels in the hyperglycemic and non-hyperglycemic groups. The bottom and top of the box showed the first and third quartiles, while whisker shows the range between + 1.5 Inter Quartile Range (IQR) and the first quartile -1.5 IQR. The median was shown as a horizontal line in the box as well as a number beside the box

Subsequent analysis with the Mann-Whitney test indicated that the hyperglycemic group had a higher sum of the rank of MMP-9 levels than the ones in the non-hyperglycemic group (1596.5 and 959.5 ng/mL, respectively), although the difference was statistically not significant (95% CI, 191.24-2849.53; p = 0.07).

Next, by using the ROC curve, the cut-off point for MMP-9 was obtained, i.e., 600.99 ng/mL (Figure 2). Table 2 shows the association of the MMP-9 levels based on the cut-off point. The proportion of MMP-9 levels > 600.99 ng/mL in hyperglycemia group was 82.5%, higher than the ones in the non-hyperglycemia group (54.8%). This was significantly different based on the value of risk estimated by the calculated odds ratio (OR) = 3.88 (95% CI, 1.319-11.428; p = 0.011).

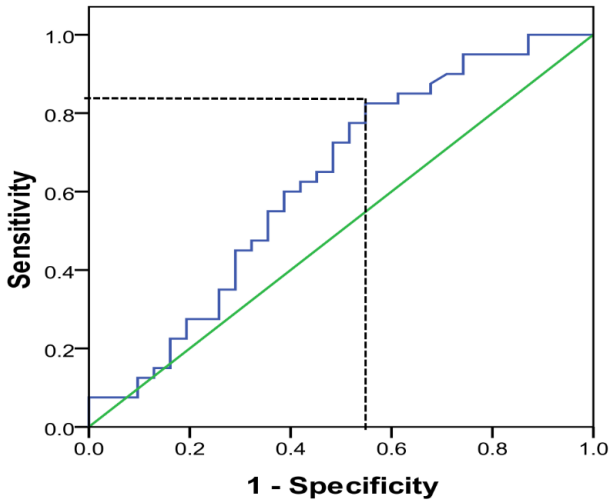

Figure 2: Receiver Operating Characteristic (ROC) curve to determine the cutoff point of the MMP-9 levels

Previous studies have shown adverse effects of MMP-9 levels on stroke outcomes [8]. However, no significant association between the MMP-9 levels with the stroke outcomes (as assessed with GMSS) was observed in this study (p = 0.214).

Table 2: Bivariate analysis of MMP-9 to the Hyperglycemic and Non-hyperglycemic Group

MMP-9 (ng/mL)	Hyperglycemia		Non-hyperglycemia		χ^2	p	OR	95%CI	
	n	%	n	%				Lower limit	Upper limit
≥600.99	33	82.5	17	54.8	6.416	0.011	3.88	1.319	11.428
<600.99	7	17.5	14	45.2					

The patients subsequently were divided into two groups based on the MMP-9 levels, i.e., one group above the cut-off point (≥ 600.99 ng/mL) and another one below the cut-off point. However, no significant difference between the two groups regarding clinical outcomes according to the GMSS was observed based on the Mann-Whitney analysis (p = 0.91).

Discussion

In our study, the MMP-9 levels ≥ 600.99 ng/mL were observed more frequently in the hyperglycemic group than in the non-hyperglycemic group. These results support previous studies which demonstrated that the incidence of hyperglycemia is associated with elevated MMP-9 levels [17].

Hyperglycemia is a common phenomenon among acute stroke patients, with the incidence rate of approximately 60% [2]. An increased blood glucose level is associated with various complications affecting the central nervous system. Hyperglycemia during an ischemic stroke has been associated with an increased risk of mortality and poor functional outcome [17] [18]. This can be due to the up-regulation of many neurotoxic mediators (e.g., MMP-9) released by cells that are stimulated by high blood glucose levels. Previous studies have shown that hyperglycemia will increase the incidence of oxidative stress and the activation of MMP-9, which subsequently exacerbating the dysfunction of the blood-brain barrier after ischemic injury / re-perfusion and cerebral oedema [19]. However, it is elusive yet of how high of blood glucose level to trigger an elevation in MMP-9 level and of how high the MMP-9 level could rise as a result of hyperglycemia [10] [21].

We proposed in this study that the cut-off point of the MMP-9 level was 600.99 ng/mL. MMP-9 levels above the cut-off point were more frequently encountered in the hyperglycemic group than in the non-hyperglycemic group. These results have some significant impacts. First, our study reconfirms findings from previous studies showing that hyperglycemia is associated with increased levels of MMP-9 [17]. This elevation of MMP-9 in hyperglycemia was also found in another condition such as severe sepsis, indicating that the process does not depend on the diabetic status of the patient before a stroke [22]. Second, this study showed a cut-off point for the elevation of MMP-9 levels due to hyperglycemia. This implies in clinical practice. Clinicians should be aware that when a hyperglycemic patient has an MMP-9 level above the cut-off point, oxidative stress has already occurred. This, in turn, will affect the outcome of stroke and other complications that may arise. In summary, we hypothesise that MMP-9 level ≥ 600.99 ng/mL can be a predictor for poor outcomes of ischemic stroke.

Several studies have demonstrated an association of elevated MMP-9 levels with poor outcomes in stroke patients. In addition to the exacerbation of blood-brain barrier dysfunction after injury ischemic injury/re-perfusion, MMP-9 is also known to be involved in the mechanism of the central depression within acute stroke patients, and it contributes to the destruction of brain cells, thus aggravating the incidence of brain oedema [19]. The central depression is characterised by the neuronal and glial depolarisation, followed by an increased expression of MMP-9 after 3-6 hours. There is also evidence that shows the detrimental role of MMP-9, which leads to the expansion of brain injury after focal ischemia [23]. The increased activity of MMP-9 is characterised by the simultaneous reduction in the extracellular matrix changing the adhesive contact of neuronal cells to the extracellular matrix and by events that underlie their contributions to neuronal degeneration in the ischemic penumbra. This lead to migration of neutrophils to brain parenchyma and stimulates another release of MMP-9 of resident brain cells (neurons and glial cells) which resulted in apoptosis of these cells [24]. A recent study demonstrated that MMP-9 could directly trigger anoikis, such as neuronal death due to the occurrence of signal interference between cells and the matrix [13]. Indeed, activation of MMP-9 is associated with the size of the brain infarct. Also, levels of pro-MMP-9 in plasma and levels of activated MMP-9 in the murine cerebral tissue increased after 24-hours of permanent occlusion of the middle cerebral artery. The increment of pro-MMP-9 levels in plasma at 24 hours was associated with the infarct size [25]. In human study, increased MMP-9 plasma level correlated with infarct volume increment at baseline (0-6th hour), 12, 24, and 48th hour after stroke and correlated with higher NIHSS scores at 12, 24 and 48th hour [26]. Another study showed increased MMP-9 plasma level on admission was positively correlated with NIHSS score after 1 month and 12% increased the risk for major disability, and 29% increased the risk for death in the 3-month interval after stroke [27] [28]. It is worth to mention that these 2 studies did not find any significant correlation between blood glucose and MMP-9 plasma level, showing that another mechanism besides hyperglycemia could affect the association between MMP-9 levels and stroke outcome [27] [28].

Researchers are attempting to confirm a link between increased levels of MMP-9 with the poor outcomes of ischemic stroke. However, we did not observe any significant correlation between MMP-9 levels and the stroke outcomes as assessed with the GMSS ($p = 0.214$). An analysis using the Mann-Whitney test to see any significant difference in clinical outcomes between patients with MMP-9 levels above and below the cut-off point also showed no significant difference. This is in contrast to results of other studies that have been previously described. The difference could be partly explained by one of the limitations of this study, i.e., no stratification of patients based on their medical history of diabetes mellitus. Previous studies have shown that an up-regulation of MMP-9 activity contributes to the exacerbation of vascular damage in diabetic patients, but not in patients with acute hyperglycemia [29]. This vascular damage disrupted the blood-brain barrier and led to an unfavourable outcome in diabetic patients [30].

We acknowledge several limitations of this study: (1) no stratification of patients based on their medical history of diabetes mellitus; (2) no stratification of patients by the subtype of ischemic stroke, such as atherosclerosis in large arteries or cardiac embolism; (3) no distinction of size and location of the infarct's lesion and the involvement of cerebral arteries; and (4) no significant difference on comorbidity factors between the hyperglycemic and the non-hyperglycemic subjects was observed. Based on these limitations, we recommend conducting a more comprehensive study to improve these weaknesses.

We conclude that based on the calculated cut-off point (600.99 ng/mL), the proportion of higher MMP-9 levels were significantly more in the hyperglycemic than in the non-hyperglycemic group of acute ischemic stroke patients.

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References

1. A. D. Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2012; 35:S64-71. https://doi.org/10.2337/dc12-s064 PMid:22187472 PMCid:PMC3632174

2. Williams LS, Rotich J, Qi R, Fineberg N, Espay A, Bruno A, Fineberg SE, Tierney WR. Effects of admission hyperglycemia on mortality and costs in acute ischemic stroke. Neurol. 2002; 59:67-71. https://doi.org/10.1212/WNL.59.1.67

3. Garg R, Chaudhuri A, Munschauer F, Dandona P. Hyperglycemia, insulin, and acute ischemic stroke: a mechanistic justification for a trial of insulin infusion therapy. Stroke. 2006; 37:267-73. https://doi.org/10.1161/01.STR.0000195175.29487.30 PMid:16306459

4. Bevers MB, Vaishnav NH, Pham L, Battey TW, Kimberly WT. Hyperglycemia is associated with more severe cytotoxic injury after stroke. J Cereb Blood Flow Metab. 2016; 37:2577-83. https://doi.org/10.1177/0271678X16671730 PMid:27671250 PMCid:PMC5531353

5. Uemura S, Matsushita H, Li W, Glassford AJ, Asagami T, Lee KH, Harrison DG, Tsao PS. Diabetes mellitus enhances vascular matrix metalloproteinase activity: role of oxidative stress. Circ Res. 2001; 88:1291-8. https://doi.org/10.1161/hh1201.092042 PMid:11420306

6. Aljada A, Ghanim H, Mohanty P, Syed T, Bandyopadhyay A, Dandona P. Glucose intake induces an increase in activator protein 1 and early growth response 1 binding activities, in the expression of tissue factor and matrix metalloproteinase in mononuclear cells, and in plasma tissue factor and matrix metalloproteinase concentrations. Am J Clin Nutr. 2004; 80:51-7. https://doi.org/10.1093/ajcn/80.1.51 PMid:15213027

7. Zhao BQ, Tejima E, Lo EH. Neurovascular proteases in brain injury, hemorrhage and remodeling after stroke. Stroke. 2007; 38:748-52. https://doi.org/10.1161/01.STR.0000253500.32979.d1 PMid:17261731

8. Montaner J, Alvarez-Sabín J, Molina C, Anglés A, Abilleira S, Arenillas J, González MA, Monasterio J. Matrix metalloproteinase expression after human cardioembolic stroke: temporal profile and relation to neurological impairment. Stroke. 2001; 32:1759-66. https://doi.org/10.1161/01.STR.32.8.1759 PMid:11486102

9. Abdelnaseer M, Elfayomi N, Hassan E, Kamal M, Hamdy A, Elsawy E. Serum matrix metalloproteinase-9 in acute ischemic stroke and its relation to stroke severity. Egypt J Neurol Psychiatry Neurosurg. 2015; 52:274-8. https://doi.org/10.4103/1110-1083.170661

10. Barr TL, Latour LL, Lee KY, Schaewe TJ, Luby M, Chang GS, El-Zammar Z, Alam S, Hallenbeck JM, Kidwell CS, Warach S. Blood brain barrier disruption in humans is independently associated with increased matrix metalloproteinase-9. Stroke. 2010; 41:123-8. https://doi.org/10.1161/STROKEAHA.109.570515 PMid:20035078 PMCid:PMC2827673

11. Hao Y, Tian S, Sun M, Zhu Y, Nie Z, Yang, S. Association between matrix metalloproteinase gene polymorphisms and development of ischemic stroke. Int J Clin Exp Pathol. 2015; 11647-52. PMid:26617904 PMCid:PMC4637720

12. Buraczynska K, Kurzepa J, Ksiazek A, Buraczynska M, Rejdak K. Matrix metalloproteinase-9 (MMP-9) gene polymorphism in stroke patients. Neuromolecular Med. 2015; 17:385-90. https://doi.org/10.1007/s12017-015-8367-5 PMid:26330106 PMCid:PMC4643105

13. Gu Z, Kaul M, Yan B, Kridel SJ, Cui J, Strongin A, Smith JW, Liddington RC, Lipton SA. S-nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. Science. 2002; 297:1186-90. https://doi.org/10.1126/science.1073634 PMid:12183632

14. Chaturvedi M, Kaczmarek L. MMP-9 inhibition: a therapeutic strategy in ischemic stroke. Mol Neurobiol. 2014; 49:563-73. https://doi.org/10.1007/s12035-013-8538-z PMid:24026771 PMCid:PMC3918117

15. Pöllänen PJ, Karhunen PJ, Mikkelsson J, Laippala P, Perola M, Penttilä A, Mattila KM, Koivula T, Lehtimäki T. Coronary artery complicated lesion area is related to functional polymorphism of matrix metalloproteinase 9 gene: an autopsy study. Arterioscler Thromb Vasc Biol. 2001; 21:1446-1450. https://doi.org/10.1161/hq0901.095545 PMid:11557670

16. Lamsudin R. Reliability of Gadjah Mada Stroke Scale (GMSS) in stroke patients. In: PERDOSSI. Buku Abstrak Musyawarah Kerja dan Pertemuan Ilmiah Tahunan Perdossi, PERDOSSI: Malang, Indonesia, 1998.

17. Kamada H, Yu F, Nito C, Chan PH. Influence of hyperglycemia on oxidative stress and MMP-9 activation after focal cerebral ischemia/reperfusion in ats: relationship to blood-brain barrier dysfunction. Stroke. 2007; 38:1044-9. https://doi.org/10.1161/01.STR.0000258041.75739.cb PMid:17272778 PMCid:PMC1828129

18. Sapojnikova N, Kartvelishvili T, Asatiani N, Zinkevich V, Kalandadze I, Gugutsidze D, Shakarishvili R, Tsiskaridze A. Correlation between MMP-9 and extracellular cytokine HMGB1 in prediction of human ischemic stroke outcome. Biochim Biophys Acta. 2014; 1842:1379-84. https://doi.org/10.1016/j.bbadis.2014.04.031 PMid:24815357

19. Gursoy-Ozdemir Y, Qiu J, Matsuoka N, Bolay H, Bermpohl D, Jin H, Wang X, Rosenberg GA, Lo EH, Moskowitz MA. Cortical spreading depression activates and upregulates MMP-9. J Clin Invest. 2004; 113:1447-55. https://doi.org/10.1172/JCI200421227 PMid:15146242 PMCid:PMC406541

20. Hsieh HL, Chi PL, Lin CC, Yang CC, Yang CM. Up-regulation of ROS-dependent matrix metalloproteinase-9 from high-glucose-challenged astrocytes contributes to the neuronal apoptosis. Mol Neurobiol. 2014; 50:520-33. https://doi.org/10.1007/s12035-013-8628-y PMid:24395134

21. Tsai WC, Liang FC, Cheng JW, Lin LP, Chang SC, Chen HH, Pang JH. High glucose concentration up-regulates the expression of matrix metalloproteinase-9 and -13 in tendon cells. BMC Musculoskelet Disord. 2013; 14:255. https://doi.org/10.1186/1471-2474-14-255 PMid:23981230 PMCid:PMC3765930

22. Sachwani GR, Jaehne AK, Jayaprakash N, Kuzich M, Onkoba V, Blyden D, Rivers EP. The association between blood glucose levels and matrix-metalloproteinase-9 in early severe sepsis and septic shock. J Inflamm. 2016; 13:1-8. https://doi.org/10.1186/s12950-016-0122-7 PMid:27110221 PMCid:PMC4840979

23. Planas AM, Solé S, Justicia C. Expression and activation of matrix metalloproteinase-2 and -9 in rat brain after transient focal cerebral ischemia. Neurobiol Dis. 2001; 8:834-46. https://doi.org/10.1006/nbdi.2001.0435 PMid:11592852

24. Turner RJ, Sharp FR. Implications of MMP9 for blood brain barrier disruption and hemorrhagic transformation following ischemic stroke. Front Cell Neurosci. 2016; 10:1-13. https://doi.org/10.3389/fncel.2016.00056 PMid:26973468 PMCid:PMC4777722

25. Park KP, Rosell A, Foerch C, Xing C, Kim WJ, Lee S, Opdenakker G, Furie KL, Lo EH. Plasma and brain matrix metalloproteinase-9 after acute focal cerebral ischemia in rats. Stroke. 2009; 40:2836-42. https://doi.org/10.1161/STROKEAHA.109.554824 PMid:19556529 PMCid:PMC3712850

26. Demir R, Ulvi H, Ozel L, Özdemir G, Güzelcik M, Aygül R. Relationship between plasma metalloproteinase-9 levels and volume and severity of infarct in patients with acute ischemic stroke. Acta Neurol Belg. 2012; 112:351-6. https://doi.org/10.1007/s13760-012-0067-4 PMid:22581515

27. Abdelnaseer MM, Elfauomy NM, Esmail EH, Kamal MM, Elsawy EH. Matrix metalloproteinase-9 and recovery of acute ischemic stroke. J Stroke Cerebrovasc Dis. 2017; 26:733-40. https://doi.org/10.1016/j.jstrokecerebrovasdis.2016.09.043 PMid:28063771

28. Zhong C, Yang J, Xu T, Xu T, Peng Y, Wang A, Wang J, Peng H, Li Q, Ju Z, Geng D, Zhang Y, He J; CATIS Investigators. Serum matrix metalloproteinase-9 levels and prognosis of acute ischemic stroke. Neurol. 2017; 89:805-12. https://doi.org/10.1212/WNL.0000000000004257 PMid:28747453 PMCid:PMC5580861

29. Elgebaly MM, Ogbi S, Li W, Mezzetti EM, Prakash R, Johnson MH, Bruno A, Fagan SC, Ergul A. Neurovascular injury in acute hyperglycemia and diabetes: a comparative analysis in experimental stroke. Transl Stroke Res. 2011; 2:391-8. https://doi.org/10.1007/s12975-011-0083-3 PMid:21909340 PMCid:PMC3169178

30. Yu X, Xu X, Jackson A, Sun J, Huang P, Mao Y, Chen Z, Lou M, Jiang Q, Zhang M. Blood brain barrier disruption in diabetic stroke related to unfavorable outcome. Cerebrovasc Dis. 2016; 42:49-56. https://doi.org/10.1159/000444809 PMid:26986824

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Tumescent Local Infiltration Anesthesia for Mini Abdominoplasty with Liposuction

Ahmed Abdalla Mohamed[1], Tamer Fayez Safan[1], Hamed Fathy Hamed[1], Maged Abdelwahab Abdelaziz Elgendy[2*]

[1]Faculty of Medicine, Cairo University, Cairo, Egypt; [2]Department of Anesthesiology & ICU, Faculty of Medicine, Cairo University, Cairo, Egypt

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***Correspondence:** Maged Abdelwahab Abdelaziz Elgendy. Department of Anesthesiology & ICU, Faculty of Medicine, Cairo University, Cairo, Egypt. E-mail: magedabdelwahab@yahoo.com

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Abstract

AIM: To evaluate the feasibility and safety of mini abdominoplasty with liposuction under local tumescent anaesthesia (LA) as the sole anaesthetic modality.

METHODS: The study included 60 female patients with a mean age of 33.3 ± 5.6 years. Local infiltration using a mixture of 1:1000 epinephrine (1 ml), 2% lidocaine (100 ml) and 0.5% Levobupivacaine (50 ml) in 2500 ml saline was started with Local infiltration started with the abdomen, outer thigh, hips, back, inner thighs and knees. After Mini Abdominoplasty with supplemental liposuction was conducted and application of suction drains wound closure was performed, and the tight bandage was applied. Pain during injection, incision and surgical manipulations was determined. Duration of postoperative analgesia, till oral intake and return home, patients and surgeon satisfaction scores were determined.

RESULTS: All surgeries were conducted completely without conversion to general anaesthesia. Injection pain was mild in 46 patients, moderate in 10 and hardly tolerated in 4 patients. Incision pain was mild in 16 patients, while 44 patients reported no sensation. During the surgical procedure, 6 patients required an additional dose of LA. Meantime till resumption of oral intake was 1.6 ± 0.9 hours. Meantime till home return was 5.6 ± 2.4 hours. Twelve patients were highly satisfied, 18 patients were satisfied, and these 42 patients were willing to repeat the trial if required. Eight patients found the trial is good and only one patient refused to repeat the trial and was dissatisfied, for a mean total satisfaction score of 3.1 ± 0.9.

CONCLUSION: Mini Abdominoplasty with liposuction could be conducted safely under tumescent LA with mostly pain-free intraoperative and PO courses and allowed such surgical procedure to be managed as an office procedure. The applied anaesthetic procedure provided patients' satisfaction with varying degrees in about 97% of studied patients.

Introduction

Limited abdominoplasty is commonly performed under general anaesthesia with endotracheal intubation or manually controlled intravenous anaesthesia. Despite the surgeons' preference of performing the procedure on patients under general anaesthesia; patients have anxiety over complications due to general anaesthesia. On the other hand, manually controlled infusion of intravenous anaesthesia had disadvantages including inefficient control of anaesthesia [1].

There was the widespread application of local anaesthesia for multiple surgical procedures and was proved effective with advantages extending to the postoperative period; Agbakwuru et al., [2] reported that hydrocelectomy under local anaesthesia and sedation is practicable and was tolerated and accepted by the adult patients. Parirokh et al., [3] found combining an inferior alveolar nerve block, and a buccal infiltration injection provided more effective anaesthesia in mandibular molars with irreversible pulpitis Also, Gun et al., [4] found the application of LA reduces bleeding during rhinoplasty and pain control postoperatively. Chia & Theodorou [5] reported excellent safety profile and short recovery period using laser-assisted liposuction and suction-assisted lipectomy with the patient under local anaesthesia and

documented that the awake patient can participate in body positioning and to provide physiologic monitoring.

Through the development of instruments and the introduction of new drugs, various means of anaesthesia for breast surgery have been reported. More frequent use of a local anaesthetic combined with some form of intravenous sedation in office-based facilities has been reported. This change keeps hospital costs down as well as saving time for patients [6] [7].

The current study aimed to evaluate the feasibility and safety of performing reduction mastectomy under tumescent local infiltration anaesthesia as the sole anaesthetic modality.

Methods

The current prospective study was conducted at Anesthesia and General Surgery departments, Kasr Al-Eini University Hospital From Jan 2018 till June 2018. After approval of the study protocol and obtaining fully informed patients' written consents, patients assigned for Mini Abdominoplasty with liposuction were enrolled in the study.

Patients with hormonal disturbances had body mass index (BMI) > 35 kg/m^2 or required other plastic surgeries for contour configuration were excluded from the study. Also, patients had pathologies requiring surgical interference under any anaesthetic technique other than local anaesthesia was not enrolled in the study.

All patients underwent determination of body weight and height and calculation of BMI according to the equation: BMI = Wt (kg)/(Height in meter)^2 [8]. A BMI of < 24.9 kg/m^2 is considered normal; a person with a BMI of 25-30 kg/m^2 is considered overweight but at low risk of serious medical complications, while those with a BMI of > 30, > 35 and > 55 kg/m^2 are considered obese, morbidly obese and super-morbidly obese, respectively. Morbidity and mortality rise sharply when the BMI is > 30 kg/m^2 [9]. Then, all patients had full clinical examination including general and abdominal examinations and were photographed preoperatively in lateral and front positions. The planned incisions were marked with an irremovable marker pen.

The local anaesthetic solution was prepared using a mixture of 5 ml of 1:1000 of epinephrine, 100 ml of 2% lidocaine, 50 ml of 0.5% Levobupivacaine in a 2500 ml saline solution. All patients were premedicated with midazolam in a dose of 0.05 mg/kg, and ondansetron 4mg and dexamethasone (0.3 mg/kg, maximal dose 8 mg) were administered IV to prevent nausea and vomiting.

Local infiltration started with the abdomen, outer thigh, hips, back, inner thighs and knees. After Mini Abdominoplasty with supplemental liposuction was conducted and application of suction drains wound closure was performed, and the tight bandage was applied.

Evaluated intraoperative data included severity of injection pain, incision pain and pain during surgical manipulation using 4-points verbal analogue scale with 0: no pain, 1: mild pain not required additional dose of LA, 2: moderate pain required additional doses of LA and 3: severe pain not responding to additional doses of LA and required intravenous sedation or conversion to general anaesthesia.

Throughout operative procedure; heart rate, systolic, diastolic and mean blood pressures were non-invasively monitored and recorded before the start of injection of LA (baseline), after full injection, at time of skin incision and closure and after transfer to post-anaesthetic care unit (PACU).

Postoperative evaluation included determination of the duration of analgesia determined as duration elapsed since the time of start of surgery till request of postoperative rescue analgesia. The frequency of postoperative nausea and vomiting (PONV) and requirement of additional doses of antiemetics. Total postoperative hospital stay was determined.

Patients' satisfaction was evaluated using 5-point satisfaction score with 4: Highly satisfactory and will request a similar anaesthetic procedure whenever required, 3: Satisfactory and will request it once again if required, 2: Good and may request it once again if required, 1: unsatisfactory and may or may not request it once again if required, 0: Dissatisfactory and will not request it once again if required.

Surgical outcome included the frequency of seroma collection, hematoma formation and development of wound infection. Surgeon' satisfaction was evaluated using a 5-point satisfaction score with 4: Highly satisfactory and will request for similar procedures, 3: Satisfactory and will request it once again if required, 2: Good and may request it once again if required, 1: Bad and will not request it once again if required, 0: unsatisfactory and will not request it once again if required.

Obtained data were presented as mean±SD, ranges, numbers and ratios. Results were analysed using Wilcoxon's ranked test for unrelated data (Z-test) and Chi-square test (X^2 test) for numerical data. Statistical analysis was conducted using the SPSS (Version 15, 2006) for Windows statistical package. P value < 0.05 was considered statistically significant.

Results

The study included 60 female patients with a mean age of 33.3 ± 5.6; 23-43 years. Only 16 patients were overweight, and 44 patients were obese, with a total BMI of 31.7 ± 2.1; range: 26.3-33.9 kg/m^2. 14 patients were of ASA grade II, and 46 patients were of ASA grade I, (Table 1).

Table 1: Patients' demographic data

Data			Findings	
Age (years)	Strata	≤ 25	6 (10%)	23.3 ± 0.6 (23-24)
		> 25-30	12 (20%)	28 ± 1.1 (26-29)
		> 30-35	24 (40%)	32.5 ± 1.9 (30-35)
		> 35-40	8 (13.3%)	38 ± 1.2 (37-39)
		> 40	10 (16.7%)	42 ± 1 (41-43)
	Total		60 (100%)	33.3 ± 5.6 (23-43)
ASA grade	I		46 (76.7%)	
	II		14 (23.3%)	
Weight (kg)			88.5 ± 5.3 (75-95)	
Height (cm)			167.2 ± 2.9 (162-172)	
BMI (kg/m^2)	Strata	25-30	16 (26.6%)	28.7 ± 1.1 (26.3-29.8)
		> 30	44 (70.4%)	32.7 ± 1 (30.4-33.9)
	Total		60 (100%)	31.7 ± 2.1 (26.3-33.9)

Data are presented as numbers & mean ± SD; percentages & ranges are in parenthesis.

All anaesthetic procedures were conducted uneventfully, and no patient was excluded because of the intraoperative refusal of injection. 46 patients found injection pain was mild, 10 patients found it moderate and only 4 patients found it severe but could be hardly tolerated. On the start of the surgical procedure, 16 patients reported the mild sensation of the incision, while the remaining 44 patients reported no sensation and could not notify if the wound was made or not.

During the surgical procedure, no patient required conversion to general anaesthesia and all surgeries were completed under LA. Only 6 patients required an additional dose of LA; 4 during the procedure and 2 during wound closure, while 26 patients had mild tension pain during the procedure but not required additional anaesthesia and 28 patients had no sensation either during dissection or during wound closure, (Table 2 and Figure 1).

Table 2: Procedural pain data

Data		Findings
Injection pain	Mild (score = 1)	46 (76.7%)
	Moderate (score = 2)	10 (16.7%)
	Severe (score = 3)	4 (6.6%)
	Total score	1.3 ± 0.6 (1-3)
Incision pain	No (score = 0)	44 (73.3%)
	Mild (score = 1)	16 (26.7%)
Surgical procedural pain	No (score = 0)	28 (46.7%)
	Mild (score = 1)	26 (43.3%)
	Moderate (score = 2)	6 (10%)

Data are presented as numbers & mean ± SD; percentages & ranges are in parenthesis

Throughout the study period, hemodynamic parameters were stable, despite the increased levels detected at the time of start of injection. However, at the time of skin incision, hemodynamic measures started to re-stabilise, and at the time of admission to PACU, all measures were non-significantly different compared to baseline measures, (Table 3).

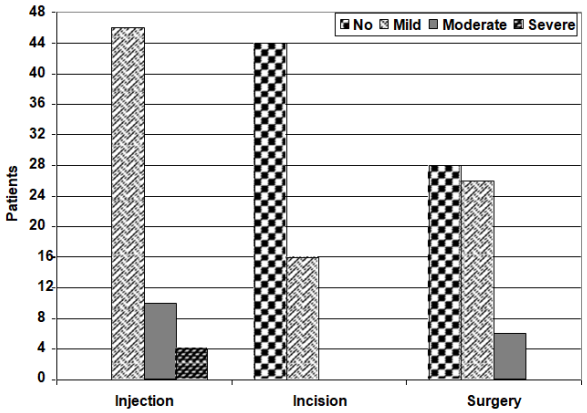

Figure 1: Patients distribution according to pain scores during infiltration anaesthesia and surgery

Eight patients (13.3%) complained of postoperative nausea, and one of them had one attack of vomiting, these patients received metoclopramide injection, and their oral intake was postponed till relieve of nausea sensation, while the remaining 52 patients (86.7%) resumed oral intake within their first 2-hr PO. Meantime till resumption of oral intake of 1.6 ± 0.9; 1-4 hours.

Table 3: Mean levels of hemodynamic measures estimated throughout the observation period

Parameter	Baseline	1-min after full injection	Skin incision	Wound closure	Admission to PACU
HR (beat/min)	86.7 ± 2.2	89.7 ± 3.9	88 ± 5.6	87.5 ± 3.2	87.1 ± 2.2
SBP (mmHg)	107.3 ± 9.1	118.9 ± 5.6	113.4 ± 3.9	108.3 ± 8.6	108.8 ± 8
DBP (mmHg)	72.5 ± 3.8	74.4 ± 4.5	70 ± 2.9	70.5 ± 2.5	72.1 ± 3.5
MAP (mmHg)	84.1 ± 4.8	89.2 ± 4.4	84.5 ± 2.1	83.1 ± 3.5	84.3 ± 3.9

Data are presented as mean ± SD.

A group of 18 patients (30%) did not request rescue analgesia during their PACU stay and were discharged within the first 4-hr PO. 26 patients (43.3%) required PO rescue analgesia once after PACU transfer and were discharged within the first 6-hr PO. Six patients (10%) required two doses of rescue analgesia and were discharged within 9 hours PO. Only 4 patients (6.7%) requested rescue analgesia for three times and had delayed home return till 12 hours PO, (Figure 2).

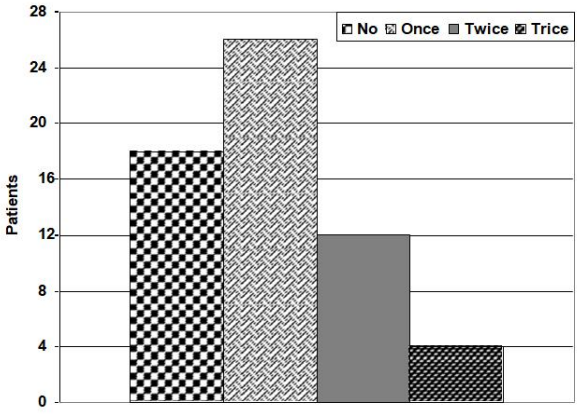

Figure 2: Patients distribution according to the frequency of requests of rescue PO analgesia

Meantime till home return was 5.6 ± 2.4; 3-12 hours. Despite all patients completed their operative course without conversion to general anaesthesia; 24 patients were highly satisfied; 18 patients were satisfied, and these 42 patients were willing to repeat the trial if required. 16 patients found the trial is a good one and may repeat it if required, while only 2 patients refused to repeat the trial and were dissatisfied, for a mean total satisfaction score of 3.1 ± 0.9; range: 1-4, (Table 4 and Figure 3).

Table 4: Postoperative data

Data			Findings	
PONV	Yes	Nausea	6 (10%)	
		Vomiting	2 (3.3%)	
	No		52 (86.7%)	
Time till resumption of oral intake (hours)	1		38 (63.3%)	
	2		14 (23.3%)	
	3		4 (6.7%)	
	4		4 (6.7%)	
	Total		1.6 ± 0.9	
PO hospital stay (hours)	< 6 hours		42 (70%)	4.4 ± 0.8 (3-5)
	6-9 hours		12 (20%)	8 ± 0.7 (7-9)
	9-12 hours		6 (10%)	10.7 ± 1.5 (9-12)
	Total		5.6 ± 2.4 (3-12)	
Patients' satisfaction scoring	Highly satisfied (Score = 4)		24 (40%)	
	Satisfied (Score = 3)		18 (30%)	
	Good (Score = 2)		16 (26.7%)	
	Unsatisfied (Score = 1)		2 (3.3%)	
	Dissatisfied (Score = 0)		0	

Data are presented as numbers & mean ± SD; percentages & ranges are in parenthesis

Mean duration of wound drainage was 6 ± 1.1; 4-8 days; drain was removed after a mean duration of 4.8 days in 10 patients, and in 18 patients after a mean duration of 6.4 days and in 2 patients wound drainage was delayed for 8 days.

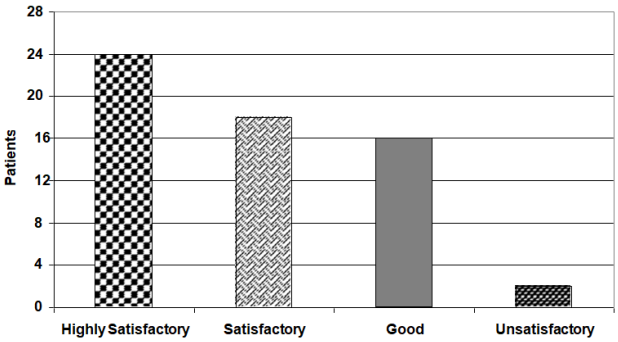

Figure 3: Patients' distribution according to PO satisfaction by the anaesthetic procedure

No wound infection or dehiscence was detected and wound healing was complete after a mean duration of 10 ± 1.1; range: 9-12 days; however, in 13 patients wound healing was complete, and stitches were removed on the 9th PO day, in 7 patients wound healing was complete, and stitches were removed on the 10th PO day, in another 7 patients wound healing was complete, and stitches were removed on the 11th PO day and in only 3 patients on the 12th PO day. No seroma was detected before the removal of stitches, (Table 5).

Table 5: Postoperative wound data

Data		Findings	
PO wound drainage (days)	< 6 days	20 (33.3%)	4.8 ± 0.4 (4-5)
	6-7 days	36 (60%)	6.4 ± 0.5 (6-7)
	> 7 days	4 (6.7%)	8
	Total	8 ± 2.2 (8-16)	
Duration till complete wound healing	9 days	26 (43.4%)	
	10 days	14 (23.3%)	
	11 days	14 (23.3%)	
	12 days	6 (10%)	
	Total	20 ± 2.2 (18-24)	

Data are presented as numbers & mean ± SD; percentages & ranges are in parenthesis.

Discussion

Tumescent local anesthesia (LA) infiltration was proved effective as the sole anesthetic modality for women undergoing Mini Abdominoplasty with supplemental liposuction as manifested by the findings that all surgeries were conducted completely without conversion to general anaesthesia and the high frequency of patients reporting mild or no pain during skin incision and/or surgical procedure with a minimal number required booster dose of LA during surgery. Additionally, 42 patients were satisfied by the applied anaesthetic procedure and willing to use the same procedure if required later on and the other 16 patients found it good anaesthetic modality and may request it if required, while only 2 patients were unsatisfied and refused similar procedure once again.

Patients attributed their satisfaction to many different causes; the sparing of exposure to general anesthesia and its possible risks, the sense of being awake during surgery, management as office procedure with resumption of oral intake and return home within few postoperative hours, the significant reduction of the cost of anesthesia and subsequent reduction of the hospital resource.

The reported findings supported that previously reported concerning breast surgery under LA; Carlson et al., [10] described unilateral total mastectomy for cancer under LA using the tumescent technique of infiltrating dilute lidocaine with epinephrine and reported no morbidity related to the surgery, patients were discharged 1-4 days after surgery, anaesthesia was adequate in all cases and there was no deviation from the described technique and concluded that the tumescent technique is a safe, effective method for performing a total mastectomy especially in patients who would not be considered candidates for general anaesthesia. Sleth et al., [11] found breast surgery under local infiltration anaesthesia provides adequate perioperative analgesia and is a technically low-risk procedure. Ranieri et al., [12] reported the feasibility of quadrantectomy and sentinel lymph node removal under LA in patients with ultrasound negative axillary lymph nodes. Groetelaers et al., [13] confirmed the safety of the sentinel lymph node biopsy under local

anaesthesia in selecting patients for axillary lymph node dissection in breast cancer

Habbema [14] and Habbema & Alons [15] documented that breast liposuction using tumescent LA and powered cannulas is a safe and effective treatment modality for breast reduction. Kitowski et al., [16] retrospectively studied patients had breast cancer surgery including mastectomy, full axillary dissections, and expander or implant reconstruction and found that 74% of patients were able to undergo breast cancer surgery with local or paravertebral block regional anesthesia with no conversions to general anesthesia and no unplanned overnight admissions and only 10% of them developed postoperative nausea or vomiting, and concluded that most elective outpatient breast cancer surgery operations can be performed with the patients given local or regional anesthesia.

Kashiwagi et al., [17] reported that radical surgery for breast cancer could be performed under LA in all of the studied 42 patients and were not demanded to shift from local to general anaesthesia, and none of the serious complications was encountered and concluded that today's radical operation under LA for breast cancer is a useful procedure as minimally invasive surgery.

The used anaesthetic solution was a mixture of one ml of 1:1000 of epinephrine, 100 ml of 2% lidocaine, 50 ml of 0.5% Levobupivacaine in a 2500 ml saline solution. The addition of adrenaline significantly reduced intraoperative blood loss allowing meticulous dissection of the excised lipoma so minimised seroma formation and so motivated the surgeon' satisfaction by the applied anaesthetic procedure. In line with these data, Sleth et al., [11] used a similar mixture of lidocaine, ropivacaine and adrenaline during breast surgery and did not require conversion to general anaesthesia or supplementation with LA. Hardwicke et al., [18] reported that a meta-analysis of operative blood loss during reduction mastectomy showed a highly significant drop in operative blood loss in breasts infiltrated with epinephrine with a reduction in the need for blood transfusion and recommended the use of dilute epinephrine infiltration before reduction mammaplasty. In support of adding ropivacaine to the anaesthetic mixture, Manfè et al., [19] compared the efficacy of ropivacaine versus levobupivacaine for postoperative pain control in patients who underwent minor breast surgery and found ropivacaine results in more effective pain relief, while levobupivacaine provided long-term postoperative analgesia

Klein JA [20] found that. Tumescent technique for local anaesthesia improves safety in large-volume liposuction.

Kendler M et al., [21] use electrochemotherapy under local tumescent anaesthesia for treatment of cutaneous metastases particularly in elderly patients, in whom general anesthesia can be difficult because of comorbidity

Coldiron B et al., [22] give guidelines of care for tumescent liposuction.

It could be concluded that bilateral reduction mastectomy could be conducted safely under tumescent LA with mostly pain-free intraoperative and postoperative courses and allowed such surgical procedure to be managed as an office procedure. Moreover, the anaesthetic procedure provided patients' satisfaction with varying degrees in about 97% of studied patients.

References

1. Bitar G, Mullis W, Jacobs W, et al. Safety and efficacy of office-based surgery with monitored anesthesia care/sedation in 4778 consecutive plastic surgery procedures. Plast Reconstr Surg. 2003; 111:150–6. https://doi.org/10.1097/00006534-200301000-00025 PMid:12496575

2. Agbakwuru EA, Salako AA, Olajide AO, Takure AO, Eziyi AK: Hydrocelectomy under local anaesthesia in a Nigerian adult population. Afr Health Sci. 2008; 8(3):160-2. PMid:19357743 PMCid:PMC2583265

3. Parirokh M, Satvati SA, Sharifi R, Rekabi AR, Gorjestani H, Nakhaee N, Abbott PV: Efficacy of combining a buccal infiltration with an inferior alveolar nerve block for mandibular molars with irreversible pulpitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010; 109(3):468-73. https://doi.org/10.1016/j.tripleo.2009.11.016 PMid:20219602

4. Gun R, Yorgancılar E, Yıldırım M, Bakır S, Topcu I, Akkus Z: Effects of lidocaine and adrenaline combination on postoperative edema and ecchymosis in rhinoplasty. Int J Oral Maxillofac Surg. 2011; 40(7):722-9. https://doi.org/10.1016/j.ijom.2011.02.022 PMid:21458231

5. Chia CT, Theodorou SJ: 1,000 consecutive cases of laser-assisted liposuction and suction-assisted lipectomy managed with local anesthesia. Aesthetic Plast Surg. 2012; 36(4):795-802. https://doi.org/10.1007/s00266-012-9885-2 PMid:22447150

6. Tahiri Y, Tran de QH, Bouteaud J, et al. General anaesthesia versus thoracic paravertebral block for breast surgery: a meta-analysis. J Plast Reconstr Aesthet Surg. 2011; 64:1261–1269. https://doi.org/10.1016/j.bjps.2011.03.025 PMid:21486711

7. Kashiwagi S, Asano Y, Watanabe M, Takii M, Morisaki T, Aomatsu N, Nakamura M, Kawajiri H, Takashima T, Onoda N, Ishikawa T, Wakasa K, Hirakawa K: Day surgery of early breast cancer treated with breast-conserving operation following sentinel lymph node navigation biopsy under local anesthesia. Gan To Kagaku Ryoho. 2012; 39(12):1914-6. PMid:23267928

8. Bray GA: Pathophysiology of obesity. Am J Clin Nutr, 1992; 55:488S-94S. https://doi.org/10.1093/ajcn/55.2.488s PMid:1733117

9. Vella M, Galloway DJ: Laparoscopic adjustable gastric banding for severe obesity. Obes Surg. 2003; 13(4):642-8. https://doi.org/10.1381/096089203322190899 PMid:12935369

10. Carlson GW: Total mastectomy under local anesthesia: the tumescent technique. Breast J. 2005; 11(2):100-2. https://doi.org/10.1111/j.1075-122X.2005.21536.x PMid:15730454

11. Sleth JC, Servais R, Saizy C: Tumescent infiltrative anaesthesia for mastectomy: about six cases. Ann Fr Anesth Reanim. 2008; 27(11):941-4. https://doi.org/10.1016/j.annfar.2008.08.011 PMid:19004607

12. Ranieri E, Larcinese A, Barberi S, Caprio G, Naticchioni E, Civitelli L, Paglicci C, Pagni P, Zancla S, Rengo M, Di Giorgio A: Quadrantectomy and removal of the sentinel lymph node under

local anaesthesia in the day hospital setting. Chir Ital. 2008; 60(3):391-4. PMid:18709777

13. Groetelaers RP, van Berlo CL, Nijhuis PH, Schapers RF, Gerritsen HA: Axillary recurrences after negative sentinel lymph node biopsy under local anaesthesia for breast cancer: a follow-up study after 5 years. Eur J Surg Oncol. 2009; 35(2):159-63. https://doi.org/10.1016/j.ejso.2008.07.017 PMid:18789841

14. Habbema L: Breast reduction using liposuction with tumescent local anesthesia and powered cannulas. Dermatol Surg. 2009; 35(1):41-50. PMid:19076201

15. Habbema L, Alons JJ: Liposuction of the female breast: a histologic study of the aspirate. Dermatol Surg. 2010; 36(9):1406-11. https://doi.org/10.1111/j.1524-4725.2010.01649.x PMid:20629687

16. Kitowski NJ, Landercasper J, Gundrum JD, De Maiffe BM, Chestnut DH, Bottcher ML, Johnson JM, Johnson RL: Local and paravertebral block anesthesia for outpatient elective breast cancer surgery. Arch Surg. 2010; 145(6):592-4. https://doi.org/10.1001/archsurg.2010.77 PMid:20566982

17. Kashiwagi S, Takashima T, Asano Y, Morisaki T, Aomatsu N, Matsuoka J, Nakamura M, Kawajiri H, Onoda N, Ishikawa T, Hirakawa K: Lumpectomy and sentinel lymph node navigation surgery for breast cancer under local anesthesia. Gan To Kagaku Ryoho. 2011; 38(12):2017-9. PMid:22202270

18. Hardwicke JT, Jordan RW, Skillman JM: Infiltration of epinephrine in reduction mammaplasty: a systematic review of the literature. Plast Reconstr Surg. 2012; 130(4):773-8. https://doi.org/10.1097/PRS.0b013e318262f085 PMid:23018690

19. Manfè AZ, Marchesini M, Bortolato A, Feltracco P, Lumachi F: Ropivacaine versus levobupivacaine for minor breast surgery in outpatients: inversion of postoperative pain relief efficacy. In Vivo. 2012; 26(6):1075-7. PMid:23160696

20. Klein JA. Tumescent technique for local anesthesia improves safety in large-volume liposuction. Plast Reconstr Surg. 1993; 92:1085–98. https://doi.org/10.1097/00006534-199311000-00014 PMid:8234507

21. Kendler M, Micheluzzi M, Wetzig T, Simon JC. Electrochemotherapy under tumescent local anesthesia for treatment of cutaneous metastases. Dermatolog Surg. 2013; 39:1023–32. https://doi.org/10.1111/dsu.12190 PMid:23464631

22. Coldiron B, Coleman WP III, Cox SE, Jacob C, Lawrence N, Kaminer M, Narins RS. ASDS guidelines of care for tumescent liposuction. Dermatol Surg. 2006; 32:709–16. https://doi.org/10.1111/j.1524-4725.2006.32159.x PMid:16706767

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Treatment of Depressive Conditions in Pregnancy

Slavica Arsova[1*], Kadri Hadzhihamza[1], Stojan Bajraktarov[1], Viktor Isjanovski[2]

[1]University Clinic of Psychiatry, Skopje, Republic of Macedonia; [2]JZU Psychiatric Hospital Skopje, Skopje, Republic of Macedonia

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***Correspondence:** Slavica Arsova. University Clinic of Psychiatry, Skopje, Republic of Macedonia. E-mail: arsova_s@yahoo.com

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Abstract

BACKGROUND: Mother's mental state during pregnancy is of substantial importance for the mother, but also for the infant and his/her future growth and development. Depressive maternal disorders during pregnancy have a significant influence on the development of the baby during pregnancy as well as on the future development and mother-baby relation, the breastfeeding process and care for the baby.

AIM: This study aimed to determine the influence of SSRI antidepressant therapy and psychosocial and therapeutic interventions on depression during pregnancy. It was also our aim to determine the relation between severity of depression and sociodemographic characteristics.

METHODS: The study included 40 women, with diagnosis F32 and F33 according to ICD 10, that is, with severe depressive disorder within depressive episodes or recurrent depressive disorder. Patients were evaluated at the beginning of the treatment and 3 months after antidepressant treatment. They were followed-up for two years.

RESULTS: The results obtained have shown that a larger number of mothers treated with antidepressant medications, had normal childbirth with the unremarkable condition of both, the mother and the newborn baby.

CONCLUSION: A well-combined treatment of maternal depression during pregnancy reduces the risk of postpartum depression, which is by itself a prerequisite for normal emotional and behavioural development of the child.

Introduction

The mental state of the mother during pregnancy is of substantial importance for the mother, but also for the newborn baby and his/her future development.

Depressive maternal disorders during pregnancy have a significant influence on the development of the baby during pregnancy as well as on the future development and mother-baby relation, the breastfeeding process and care for the baby.

It is estimated that in about 7% of women during pregnancy psychic disorders have been detected, and in ¾ of them, a severe depressive condition has been observed.

Although it is considered that the percentage of women with depression during pregnancy is about 18%, still there is no sufficient information for adequate diagnosing and treatment of depression during pregnancy.

Patients who were screened during pregnancy and diagnosed with depression were significantly more likely to ask for an expert opinion and help earlier during the postpartum period than those who were not recognised [1].

An estimated 1-6% of women suffer from major depression while 15-25% have a milder form of depression. Postnatal or postpartum depression is usually associated with depression during pregnancy, especially if it has not been properly treated.

Antidepressant maternal treatment reduces the risk of relapse or disease exacerbation during pregnancy [2].

Over the last two decades, the use of SSRI antidepressant medications during pregnancy has increased and has been given to 2-8% of pregnant women [3].

Nancy Melville in her study about the risk factors for suicide in women reported that 4% of women who died by suicide in Great Britain during the period 1997-2012 were in their perinatal period (a period that is a few weeks before delivery). The most common diagnoses in these women were depression, bipolar disorders and personality disorders [4] [5]. The most common symptoms included depressed mood, self-harm, suicidal ideas and hopelessness. It has to be emphasised that in one-fifth of these women depression was not recognised, that is, they were not examined during their last month of pregnancy.

Therefore, the question of increased psychiatric and medical care of patients during pregnancy and postpartum is imposed.

This study aimed to determine the influence of SSRI antidepressant therapy and psycho-social therapeutic interventions on depression during pregnancy. It was also our aim to define the relation between severity of depression and sociodemographic characteristics.

Material and Methods

The investigation comprised 40 women with dg. F32 or F33 according to ICD 10, that is depressive condition with expressed characteristics manifested during depressive episodes and recurrent depressive disorder.

Patients were of different age, different environment, working status, education, and marital status. They were also tested for heredity presence.

Patients were examined by using the scales for assessment of depression and anxiety, HAMD rating scale for the severity of depression, and HAMA rating scale for the severity of anxiety. In addition to these two scales, the clinically structured interview was used for obtaining data about pregnancy, heredity, previous treatment or use of antidepressant medications and other characteristics.

Patients were treated with SSRI antidepressant therapy initiated in the 10-12 week with gradual reduction or discontinuation of medications by the end of the pregnancy, i.e., in the last 8 gestational weeks.

They were evaluated at the beginning of the treatment and 3 months after antidepressant treatment.

All patients were informed about the treatment and the risk/benefit ratio regarding their physic condition and pregnancy. All of them paid regular visits to a gynaecologist. The follow-up period was two years.

Results

Table 1 presents the sociodemographic characteristics of patients. The larger number of them was at the age over 30 years, with completed higher education, term delivery and without heredity inheritance.

Table 1: Sociodemographic characteristics of patients

Age	**Count**	**Per cent**
< 30	10	24.4
> = 30	29	70.7
> = 40	2	4.9
Pregnancy		
I	29	70.7
II	8	19.6
III	3	7.3
IV	1	2.4
Education		
Faculty	22	53.8
Higher	16	39.0
Basic	3	7.2
Employees		
Yes	22	53.7
No	19	46.3
Delivery		
Time	34	82.9
Caesarean section	4	9.8
Before time	3	7.3
Therapy		
With	4	9.8
Without	37	90.2
Heredity		
-	32	78.0
+	9	22.0

They were treated with antidepressant medications and the largest number with sertraline and escitalopram. In a smaller number of patients, antipsychotic medications at small doses were added (up to 1 mg risperidone and up to 5 mg olanzapine) as adjuvant therapy (Table 2).

Table 2: Therapy administered in patients

Therapy	Count	Per cent
Escitalopram	9	21.6
Sertraline	15	36.6
Without therapy	4	9.8
Escitalopram, risperidone	3	7.3
Sertraline, risperidone	5	12.2
Escitalopram, risperidone, alprazolam	1	2.4
Venlafaxine	1	2.4
Citalopram	2	4.9
Olanzapine, sertraline	1	2.4
Total	41	100.0

The average HAM anxiety score in patients on 0 days was 36.0 ± 5.2 (severe anxiety), and after 3 months the score reduced to 22.2 ± 3.3 (moderate). The difference was 13.8 units. According to t-test the difference was statistically significant for $p < 0.05$ ($p = 0.00000$) (Table 3 and Figure 1).

Table 3: Average HAMA score, 0 days and after 3 months

Hama	**Mean**	**Std.dv.**	**N**	**Diff.**	**Std.dv. - diff.**	**T**	**Df**	**P**	**Confidence - 95.0%**	**Confidence +95.0%**
0 day	36.0	5.236								
After 3m	22.2	3.285	41	13.8	3.831	23.153	40	<0.001	12.644	15.062

HAM severe anxiety disorder was registered in 71.0% (22) of patients older than 30 years, and in 30.0% (3) younger than 30 years.

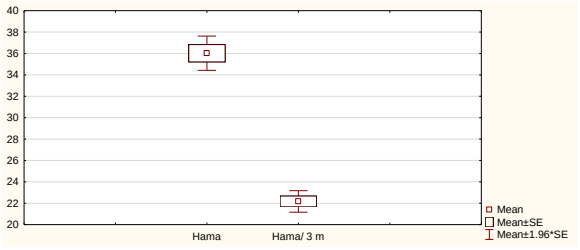

Figure 1: Average HAMA score, 0 days and after 3 months

A statistically significant relation was registered between age (over and under 30 y.) and the HAM anxiety score for p < 0.05 (Pearson Chi-square: 6.384, df = 2, p = 0.0410) (Table 3).

HAM severe anxiety disorder was registered in 63.6% (14) of patients with an obtained university degree, in 62.5% (10) with completed high education and 33.3% (1) with primary education. No statistically significant relation was observed between education and HAM anxiety score for p < 0.05 (Pearson Chi-square: 0.893, df = 2, p = 0.639) (Table 3).

HAM severe anxiety disorder was registered in 100% (4) of patients who were delivered with a Cesarean section, in 100% [3] with preterm delivery and 52.9% (18) with term delivery.

No statistically significant relation was registered between mode of delivery and HAM anxiety score for p<0.05 (Pearson Chi-square: 5.402, df=4, p= 0.248) (Table 3).

HAM severe anxiety disorder was registered in 56.3% (18) of patients who were with negative heredity, and in 77.8% (7) in those with positive heredity.

No statistically significant relation was registered between heredity and HAM anxiety score for p < 0.05 (Pearson Chi-square: 1.704, df = 2, p = 0.426) (Table 3).

The average HAM depression score was 33.4 ± 3.5 (severe anxiety) on day 0, and after 3 months it decreased to 20.8 ± 2.1 (moderate). The difference was 12.6 units. According to t-test the difference was statistically significant for p < 0.05 (p = 0.00) (Table 4 and Figure 2).

HAM severe depression disorder was registered in 90.3% (28) of patients older than 30 years and in 60.0% (6) younger than 30 years.

A statistically significant relation was found between age (over and under 30 y.) and HAM depression score for p < 0.05 (Pearson Chi-square: 4.910, df = 1, p = 0.0266) (Table 4).

Table 4: Average HAMD score, 0 days and after 3 months

Hamd	Mean	Std.dv.	N	Diff.	Std.dv. - diff.	T	Df	P	Confidence - 95.0%	Confidence +95.0%
0 day	33.4	3.498								
After 3m	20.8	2.079	41	12.6	2.408	33.464	40	0.00	11.825	13.345

HAM severe depression disorder was registered in 86.4% (19) of patients with a university degree, in 87.5% (14) of those with completed high education and 33.3% (1) of patients with primary education.

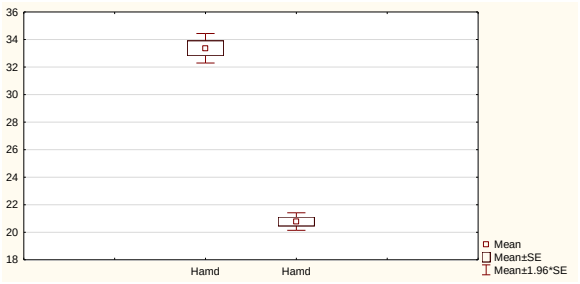

Figure 2: Average HAMD score, 0 days and after 3 months

No statistically significant relation was found between level of education and HAM depression score for p < 0.05 (Pearson Chi-square: 5.631, df = 2, p = 0.059) (Table 5).

HAM severe depression disorder was registered in 86.2% (25) of primipara patients and in 75.0% (9) of multipara patients.

No statistically significant relation was found between number of pregnancies and HAM depression score for p < 0.05 (Pearson Chi-square: 0.752, df = 1, p = 0.385) (Table 5).

HAM severe depression disorder was registered in 100% (4) of patients delivered with a Cesarean section, in 100.0% (3) of those who gave preterm childbirth and in 79.4% (27) of patients with normal delivery.

No statistically significant relation was found between mode of delivery and HAM depression score for p < 0.05 (Pearson Chi-square: 1.737, df = 2, p = 0.419) (Table 5).

HAM severe depression disorder was registered in 81.2% (26) of patients with negative heredity and in 88.8% (8) of patients who were found to have positive heredity.

No statistically significant relation was found between heredity and HAM depression score for p < 0.05 (Pearson Chi-square: 0.289, df = 1, p = 0.590) (Table 5).

Table 5: Relation between HAMA and HAMD *versus* sociodemographic characteristics

Characteristics/ Pearson Chi-square	HAMA	HAMD
Age	P = 0.041080*	P = 0.026698*
Pregnancy	P = 0.64087	P = 0.385549
Education	P = 0.639839	P = 0.059864
Delivery	P = 0.248447	P = 0.419394
Heredity	P = 0.426384	P = 0.590536

Discussion

The results obtained have shown a significant improvement in patients about the reduction of depression and anxiety after a 3-month psychopharmacological treatment. A statistically significant relation was found between the severity of depression and anxiety, on the one hand, and age, on the other. Patients older than 30 years presented with more expressed anxiety and depression than the younger pregnant women.

In general, the largest number of primipara patients with depression was at the age over 30 years, which coincides with the trend of getting married after the age of 30/later in life/and raising a family, but it might also be due to the need of secure socioeconomic status.

Although there were more severe depression and anxiety disorders in patients with a university degree in comparison with those with a lower level of education, the difference was not statistically significant. More severe depression manifested in mothers with completed higher education might be a result of their higher awareness and consciousness about their psychological disorders and the need of expert help.

The results have shown that a larger number of pregnant patients treated with antidepressant therapy had a normal delivery with the subsequent normal/unremarkable condition in both, the mother and the newborn infant; only one woman had preterm childbirth, and one had a Cesarean section.

More than 80% of mothers were treated with SSRI antidepressant therapy, and they had normal/uncomplicated pregnancy and delivery, which speaks in favour of the risk/benefit ratio of applying antidepressant therapy.

The task of each therapist/medical expert is to make an individual assessment of the severity of depression during pregnancy, the assessment of the risk for the mother and the newborn infant regarding the eventual damage or benefit from antidepressant medications [1] [2] [6] [7].

Majority of studies point out the differences in psychiatric treatment applied in women with a higher socioeconomic status compared to those with a lower one. Indicators show that women with a higher socioeconomic status were more likely to see a psychiatrist for depression during pregnancy and were treated with SSRI antidepressant medications, whereas those with a lower socioeconomic status were more likely to visit their family/general physicians asking for help about their depression during pregnancy [2].

The results of one research conducted across several European countries with different socioeconomic status indicated that major depression during pregnancy and after delivery was observed in women living in countries with lower socioeconomic status. This leads to the conclusion that these women rarely consult a psychiatrist during pregnancy, which consequently results in a more severe postnatal depression or delayed treatment of postpartum depression. The research has demonstrated that patients who were treated with SSRI medications during pregnancy or patients who had not discontinued their previously started SSRI treatment were at a lower risk of onset or deterioration/ exacerbation of the condition in the postnatal period. It can be concluded that the optimum treatment with SSRI is indispensable in patients with the major depressive disorder [4].

Literature data show that depression during pregnancy is associated with poor care of the mother for her health during pregnancy, including rare visits to a doctor's office, irregular use of medications, substance abuse, increased risk for preterm birth and low birth weight, and emotional developmental difficulties in the children whose mothers suffered from depression during pregnancy or postpartum [3].

It is considered that depression before and during pregnancy is one of the larger risks for development of postpartum depression, which has a negative effect on the mother/child relation as well as on adequate care for herself and the child [8].

National Institute for Health and Clinical Excellence in the United Kingdom (NICE) guidelines and American Psychiatric Association (APA) recommend CBT (cognitive behavioural therapy) and SSRI antidepressant therapy [9].

Literature suggests that SSRI treatment during pregnancy has no statistically increased risk of giving birth to a child with congenital anomalies than other women [5] [9] [10].

In conclusion, the optimal combined treatment of peripartum depression reduces the risk of postpartum depression, which is a prerequisite for normal emotional and behavioural development of the child.

References

1. Venkataesh KK, Nadel H, Blewett D, Freeman MP, Kaimal AJ, Riley LE. Implementation of universal screening for depression during pregnancy: feasibility and impact on obstetric care. Am J Obstet Gynecol. 2016; 215(4):517.e1-8. https://doi.org/10.1016/j.ajog.2016.05.024 PMid:27210067

2. Hanley GE. Socioeconimic status and treatment of depression during pregnancy: a retrospective population – based cohort study in British Columbia, Canada. Arch Womens Ment Health, 2018.

3. Anderson P. Untreated Depression in pregnancy Linked to Low Birth Weight. JAMA Psychiatry. 2016. Available from:

https://www.medscape.com/viewarticle/864910

4. Lupattelli A, Twigg MJ, Zagorodnikova K, Moretti ME, Drozd M, Panchaud A, Rieutord A, Juraski RG, Odalovic M, Kennedy D, Rudolf G. Self-reported perinatal depressive symptoms and postnatal symptom severity after treatment with antidepressants in pregnancy: a cross-sectional study across 12 European countries using the Edinburgh Postnatal Depression Scale. Clinical Epidemiology. 2018; 10:655. https://doi.org/10.2147/CLEP.S156210 PMid:29922092 PMCid:PMC5997125

5. Melville NA. Antidepressant Use in pregnancy and Heart Defects: No Links. J Clin Psychiatry. 2016; 77:e36-e42

6. Brooks M. Antidepressants during pregnancy and psych disorders in kids. 2018. Available from: https://www.medscape.org/viewarticle/887606

7. Melville NA. Risk factors for suicide in pregnancy identified. 2016. Available from: https://www.medscape.com/viewarticle/857648

8. Swift D. Pospartum depression affects 1 in 9 new mothers. MMWR. 2017; 66(6):153-158.

9. Koren G, Nordeng H. Antidepressant use during pregnancy and lactation: the benefit-risk ratio. Am J Obstet Gynecol. 2012; 207(3):157-163. https://doi.org/10.1016/j.ajog.2012.02.009 PMid:22425404

10. Molenaar NM, Brouwer ME, Bockting CL, Bonsel GJ, van der Veere CN, Torij HW, et al. Stop or go? Preventive Cognitive Therapy with Guided Tapering of Antidepressants during Pregnancy. BMC Psychiatry. 2016; 16:72. https://doi.org/10.1186/s12888-016-0752-6 PMid:26993629 PMCid:PMC4797115

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Clinical Science

Does the Presence of Diabetes Mellitus Make a Difference in Pharmacological Stress Echocardiography Outcome Results?

Valentina Andova[1*], Ljubica Georgievska-Ismail[1], Elizabeta Srbinovska[1], Biljana Janeska[2]

[1]University Clinic of Cardiology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]Institute of Forensic Medicine, Criminalistic and Deontology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

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***Correspondence:** Valentina Andova. University Clinic of Cardiology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: andovav@yahoo.com

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Abstract

BACKGROUND: Coronary artery disease (CAD) is the leading cause of mortality and morbidity in patients with diabetes (DM).

AIM: The aim of our study was to analyse the ability of pharmacological SE to risk stratify patients with DM using qualitative and quantitative assessment of LV function.

METHODS: We prospectively assessed 105 consecutive patients (58.7 ± 9.5 y, 39 male) with known or suspected CAD who underwent dipyridamole or dobutamine SE.

RESULTS: Change of systolic LV function at maximal SE was less pronounced in patients with DM, while parameters of the diastolic function and its change with stress were almost insignificant. WMSI in comparison to GLS% didn't make a difference in SE outcome regarding DM presence. WMSI was almost unchanged at maximal stress in diabetic patients. Conversely, GLS% showed significant worsening at maximal stress in diabetic patients. However, only WMSI at maximal stress along with DM presence appeared as independent predictors of the presence of new and worsening CAD during SE. Longitudinal strain assessed using speckle tracking during pharmacological stress echocardiography was superior to conventional echocardiography expressed by wall motion analysis in making a difference regarding DM presence.

CONCLUSION: We confirmed the usefulness of stress echocardiography using qualitative and/or quantitative parameters in the detection of CAD in patients with DM.

Introduction

Coronary artery disease (CAD) is the leading cause of mortality and morbidity in patients with diabetes. Approximately one-half of deaths are attributed to CAD in diabetic patients [1], whose risk of myocardial infarction or cardiac death is two-to four-fold as great compared with nondiabetic patients [2] [3]. Moreover, cardiac events are as frequent in diabetic patients without evidence of CAD as in nondiabetic patients with known CAD [4]. The increased risk associated with diabetes calls for effective prevention [5], [6], [7], [8] and risk stratification strategies to optimise therapeutic interventions [9]. Exercise testing is of limited value in the diabetic population because exercise capacity is often impaired by peripheral vascular [10] or neuropathic disease [11]. Furthermore, test specificity is less than ideal [12] because of the high prevalence of hypertension [13] and microvascular disease[14]. Stress echocardiography (SE) represents an established diagnostic [15] and prognostic modality [16], [17], [18], [19] in diabetic patients. However, it is still undefined whether it retains the same prognostic value in diabetic and nondiabetic patients.

The aim of our study was to analyse the ability of pharmacological (dobutamine or

dipyridamole) stress echocardiography (SE) to risk stratify patients with DM using qualitative and/or quantitative assessment of LV function.

Methods

We prospectively assessed 105 consecutive patients, with a mean age of 58.7 ± 9.5 years, who underwent dipyridamole or dobutamine stress echocardiography between January 2016 and May 2018 in a University Clinic of cardiology in Skopje. Before the study, patients' demographic characteristics were obtained, and patients were questioned about the presence of atherosclerotic risk factors, previous CAD and angioplasty. Subjects were classified as having diabetes when treated for insulin-dependent or non-insulin-dependent diabetes or having elevated fasting glucose levels according to issued up-to-date standards from professional organisations.

The indications for the exam was a referral from a physician according to the complaints of the participating patient or was a result of inconclusive results of treadmill stress testing. Written informed consent to undergo stress testing and to participate in the study was obtained from each patient.

Echocardiographic examination was performed using GE, Vivid 7 with a recording of the views and later analysing on the machine itself or acoustic-tracking software (Echo Pac, GE). All measurements were performed according to guidelines suggested by professional echocardiographic societies [20], [21].

An accelerated high-dose dipyridamole protocol was used for all patients. Dipyridamole was infused intravenously at a dose of 0.84 mg/kg body weight over 6 min. Aminophylline (up to 240 mg) was routinely administered to patients 5 min after the end of the test or immediately if there were obvious clinical and/or ECG signs of ischemia. Dobutamine was administered intravenously beginning at a dose of 10 μg/kg body weight and minute and increased in steps every 3 minutes to 20, 30, and 40 μg/kg.

Two-dimensional echocardiography, blood pressure measurements and 12-lead electrocardiography (ECG) were used for continuous monitoring during the test and the recovery phase. Echocardiographic images were semi-quantitatively assessed using 17 segments, and wall motion score index (WMSI) was derived by dividing the sum of individual segment scores by the number of interpretable segments. Ischemia was defined as stress-induced new and/or worsening of pre-existing wall motion abnormality, or biphasic response (i.e. low-dose improvement followed by high-dose deterioration) [22], [23].

Global longitudinal strain (GLS%) for the LV was automatically provided as the average value of the regional peak systolic longitudinal strain of the three apical views by the software [24], [25], [26]. The images for analysis were obtained out of stress-echo protocol. All images were recorded with a high frame rate (> 50 frames/s). The LV was divided into 17 segments, and each segment was analysed individually. Only myocardial segments considered to be of adequate quality by both the automatic system and the operator were included in the analysis. All examinations were assessed by the same cardiologist.

Sixty-one patients underwent coronary angiography within a few days of the stress echocardiography tests. Angiographic assessments involved presence and quantification of stenosis severity as well as the calculation of Syntax score.

The study protocol was approved by the Medical Ethics Committee of Medical School, University "St. Cyril & Methodius), Skopje.

Categorical parameters were summarised as percentages and continuous parameters as mean ± SD. Comparisons between the two groups with and without DM was performed using the Mann-Whitney nonparametric test for continuous parameters and Pearson's chi-square test for categorical parameters. Comparisons between the two groups before and after stress were performed using Wilcoxon Signed Rank test. Assessment of correlation of various echocardiographic parameters was done using Pearson's correlation analysis. Multiple logistic regression analysis was performed in stepwise order to determine independent predictors among clinical and echocardiographic covariates of the positive stress echocardiographic result. All data analysis was performed using SPSS version 25.0 (IBM SPSS, Inc., Chicago, Illinois) and p-value ≤ 0.05 was considered significant.

Results

Out of 105 patients who underwent SE, DM was present in 36 (34.3%); 22/61,2% were taking oral antidiabetic drugs, and 14/38.9% were using insulin to control diabetes. In comparison to patients without DM, those with DM were older (p = 0.0001), with higher BMI (p = 0.034), less frequently were current smokers (p = 0.033), but more frequently had a history of hypertension (p = 0.013) and insignificantly higher percentage of the history of CAD

Table 1: Basal and functional characteristics, symptoms and ECG changes of patients divided according to the DM presence

Parameter	With DM N = 36	Without DM N = 69	p
Age (y)	63.1 ± 6.7	56.4 ± 9.9	0.0001
Gender (m/f %)	36.1/63.9	37.7/62.3	0.874
BMI (kg/m^2)	30.4 ± 4.6	28.3 ± 5.1	0.034
Current smoking (n/%)	9/25.0	32/46.4	0.033
History of hypertension (n/%)	32/88.9	46/66.7	0.013
History of dislipidemia (n/%)	25/69.4	48/69.6	0.580
History of CAD (n/%)	15/41.7	19/27.5	0.142
Beta-blocker therapy (n/%)	23/21.9	37/35.2	0.313
Calcium channel blocker therapy (n/%)	7/19.4	17/24.6	0.547
Symptoms (n%)	7/19,4	14/20.3	0,918
ECG changes (n/%)	10/27.8	13/18.8	0,293
Rhythm disorder (n/%)	4/11.1	6/8.7	0,689
Δ BPs (mmHg)	-1.2 ± 20.1	1.1 ± 17.4	0.525
ΔBPd (mmHg)	1.5 ± 10.3	2.2 ± 9.8	0.713
HR rest (Imp/min)	79.5 ± 15.2	72.0 ± 11.2	0.005
HR max (Imp/min)	105.9 ± 21.3	97.9 ± 18.1	0.048
ΔHR (Imp/min)	-26.4 ± 18.37	-25.8 ± 14.8	0.876

DM = diabetes mellitus; BMI = body mass index, CAD = coronary artery disease; ECG = electrocardiogram; BP = blood pressure; s = systolic;d=diastolic; HR = heart rate; Δ= change from rest to maximal dose of stressor; *p<0.05 for comparison between groups.

Dobutamine or dipyridamole pharmacological protocol was applied in equal half's of patients with DM, while those without DM receive more frequently dipyridamole protocol (p = 0.049). Although systolic blood pressure increase with SE in patients with DM while a decrease in patients without DM, there was no significant difference in change. With SE diastolic pressure decrease in both groups of patients without difference between them. Heart rate was significantly higher in patients with DM at rest as well as at maximal dose of the stressor (peak stress), but there was lack of significant difference in its change (delta) (Table 1).

Comparison of systolic LV functional data during SE showed an insignificant increase of LV ejection fraction (LVEF) and significant increase of indexed systolic volume (SV/BSA), indexed cardiac output (CI) and early diastolic mitral annular tissue Doppler velocity (s'TDI) in both groups of patients (Table 2). Although the change (delta value) from rest to peak stress was less pronounced in patients with DM, the difference between groups was not significant for all values (Table 2). As for parameters of diastolic function, comparison of data showed insignificant increase of left atrial volume index (LAVI), significant increase of early diastolic mitral annular tissue Doppler velocity (e'TDI) and insignificant increase of value of LV filling pressure expressed as E/e'ratio in patients with DM without significant difference between respective delta values (Table 2). Value of transmitral flow parameters showed a significant decrease in patients with DM and only for deceleration time (DT) and isovolumetric relaxation time for patients without DM, whereas delta value was border significant only for DT mostly for diabetic patients.

As for WMSI, besides it was insignificantly higher in diabetic patients at rest, it has been shown that the index was almost unchanged during stress in both groups of patients, whereas in diabetic patients GLS% showed worsening (more positive or decreased negative value) at maximal stress which was significantly different to those without DM who showed slight improvement (less positive or increased negative value) of GLS% (Table 3).

Table 2: Comparison of systolic and diastolic parameters during SE in patients divided according to the DM presence

Parameter	With DM N = 36		Without DM N = 69		p**
	At rest	Max stress	At rest	Max stress	
LVEF (%)	60.1 ± 8.7	60.4 ± 10.1	62.0 ± 8.2	63.7 ± 8.5	0.378
p*	0.922		0.076		
SV/BSA (ml/m^2)	41.8 ± 12.0	90.6 ± 21.6	43.5 ± 10.7	95.2 ± 25.0	0.486
p*	0.0001		0.0001		
CI (L/min/m^2)	3.4 ± 1.3	9.2 ± 2.7	3.0 ± 0.8	8.8 ± 2.9	0.896
p*	0.0001		0.0001		
s'TVI (cm/s)	7.7 ± 1.4	9.1 ± 2.3	7.4 ± 1.0	8.6 ± 2.0	0.461
p*	0.0001		0.0001		
LAVI (ml/m^2)	19.5 ± 5.3	19.6 ± 5.6	18.3 ± 6.0	19.2 ± 7.0	0.756
p*	0.753		0.156		
E/A	0.9 ± 0.2	0.8 ± 0.2	1.0 ± 0.2	1.0 ± 0.3	0.318
p*	0.033		0.504		
DT (ms)	195.0 ± 53.1	130.9 ± 44.3	206.0 ± 47.1	167.6 ± 52.4	0.051
p*	0.0001		0.0001		
IVRT (ms)	83.3 ± 16.3	63.1 ± 11.2	85.4 ± 15.1	65.4 ± 8.6	0.787
p*	0.0001		0.0001		
e' TDI average	9.3 ± 2.2	10.1 ± 2.4	9.6 ± 1.9	10.7 ± 2.4	0.919
p*	0.009		0.0001		
E/e' average	9.5±2.8	9.6±2.7	8.9±2.4	8.9±2.6	0.973
p*	0.912		0.754		

LVEF = left ventricular ejection fraction; SVI = systolic volume indexed to BSA; CI = cardiac output indexed to BSA; s'TDI = peak systolic mitral annular velocity by TDI; LAVI = maximum left atrial volume indexed to BSA; E velocity = early mitral inflow velocity; A velocity = late mitral inflow velocity; DT = deceleration time; IVRT = isovolumetric relaxation time; e' TDI = early diastolic mitral annular tissue Doppler velocity; *p < 0.05 for comparison between groups; **p = comparison of delta values between two groups.

However, the significant difference of GLS% delta values between diabetic and non-diabetic patients was not confirmed (Table 3). About the number of segments with longitudinal LV strain < 12% the outcome of stress was the same as for GLS%. Thus there was no a significant difference in stress outcome in diabetic patients *vs* non-diabetic (Table 3). Also, the percentage of positive results according to the worsening of WMSI and/or GLS% during SE were insignificantly more frequent in diabetic patients (Table 3).

Table 3: Comparison of wall motion score index and global longitudinal LV strain values during SE in patients divided according to the DM presence

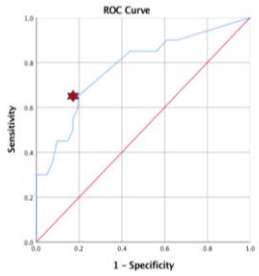
Parameter	With DM N = 36	Without DM N = 69	p
WMSI at rest	1.12 ± 0.14	1.05 ± 0.09	0.337
WMSI at peak stress	1.10 ± 0.15	1.07 ± 0.13	0.579
ΔWMSI	0.01 ± 0.17	-0.01 ± 0.12	0.126
GLS (%) at rest	-15.0 ± 3.7	-16.6 ± 5.0	0.905
GLS (%) at peak stress	-14.8 ± 3.5	-17.0 ± 4.4	0.008
Δ GLS (%)	-0.13 ± 2.85	0.36 ± 4.70	0.559
No. LS seg < -12% at rest	4.9 ± 3.0	3.4 ± 2.5	0.847
No. LS seg < -12% at peak stress	4.9 ± 3.8	4.0 ± 3.1	0.126
ΔNo. LS seg < -12%	0.00 ± 2.84	-0.60 ± 2.85	0.479
Positive results according to WMSI (n/%)	9/25	14/20.3	0.580
Positive results according to GLS% (n/%)	14/44.4	26/37.7	0.520

WMSI = wall motion score index; Δ = change from rest to maximal dose of stressor; GLS = global longitudinal strain; LG=longitudinal strain.

There was statistically significant correlation between presence of DM and decrease of E/A ratio (r = -0.278; p = 0.004), shortening of DT (r = -0.332; p = 0.001) and worsening of GLS% (r = 0.245; p = 0.012) at maximal stress, while such correlation didn't appeared regarding stress WMSI. After SE coronary angiography was done in 61 patients. Diabetic patient in comparison to those without had with borderline significance more new and/or worsening CAD (44.8% *vs*. 21.9%; p = 0.057; respectively), especially multivessel disease (34.5% *vs*. 12.5%; p = 0.044; respectively) as well as significantly greater Syntax score (8.0 *vs*. 2.5; p = 0.010, respectively) and insignificantly more frequently presence of coronary artery plaque. There was statistically significant correlation between presence of DM and angiographycaly proven new and/or worsening CAD (r = 0.244; p = 0.058), multivessel disease (r = 0.267; p = 0.037), Syntax score (r = 0.327; p = 0.010) and diseased Cx coronary artery (r = 0.306; p = 0.016). In addition presence of new and/or worsening CAD was significantly correlated with WMSI at maximal stress (r = 0.386; p = 0.002) and its change during SE (r = -0.645; p = 0.0001) along with GLS% at maximal stress (r = 0.262, p = 0.042) as well as without any correlation to functional parameters either of systolic or diastolic LV function.

To determine the independent predictors of new and/or worsening CAD among patients who were pharmacologically stressed, we performed multiple stepwise logistic regression analysis with demographic, clinical and echocardiographic covariates that showed significant relation to it. The results that were adjusted for age and gender, demonstrated that WMSI at maximal stress (OR = 375.8; 95% CI 6.2-22649.7; p = 0.005) and presence of DM (OR = 3.8; 95%CI 1.078-13.396; p = 0.038) appeared as independent predictors of presence of new and/or worsening CAD during SE. Positive predictive value of the model was 69.2%, while the negative was 77%.

Figure 1 demonstrates the receiver operator characteristics curve (ROC) for predictive probability from the model which showed an AUC value of 0.777. Also, WMSI at maximal stress and the presence of DM during pharmacological SE as a model were associated with an acceptable sensitivity of 64% and higher specificity (1-20) of 80%.



Area Under the Curve

Test Result Variable(s): Predicted probability

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.777	.066	.000	.648	.907

The test result variable(s): Predicted probability has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 1: ROC curve for the presence and/or worsening of angiographic CAD using WMSI and DM presence during pharmacological SE

Discussion

Given that the change of systolic LV functional data or rather its improvement from rest to peak stress was less pronounced in patients with DM, as well parameters of diastolic function and its change with stress were almost insignificant (slightly increase of E/e' in DM), we could not find the significant functional marker in order to make a difference in patients with and without DM. Such results mainly differ from our expectations taking into account that patients with DM were significantly older, had more frequently a history of hypertension, significantly more new and/or worsening CAD, especially multivessel disease and greater Syntax score. Although numerous studies highlighted the role of diastolic stress as predictor of CAD presence and its prognosis [27], [28], [29], [30], [31] as well in diabetic patients [32], [33], [34], we could not provide such evidence mainly due to small number of patients with DM in our study, lack of performed coronary angiography in all patients, possible good controlled risk factors as well as due to well-known technical factors regarding TDI velocity that can only be measured in one dimension and is significantly limited by angle dependence which produce difficulties in assessment of multiple wall segments (especially apical segments), along with influence of heart motion and contraction of adjacent segments on TDI data during SE, especially during dobutamine application [35]. Also, we could speculate that the results of our study were in line with those of Fang et al., [36] who detected the normal response to stress in diabetic patients without a significant difference with a control group that might be due to the early stage of diabetic cardiomyopathy.

Our study showed that visual wall motion analysis expressed as WMSI in comparison to longitudinal strain assessed using speckle tracking (GLS%) didn't make a difference in SE outcome regarding DM presence. WMSI was almost unchanged at maximal stress in diabetic patients, and paradoxically showed even slight increment in patients without DM. However, GLS% showed significant worsening at maximal stress in diabetic patients which was significantly different to those without DM who showed slight improvement. Presence of DM was significantly correlated with worsening of GLS% at maximal stress, while such correlation didn't appear regarding stress WMSI. Given that WMSI and GLS% at maximal stress were significantly correlated to new and/or worsening CAD, we were expected that two of them would appear as its predictors, but regression analysis revealed that only WMSI at maximal stress and DM appeared as independent predictors of presence of new and/or worsening CAD during SE providing sensitivity of the model of 64% and specificity of 80%.

Since Voight et al., [37] demonstrated that strain rate analysis during SE provides objective evidence of ischemia, the interest of using quantitative

assessment of LV systolic function by 2D speckle tracking during SE gained interest because it removes the subjective nature of visual assessment by wall motion analysis (WMA) in detecting CAD. Hence, a number of studies have shown that imaging GLS% was as good as [38], [39] or even superior [37], [40] [41] to conventional echocardiography, subsequently lot of them concluding that GLS% provides incremental diagnostic accuracy in combination with expert WMA [42] in detecting CAD. Our results were partly in line with such conclusions; however only WMSI appeared as independent predictor of CAD which was consistent with the results of dobutamine SE study of Celutkiene et al., [43] who stressed that none of the single quantitative parameters investigated was able to identify significant CAD with a comparable diagnostic accuracy *vs* visual assessment using WMA. Also, Nagi et al., [44] fail clearly to demonstrate the diagnostic benefit of strain analysis over expert WMA alone during contrast-enhanced SE.

Given that in diabetic patients risk stratification is a major objective considering their increased risk for CAD and its' major cardiovascular events, several well-known studies published their results that revealed prognostic ability of SE in diabetic patients on the basis of conventional WMA [45], [16], [18], [44], [33], [34]. Thus, the degree of worsening WMSI during SE, especially its' multivessel distribution correlated with the extent of CAD which was consistent with our study, but more importunately with increasing cardiac events, including death in subsequent years. In this respect, it should be born in mind that data from studies [33], [34] emphasised that regardless a negative test result of SE based solely on wall motion criteria in diabetic patients it is associated with the less benign outcome which is why assessment of GLS% using speckle tracking would be an advantage. To our knowledge, data concerning LV myocardial deformation during SE in patients with DM are available only for longitudinal deformations and are still limited to very few studies. Although we could not confirm GLS% as independent predictor of CAD either in diabetic or nondiabetic patients, the value of GLS% at maximal stress appeared as significant distinctive parameter for DM presence as well as for more extensive CAD which is consistent with the study of Wierzbowska-Drabik et al., [45] who found more impaired GLS% in patients with DM and CAD at rest as well as at maximal SE in comparison to their counterparts with CAD but without DM, hence they concluded that coexisting CAD and DM had synergistic detrimental effect on myocardial strain. Furthermore, Philouze et al., [46] confirmed in their study that dobutamine SE unmasks functional alterations expressed by myocardial mechanics in patients with DM that could be barely detectable at rest mainly in asymptomatic patients with uncomplicated DM.

The main limitation of our study was a relatively small number of patients, especially with DM. Patients with DM were older, with higher BMI, more frequent history of hypertension and previous CAD, which might influence the obtained longitudinal strain data. There was no assessment of the influence of metabolic control and therapeutic interventions in diabetic patients. Coronary angiography was not available in all patients, but this is in line with clinical guidelines, which do not recommend invasive testing in asymptomatic patients. Aiming to good spatial resolution and image quality for satisfactory speckle-tracking during SE, we included in the study and analysed only individuals with good acoustic windows.

The additional long-term analysis of the prognostic significance of reduced GLS% at rest and stress in patients with DM may potentially increase the clinical utility of our observations.

In conclusion, although wall motion score index (WMSI) was insignificantly higher and GLS% was worse in diabetic patients at rest, it has been shown that the WMSI was almost unchanged during stress in both groups of patients, whereas in diabetic patients GLS% showed significant worsening at maximal stress which. However, besides assessment of GLS% appeared superior to qualitative analysis expressed by WMSI in making difference regarding DM presence, regression analysis revealed that only WMSI at maximal stress and DM appeared as independent predictors of presence of new and/or worsening CAD during SE which lead to conclusion of usefulness of using qualitative and/or quantitative parameters in detection of CAD in patients with DM during stress echocardiography.

Referencies

1. Kannel WB, McGee DL. Diabetes and cardiovascular risk factors: the Framingham study. Circulation. 1979; 59(1):8-13. https://doi.org/10.1161/01.CIR.59.1.8

2. Consensus development conference on the diagnosis of coronary heart disease in people with diabetes: 10-11 February 1998, Miami, Florida. American Diabetes Association. Diabetes Care. 1998; 21(9):1551-9. https://doi.org/10.2337/diacare.21.9.1551 PMid:9727908

3. The BARI Investigators. Influence of diabetes on 5-year mortality and morbidity in a randomized trial comparing CABG and PTCA in patients with multivessel disease. Circulation. 1997; 96(6):1761-9. https://doi.org/10.1161/01.CIR.96.6.1761

4. Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic dubjects with and without prior myocardial infarction. N Engl J Med. 1998; 339(4):229-34. https://doi.org/10.1056/NEJM199807233390404 PMid:9673301

5. The sixth report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure national institutes of health national heart, lung, and blood institute national high blood pressure education program. http://www.sld.cu/galerias/pdf/servicios/hta/6to._reporte_del_jnc_u

sa.pdf

6. Collins R et al. MRC/BHF Heart protection study of cholesterol lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controled trial. Lancet. 2003; 361:1005-16

7. Collaborative overview of randomised trials of antiplatelet therapy--I: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. Antiplatelet trialists' Collaboration. BMJ. 1994; 308(6921):81-106. https://doi.org/10.1136/bmj.308.6921.81 PMid:8298418 PMCid:PMC2539220

8. Supplement 1. American Diabetes Association: clinical practice recommendations 2000. Diabetes Care. 2000; 23(Suppl 1):S1-116. PMid:10859117

9. Detre KM, Guo P, Holubkov R, et al. Coronary revascularization in diabetic patients: a comparison of the randomized and observational components of the Bypass Angioplasty Revascularization Investigation (BARI). Circulation. 1999; 99(5):633-40. https://doi.org/10.1161/01.CIR.99.5.633 PMid:9950660

10. Cameron M et al. Diabetes and periferal vascular disease. J Vasc Surg. 1999; 30(2);373-84. https://doi.org/10.1016/S0741-5214(99)70154-0

11. May O, Arildsen H, Damsgaard EM, Mickley H. Cardiovascular autonomic neuropathy in insulin-dependent diabetes mellitus: prevalence and estimated risk of coronary heart disease in the general population. J Intern Med. 2000; 248(6):483-91. https://doi.org/10.1046/j.1365-2796.2000.00756.x PMid:11155141

12. Koistinen MJ. Prevalence of asymptomatic myocardial ischaemia in diabetic subjects. BMJ. 1990; 301(6743):92-5. https://doi.org/10.1136/bmj.301.6743.92 PMid:2390590 PMCid:PMC1663397

13. Tarnow L, Rossing P, Gall MA, Nielsen FS, Parving HH. Prevalence of arterial hypertension in diabetic patients before and after the JNC-V. Diabetes Care. 1994; 17(11):1247-51. https://doi.org/10.2337/diacare.17.11.1247 PMid:7821162

14. Nahser PJ, Brown RE, Oskarsson H, Winniford MD, Rossen JD. Maximal coronary flow reserve and metabolic coronary vasodilation in patients with diabetes mellitus. Circulation. 1995; 91(3):635-40. https://doi.org/10.1161/01.CIR.91.3.635 PMid:7828287

15. Elhendy A, van Domburg RT, Poldermans D, et al. Safety and feasibility of dobutamine-atropine stress echocardiography for the diagnosis of coronary artery disease in diabetic patients unable to perform an exercise stress test. Diabetes Care. 1998; 21(11):1797-802. https://doi.org/10.2337/diacare.21.11.1797 PMid:9802723

16. Bigi R, Desideri A, Cortigiani L, Bax JJ, Celegon L, Fiorentini C. Stress echocardiography for risk stratification of diabetic patients with known or suspected coronary artery disease. Diabetes Care. 2001; 24(9):1596-601. https://doi.org/10.2337/diacare.24.9.1596 PMid:11522705

17. Elhendy A, Arruda AM, Mahoney DW, Pellikka PA. Prognostic stratification of diabetic patients by exercise echocardiography. J Am Coll Cardiol. 2001; 37(6):1551-7. https://doi.org/10.1016/S0735-1097(01)01199-8

18. Marwick TH, Case C, Sawada S, Vasey C, Short L, Lauer M. Use of stress echocardiography to predict mortality in patients with diabetes and known or suspected coronary artery disease. Diabetes Care. 2002; 25(6):1042-8. https://doi.org/10.2337/diacare.25.6.1042 PMid:12032112

19. Sozzi FB, Elhendy A, Roelandt JRTC, et al. Prognostic value of dobutamine stress echocardiography in patients with diabetes. Diabetes Care. 2003; 26(4):1074-8. https://doi.org/10.2337/diacare.26.4.1074 PMid:12663576

20. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging. 2015; 16:233-71. https://doi.org/10.1093/ehjci/jev014 PMid:25712077

21. Nagueh SF, Smiseth OA, Appleton CP, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr. 2016; 29:277-314. https://doi.org/10.1016/j.echo.2016.01.011 PMid:27037982

22. Pellikka PA, Nagueh SF, Elhendy AA, Kuehl CA, Sawada SG, American Society of Echocardiography. American Society of Echocardiography Recommendations for performance, interpretation, and application of stress echocardiography. J Am Soc Echocardiogr. 2007; 20(9):1021-41. https://doi.org/10.1016/j.echo.2007.07.003 PMid:17765820

23. Sicari R, Nihoyannopoulos P, Evangelista A, et al. Stress echocardiography expert consensus statement: European Association of Echocardiography (EAE) (a registered branch of the ESC). Eur J Echocardiogr. 2008; 9(4):415-37. https://doi.org/10.1093/ejechocard/jen175 PMid:18579481

24. Mor-Avi V, Lang RM, Badano LP, et al. Current and evolving echocardiographic techniques for the quantitative evaluation of cardiac mechanics: ASE/EAE Consensus statement on methodology and indications. J Am Soc Echocardiogr. 2011; 24(3):277-313. https://doi.org/10.1016/j.echo.2011.01.015 PMid:21338865

25. Yingchoncharoen T, Agarwal S, Popović ZB, Marwick TH. Normal ranges of left ventricular strain: A meta-analysis. J Am Soc Echocardiogr. 2013; 26(2):185-91. https://doi.org/10.1016/j.echo.2012.10.008 PMid:23218891

26. Sugimoto T, Dulgheru R, Bernard A, et al. Echocardiographic reference ranges for normal left ventricular 2D strain: results from the EACVI NORRE study. Eur Hear J - Cardiovasc Imaging. 2017; 18(8):833-40. https://doi.org/10.1093/ehjci/jex140 PMid:28637227

27. Burgess MI, Jenkins C, Sharman JE, Marwick TH. Diastolic stress echocardiography: hemodynamic validation and clinical significance of estimation of ventricular filling pressure with exercise. J Am Coll Cardiol. 2006; 47(9):1891-900. https://doi.org/10.1016/j.jacc.2006.02.042 PMid:16682317

28. Hyodo E, Hirata K, Hirose M, et al. Clinical use of doppler echocardiography and doppler tissue imaging in the estimation of myocardial ischemia during dobutamine stress echocardiography. J Am Soc Echocardiogr. 2008; 21(4):331-6. https://doi.org/10.1016/j.echo.2007.09.005 PMid:18029141

29. Joyce E, Delgado V, Bax JJ, Marsan NA. Advanced techniques in dobutamine stress echocardiography: focus on myocardial deformation analysis. Heart. 2015; 101:72-81. https://doi.org/10.1136/heartjnl-2013-303850 PMid:24760702

30. Agarwal R, Gosain P, Kirkpatrick JN, et al. Tissue doppler imaging for diagnosis of coronary artery disease: a systematic review and meta-analysis. Cardiovasc Ultrasound. 2012; 10(1):47. https://doi.org/10.1186/1476-7120-10-47 PMid:23199010 PMCid:PMC3542063

31. Hoffmann S, Jensen JS, Iversen AZ, et al. Tissue Doppler echocardiography improves the diagnosis of coronary artery stenosis in stable angina pectoris. Eur Hear J - Cardiovasc Imaging. 2012; 13(9):724-9. https://doi.org/10.1093/ehjci/jes001 PMid:22323549

32. Ha J-W,Lee H-C,Kang E-S, et al.Abnormal left ventricular longitudinal functional reserve in patients with diabetes mellitus:implication for detecting subclinical myocardial dysfunction using exercise tissue Doppler echocardiography. Heart. 2006; 93(12):1571-6. https://doi.org/10.1136/hrt.2006.101667 PMid:17449503 PMCid:PMC2095774

33. Cortigiani L, Bigi R, Sicari R, Landi P, Bovenzi F, Picano E. Prognostic value of pharmacological stress echocardiography in diabetic and nondiabetic patients with known or suspected coronary artery disease. J Am Coll Cardiol. 2006; 47(3):605-10. https://doi.org/10.1016/j.jacc.2005.09.035 PMid:16458144

34. Budoff MJ, Raggi P, Beller GA, et al. Noninvasive cardiovascular risk assessment of the asymptomatic diabetic patient: The imaging council of the American College of Cardiology. JACC Cardiovasc Imaging. 2016; 9(2):176-92. https://doi.org/10.1016/j.jcmg.2015.11.011 PMid:26846937

PMCid:PMC5371352

35. Picano E. Diabetic cardiomyopathy. the importance of being earliest. J Am Coll Cardiol. 2003; 42(3):454-7. https://doi.org/10.1016/S0735-1097(03)00647-8

36. You Fang Z, Najos-Valencia O, Leano R, Marwick TH, Brisbane F. Echo assessment of diabetes and mitral regurgitation patients with early diabetic heart disease demonstrate a normal myocardial response to dobutamine. J Am Coll Cardiol. 2003; 42:446-53. https://doi.org/10.1016/S0735-1097(03)00654-5

37. Voigt JU, Exner B, Schmiedehausen K, et al. Strain-rate imaging during dobutamine stress echocardiography provides objective evidence of inducible ischemia. Circulation. 2003; 107(16):2120-26. https://doi.org/10.1161/01.CIR.0000065249.69988.AA PMid:12682001

38. Ng ACT, Sitges M, Pham PN, et al. Incremental value of 2-dimensional speckle tracking strain imaging to wall motion analysis for detection of coronary artery disease in patients undergoing dobutamine stress echocardiography. Am Heart J. 2009; 158(5):836-44. https://doi.org/10.1016/j.ahj.2009.09.010 PMid:19853706

39. Uusitalo V, Luotolahti M, Pietilä M, et al. Two-dimensional speckle-tracking during dobutamine stress echocardiography in the detection of myocardial ischemia in patients with suspected coronary artery disease. J Am Soc Echocardiogr. 2016; 29(5):470-9. https://doi.org/10.1016/j.echo.2015.12.013 PMid:26852941

40. Bjork Ingul C, Stoylen A, Slordahl SA, Wiseth R, Burgess M, Marwick TH. Automated analysis of myocardial deformation at dobutamine stress echocardiography. J Am Coll Cardiol. 2007; 49(15):1651-9. https://doi.org/10.1016/j.jacc.2007.01.059 PMid:17433958

41. Rumbinaitė E, Žaliaduonytė-Pekšienė D, Vieželis M, et al. Dobutamine-stress echocardiography speckle-tracking imaging in the assessment of hemodynamic significance of coronary artery stenosis in patients with moderate and high probability of coronary artery disease. Medicina (B Aires). 2016; 52(6):331-9. https://doi.org/10.1016/j.medici.2016.11.005 PMid:27932192

42. Aggeli C, Lagoudakou S, Felekos I, et al. Two-dimensional speckle tracking for the assessment of coronary artery disease during dobutamine stress echo: clinical tool or merely research method. Cardiovasc Ultrasound. 2015; 13:43. https://doi.org/10.1186/s12947-015-0038-z PMid:26498476 PMCid:PMC4619392

43. Celutkiene J, Zakarkaite D, Skorniakov V, et al. Quantitative approach using multiple single parameters versus visual assessment in dobutamine stress echocardiography. Cardiovascular ultrasound. 2012; 10:31. https://doi.org/10.1186/1476-7120-10-31 PMid:22846395 PMCid:PMC3495225

44. Nagy AI, Sahlen A, Manouras A, et al. Combination of contrast-enhanced wall motion analysis and myocardial deformation imaging during dobutamine stress echocardiography. Eur Hear J - Cardiovasc Imaging. 2015; 16(1):88-95. https://doi.org/10.1093/ehjci/jeu171 PMid:25187604

45. Elhendy A, Arruda AM, Mahoney DW, et al. Prognostic stratification of diabetic patients by exercise echocardiography. J Am Coll Cardiol. 2001; 35:1551-7. https://doi.org/10.1016/S0735-1097(01)01199-8

46. Chaowalit N, Arruda AL, McCully RB, Bailey KR, Pellikka PA. Dobutamine stress echocardiography in patients with diabetes mellitus. J Am Coll Cardiol. 2006; 47(5):1029-36. https://doi.org/10.1016/j.jacc.2005.10.048 PMid:16516089

47. Wierzbowska-Drabik K, Trzos E, Kurpesa M, et al. Diabetes as an independent predictor of left ventricular longitudinal strain reduction at rest and during dobutamine stress test in patients with significant coronary artery disease. Eur Hear J - Cardiovasc Imaging. 2017; 0:1-11.

48. Philouze C, Obert P, Nottin S, Benamor A, Barthez O, Aboukhoudir F. Dobutamine stress echocardiography unmasks early left ventricular dysfunction in asymptomatic patients with uncomplicated type 2 diabetes: A comprehensive two-dimensional speckle-tracking imaging study. J Am Soc Echocardiogr. 2018; 31(5):587-97. https://doi.org/10.1016/j.echo.2017.12.006 PMid:29526563

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Clinical Science

Comparison of IFN-γ Levels in Children with Tuberculosis Disease (TB) and Latent Tuberculosis Infection (LTBI)

Katerina Boskovska[1*], Stojka Naceva-Fustic[2], Liljana Simonovska[3], Mirjana Dilberovska[1], Dragan Dacevski[1], Gorica Popova[1], Ivana Arnaudova[1], Irena Cakalarovska[1]

[1]Insitute for Respiratory Diseases in Children, Skopje, Republic of Macedonia; [2]University Children Hospital, Skopje, Republic of Macedonia; [3]Institute for Respiratory Diseases and Tuberculosis, Skopje, Republic of Macedonia

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***Correspondence:** Katerina Boskovska. Institute for Respiratory Diseases in Children, Skopje, Republic of Macedonia. E-mail: katerinaboss@yahoo.com

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Abstract

AIM: This study aimed to evaluate the importance of IFN-γ in the diagnosis of pediatric TB and LTBI and to compare the IFN-γ levels.

METHODS: We analysed 100 patients examined for possible M. tuberculosis infection or disease at the Institute of Respiratory Diseases in Children, Kozle, Skopje. Patients were divided into 2 groups: TB disease and LTBI. The following parameters were analyzed: demographic characteristics, history of previous exposure to active TB, BCG vaccination and presence of BCG scar, lung X-ray findings, tuberculin skin test by the Monteux method and the value of INF-γ according to the Quantiferon TB gold test, direct samples of acid-alcohol-resistant bacilli of sputum and Löwenstein Jensen cultures. Informed parental consent was obtained for each child included in the study.

RESULTS: In the LTBI group 60.9% had a scar from the vaccination while in the TB group 50% had BCG scar. TST induration diameters in children with or without BCG scar were significantly larger in patients with active TB. Children with active TB had significantly higher IFN-γ levels than children with LTBI. The IFN-γ for the cut-off of 0.35 IU/ml, has 64% sensitivity for detection of LTBI, versus 80.6% sensitivity for active disease. Children with close TB contact had significantly higher IFN-γ levels. Correlation between TST induration diameter and IFN-γ levels was stronger in the TB group.

CONCLUSION: IFN-γ levels are significantly higher in children with active TB, and children with close contact with TB patient. It has better sensitivity in active TB. Using both tests (IFN-γ and TST) can improve the diagnose of LTBI and TB in countries where vaccination with BCG is widespread.

Introduction

World Health Organization defines tuberculosis (TB) as an infectious bacterial disease caused by Mycobacterium tuberculosis (M. tuberculosis). Patients with lung tuberculosis from whose sputum M. tuberculosis bacilli are isolated are the main source of the infection. M. tuberculosis, which was discovered in 1882 by Robert Koch, is anaerobic, facultative intracellular slow-growing acidophilic bacillus, naturally pathogenic only in humans [1] [2]. In children, TB usually develops as a result of close family contact with smear-positive TB patient.

According to WHO in 2016 there were 10.4 million new cases with TB, and 1.8 million deaths [3]. Tuberculosis is a contagious/infectious disease which is characterised by a high rate of morbidity and mortality in the world. Children account for 5 to 15% of the cases with tuberculosis worldwide; they are more often infected and have more severe forms of the disease [4]. In the R. Macedonia in 2012 the prevalence was 26/100,000. In 2014, 285 new cases of tuberculosis were discovered, the rate is 13.8 per 100.000 populations, which is 38 cases less in comparison with 2013 when the number of newly discovered cases amounted to 325 persons. In 2016 the incidence was 16 per 100000 population.

Diagnosis of tuberculosis in children is based on data about the history of the disease, epidemiologic data, clinical signs, laboratory analyses, x-ray examinations and immunologic examinations, tuberculin skin test (TST) and Interferon-Gamma Release Assays (IGRA) tests, while the unique secure proof for correct diagnosis is isolation of the causer from biologic material [5] [6]. Establishing the diagnosis in children may be difficult because the symptoms are often very discreet; there is not self-recognition of the disease; direct microscopic

preparations are positive only in 10 to 15%; positive cultures are obtained in 30% of children, whereas in smaller age groups even less than in 20% [5] [6]. Latent tuberculosis infection (LTBI) is defined as a state of persistent immune response to stimulation by M. tuberculosis antigens with no evidence of clinically manifest active TB [7]. Common features for both TB and LTBI are positive TST and IGRA tests. LTBI is defined as an infection with tuberculous bacilli inside the granuloma where it remains in non-replicating condition but later it can be transformed into an active TB. However, recent experimental data support the dynamic model of LTBI presenting a continual endogenous reactivation and inflammatory response [8]. This has been supported by a Norwegian study from 2010 demonstrating that reactivation of tuberculosis is decreased over time [9]. The dynamic model offers explanation for the influence of isoniazid, a drug which influences the actively replicating bacilli only. As isoniazid prevents the episodes of reinfections with bacteria released from the resting phase, together with the delayed drainage and damaging of non-replicating bacteria in the stomach, latent infection weakens gradually [8]. The chance LTBI to develop into active tuberculosis during lifetime in infants amounts to 43%, in children at the age from 1 to 5 years it amounts to 29%, while in children aged from 11 to 15 years it is15%. In children with LTBI younger than five years the risk of TB development two years after the infection is 20-40% [10] [11]. The risk for active TB disease after infection depends on several factors, and the most important is the immunological status [7]. Prevention of active TB disease by treatment of LTBI is critical component of the WHO End TB Strategy [12]

The aim of this study was to evaluate the importance of the released IFN-γ from T lymphocytes IFN-γ in the diagnosis of pediatric TB and LTBI and to compare the IFN-γ levels between active TB and LTBI.

Material and Methods

In this study, we have analysed 100 patients with possible M. tuberculosis infection or tuberculosis disease who were admitted at the Institute for Respiratory Diseases in Children, Kozle, Skopje in the period from September 2014 to August 2017. In that period the total number of registered patients with TB disease in our Institute was 61, and 215 with LTBI.

Inclusion criteria

Children, aged from 1 to 15 years, with no history of tuberculosis disease, BCG vaccinated, with a history of exposure to active TB.

In the patients included in the study the following parameters were analyzed: demographic characteristics, history of previous exposure to active TB and the type of contact (close, distant, unknown), BCG vaccination and presence of BCG scar, lung X-ray findings, tuberculin skin test (TST) by the Mantoux method and the value of INF- γ according to the commercial Quantiferon TB gold test, direct samples of acid-alcohol-resistant bacilli of sputum and Löwenstein Jensen cultures.

Informed parental consent was obtained for each child included in the study.

Patients were divided into 2 groups:

1. TB disease (n=36): children with symptoms suggestive of TB, with abnormal lung X-ray findings, with or without positive ARB smear microscopy

2. LTBI(n=64): asymptomatic patients, with a history of exposure to active TB, or tuberculin hyperreactors, with normal lung X-ray.

The TB group consists of 36 children, 19 males, 17 females, and the LTBI group consists of 64 children, 29 females and 35 males, aged from 1 to 15 years.

Exclusion criteria

One patient with immunodeficiency and malnutrition, one with congenital cardiopathy and one with osteoarticular tuberculosis were excluded from the study

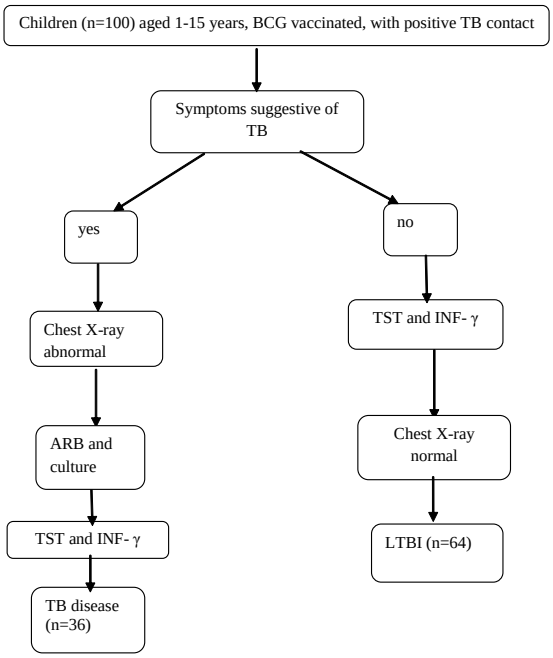

Figure 1: Included patients and study groups

Tuberculin skin test was performed on the volar aspect of the left forearm by injecting 0.1 ml

purified protein derivative (PPD) of 5 tuberculin units (TU) intracutaneously. Measuring of induration diameter was made after 72 hours. According to the National tuberculosis programme, positive TST is considered a transversal induration diameter of more than 10 mm [13].

In our study, in patients who did not have a BCG vaccination scar, the value of TST diameter ≥ 6 mm was considered as borderline for a positive skin test. In patients who had a BCG scar, the value of TST diameter ≥ 15 mm was considered as borderline for a positive skin test. In patients who had a BCG scar, the value of TST test < 15 mm was considered as negative [14] [15].

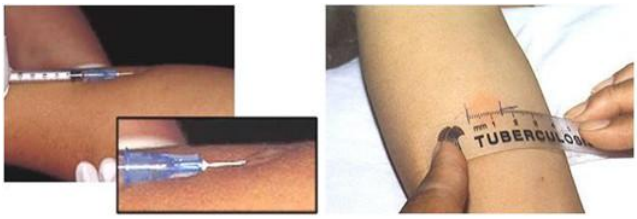

Figure 2: Description of performing and measuring induration in tuberculin skin test

Quantiferon TB Gold (QFT) analysis was performed at the Institute for Respiratory Diseases and TB in line with the guidelines of the manufacturer. The examination was performed in two phases: the incubation of the whole blood with antigens was made in the first phase, and in the second phase the measuring of IFN-γ was made by the ELISA method.

In 2-6 hours after blood taking, the test-tubes with blood were placed for incubation at 37ºC. 24 hours after incubation the test-tubes had been centrifuged, and the plasma was separated and frozen at -70ºC. The concentration of INF-γ was measured by the ELISA method (Enzyme-Linked Immunosorbent Assay) using the commercial test Quantiferon TB gold (Cellestis, QIAGEN Company). The values of INF-γ were expressed in international units on millimetre. As recommended by the manufacturer, the cut-off value for a positive test was INF- γ ≥ 0.35 IU/ml.

Results

A total of 100 children were analysed from whom 36 with TB disease and 64 with Latent tuberculosis infection. There was no significant difference between the sexes in both groups. Both sexes were equally represented. The age ranged from 1 to 15 years with an average age of 7.48 years.

In both groups, there was no significant difference between young children from 1-5 years old, and older than 5 years.

In the TB group, 55.6% were from the rural area, while in the LTBI group there was a significantly higher frequency of children from an urban area (70.3%).

The results obtained about BCG showed that all 100 children were BCG vaccinated at their birth. In the LTBI group, 60.9% had a scar from the vaccination while in 39.1% of the children the BCG scar could not be seen. In the TB group, there was an equal distribution of children with or without BCG scar (50%-50%).

Table 1: Basic demographic characteristics of the patients

Parameters	Children with LTBI (n = 64)	Children with TB (n = 36)	Significance
Gender male female	 54.7% 45.3%	 52.8% 47.2%	 $\chi2 = 0.037$ p = 0.982 (n.s.)
Age	6 (IQR = 7) (range 1 – 15)	5.5 (IQR = 8) (range 1 – 15)	F = 0.096 P = 0.953 (n.s.)
Age group Under 5 years Older than 5 years	 48.4% 51.6%	 50.0% 50.0%	 $\chi2 = 0.453$ p = 0.797 (n.s.)
Area Rural Urban	 29.7% 70.3%	 55.6% 44.4%	 $\chi2 = 6.821$ p = 0.033 (*)
BCG scar Yes No	 60.9% 39.1%	 50.0% 50.0%	 $\chi2 = 1.587$ p = 0.452 (n.s.)

According to the contact with a patient infected with active TB, our results showed that there is a significantly higher per cent of children with very close contact with active TB in the TB group. All of the children had a chest X-ray. In the LTBI group, all of them showed normal findings. In the TB group, chest X-rays were mostly with primary TB.

Direct specimens for acid-alcohol-resistant bacilli (ARB) from sputum as well as culture in the solid medium by Löwenstein Jensen were analysed in all children with active TB. Most of them, 88.9% were ARB negative, and only 11.1% ARB positive. Löwenstein Jensen cultures were positive in 22.2% of the TB cases and negative in the rest of them (77.8%). In the group of patients with LTBI most of the children, 89.1% were not analysed for ARB and Löwenstein Jensen culture. In only 10.9% of children, these analyses were performed and were all negative.

Table2: Specific characteristics in children with TB and LTBI

Parameters	Children with LTBI (n = 64)	Children with TB (n = 36)	Significance
Contact with active TB Close Distant Unknown	 48.4% 46.9% 4.7%	 75.0% 19.4% 5.6%	 $\chi2 = 7.523$ p = 0.023 (*)
Chest X-ray findings Normal TB pleuritis Primary TB Cavities Hilar lymphadenitis	 100.0% 0.0% 0.0% 0.0% 0.0%	 0.0% 8.3% 77.8% 5.6% 8.3%	 $\chi2 = 100.000$ $p = 9.87 \cdot 10^{-21}$(**)
ARB positive negative Not analyzed	 0.0% 10.9% 89,1%	 11.1% 88.9% 0,0%	 $\chi2 = 75.071$ $p = 4.99 \cdot 10^{-17}$(**)
Löwenstein–Jensen culture Positive Negative Not analyzed	 0.0% 10.9% 89.1%	 22.2% 77.8% 0.0%	 $\chi2 = 75.694$ $p = 3.66 \cdot 10^{-17}$(**)

TST induration diameters in children with or without BCG scar were significantly larger in patients with active TB (Table 3 and 4).

Table 3: TST in children with BCG scar

Parameter	Children with LTBI (n = 39)	Children with TB (n = 18)	Significance
Children with BCG scar			
TST induration diameter (mm)	8 (IQR = 11, range 3 – 24)	20 (IQR = 5, range 4 – 27)	Mann-Whitney test: *U* = 121.0 p = 0.00008(**)

In all 100 children, IFN-γ was measured with commercial Quantiferon TB gold test.

Table 4: TST in children without BCG scar

Parameter	Children with LTBI (n = 25)	Children with TB (n = 18)	Significance
Children without BCG scar			
TST induration diameter (mm)	9 (IQR =13; range 2 – 34)	15 (IQR = 7; range 3 – 24)	Mann-Whitney test:*U* = 135.5 p = 0.027(*)

In this study, the IFN-γ for the cutoff of 0.35 IU/ml, had 64% sensitivity for detection of LTBI, versus 80.6% sensitivity for active disease.

In our results, the Kruskal-Wallis test showed statistically highly significant differences in the levels of IFN-γ (IU/ml) among the two groups of patients. The Mann-Whitney tests confirmed that children with active TB have significantly higher IFN-γ levels than children with LTBI, (*U* = 649.5; **p = 0.0003).

Table 5: IFN-γ levels (IU/ml)

Parameter	Children with LTBI (n = 64)	Children with TB	Significance
Levels of IFN-γ (IU/mL)	0.475 (IQR = 1.082) (range 0.030 – 3.042)	2.037 (IQR = 2.246) (range 0.024 – 4.764)	Kruskal-Wallis test: F = 46.042 p = $1.00 \cdot 10^{-10}$(**)

The test was considered positive with titer ≥ 0.35 IU/ml.

According to that cutoff, there was the significantly higher frequency of patients with a positive test in the TB group (80.6%) compared with the LTBI group (64.1%; χ^2 = 3.984, *p = 0.047)

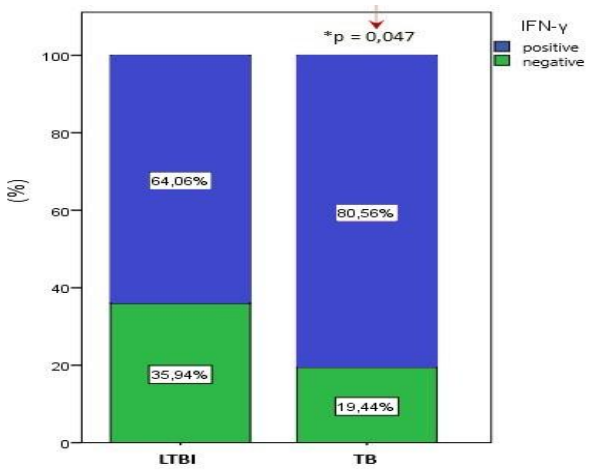

*Figure 3: significantly higher frequency of patients with a positive test in the TB group (*p < 0.05)*

In this study, we determined a new cut-off diagnostic value of IFN-γ level and its sensitivity and specificity for distinguishing LTBI from active TB in children in R. Macedonia. This was analysed with Receiver operating characteristic (ROC) curve.

Our analyses showed that IFN-γ level ≥ 0.822 IU/mL is optimal to discriminate children with LTBI and TB disease, with 74.3% sensitivity and 67.2% specificity (25.7% false negative and 32.8% false positive findings).

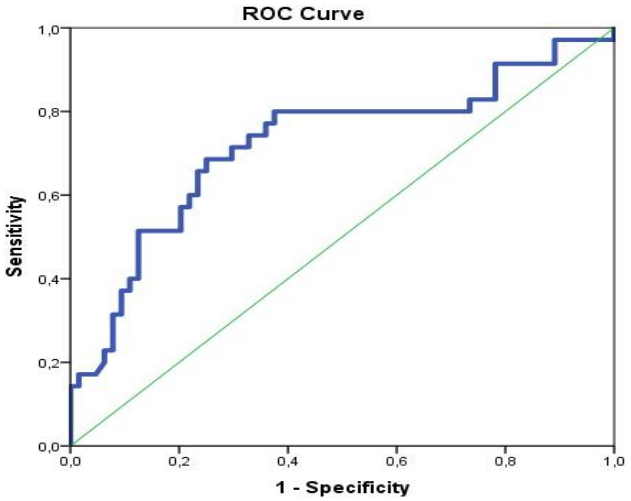

Figure 4: ROC analyses for determination of the cutoff diagnostic value of IFN-γ (IU/mL) for distinguishing children with TB disease from LTBI

About the influence of the age on IFN-γ levels, our results showed weak but statistically significant (*p = 0.013) and a positive correlation between the age and IFN-γ levels in both groups Correlation between TST induration diameter and IFN-γ levels was stronger in the group of children with active TB. We also compared the IFN-γ levels according to the contact with active TB patient.

As we can see from Table 6, patients with close contact have significantly higher IFN-γ levels (*p = 0.002).

Table 6: Comparison of the IFN-γ levels according to the contact with active TB patient

Parameter	Close contact (n = 58)	Distant contact (n = 37)	Significance
IFN-γ (IU/mL)	1.54 (IQR = 2.44) (range 0.04 – 4.76)	0.41 (IQR = 0.70) (range 0.02 – 4.47)	Mann-Whitney test: U = 666.0 p = 0.002 (*)

Discussion

When we speak about the eradication of TB, it is not enough only to treat patients with active tuberculosis but to diagnose and to treat adequately

those with LTBI.

Until recently, tuberculin skin test by the Mantoux method has been the uniquely available immunologic test for diagnosis of LTBI. It is an *in vivo* test that is based on measurement of the reaction of postponed hypersensitivity after injecting a mixture of mycobacterial antigens, PPD subcutaneously on the forearm [16]. PPD of 5 tuberculin units (TU) is used in our country. The size of induration on the site of injection is proportional to the strength of the immunologic response to competent cells. Induration diameter is read after 72 hours [17].

A positive outcome can be expected if two to eight weeks have passed after the infection with *M. tuberculosis*. Since the solution of PPD contains more than 200 protein components that are common for most of the mycobacteria, tuberculin test may give false-positive results in persons vaccinated with BCG or who were in contact with nontuberculous mycobacteria [18].

False-negative results may be found in persons with damaged or immature cell immunity such as patients infected with HIV, patients with iatrogenic-caused immunosuppression, children in younger age due to weak reactivity on the skin or if the result is falsely read as negative [19].

According to the above said it is clear that TST is not a secure test for detecting LTBI especially in countries where there is BCG vaccination such as in our country. In our study, TST induration diameters in children with or without BCG scar were significantly larger in patients with active TB.

Since 2004 the Quantiferon TB gold test has been used, and it has largely contributed to the diagnosis of LTBI and TB. The specific antigens that are used in this test are early secreted antigenic target-6KD (ESAT-6), culture filtrate protein-10KD (CFR-10) and TB 7.7. These antigens do not exist in BCG (Bacillus Calmette-Guerin) and most of the nontuberculous mycobacteria, except in M. Kansai, M. szulgai, M. marinum, M. flavescens and M. gastric [20]. Therefore, the chance of having false-positive results of IGRA tests is very small since T lymphocytes in healthy BCG-vaccinated uninfected persons as well as in those infected by nontuberculous mycobacteria do not secrete gamma interferon after stimulation with the mixture of antigens ESAT-6, CFP-10 and TB 7.7 [21].

The positive features of IGRA tests are their high diagnostic sensitivity and specificity, reproducibility and possible standardisation. The research of Pai *et al.,* (2008) showed 99% specificity of Quantiferon TB gold test in persons who were not BCG-vaccinated and 96% in BCG-vaccinated persons, while the sensitivity reached 78% [22]. Sun et al., found a sensitivity for all TB disease in children of 70% for ELISA (range: 57%–96%), 62% for ELISPOT (range: 40%–100%), and 71% for the TST (range: 43%–100%) [23]. In our study, the IFN-γ for the cutoff of 0,35 IU/ml, had 64% sensitivity for detection of LTBI, versus 80,6% sensitivity for active disease.

Regarding IFN-γ levels differences in children with LTBI and active TB, Whittaker et al., [24] described a lower amount of IFN-γ in LTBI patients compared to active TB, whereas Latorre et al. described no significant difference between these two groups [25]. Our results showed statistically highly significant differences in the levels of IFN-γ (IU/ml) among the two groups of patients. Children with active TB had significantly higher IFN-γ levels than children with LTBI (U = 649.5; **p = 0.0003). Connell et al., in 2 subsequent studies, showed a good agreement between positive IGRAs and TB disease, whereas a poor correlation between IGRAs and TST for the diagnosis of LTBI in children was found [26] [27].

About the influence of the age on IFN-γ levels, our results showed weak but statistically significant (*p = 0.013) and a positive correlation between the age and IFN-γ levels in both groups. Nakaoka et al. reported a decreased response to QFT in children < 5 years of age [28].

Correlation between TST induration diameter and IFN-γ levels was stronger in the group of children with active TB. Onur at al. reported that QFT positivity differed significantly according to TST induration diameter and the positivity rate was significantly higher in patients with an induration diameter of 10–14 and ≥ 15 mm compared to other diameters [29]. In our study, we also compared the IFN-γ levels in patients with close or distant contact with active TB. The results showed that patients with close contact have significantly higher IFN-γ levels (*p = 0.002). Kang et al., found the positive QFT rates 4%, 10%, 44% and 81% in low risk, casual contact, close contact, and active TB patients, respectively [30].

The limitations of this study are that we hadn't investigated children without risk of TB infection, so we couldn't make a correlation between IFN-γ levels in healthy children and children with LTBI and determine a new cut-off value of IFN-γ that can distinguish children with LTBI from healthy controls. The specificity of IFN-γ couldn't be analysed too.

In conclusion, the present study indicates that IFN-γ levels are significantly higher in children with active TB, and children with close contact with TB patient. It has better sensitivity in active TB, and the correlation between TST induration diameter and IFN-γ levels is stronger in children with active TB versus children with LTBI. Using both tests (IFN-γ and TST) can improve the diagnose of LTBI in children in countries like R. Macedonia where vaccination with BCG is widespread. According to our results that IFN-γ has better sensitivity and stronger correlation with TST inactive TB, these tests can contribute to better and certain diagnose of TB disease, because of a lack of microbiological proof for M. tuberculosis in children.

References

1. Report WHO 2010, Global tuberculosis control-surveillance, planning, financing. Geneva, Switzerland, 2010.

2. World Health Organization. Global Tuberculosis Control: Surveillance, Planning, Financing. Geneva, Switzerland: World Health Organization, 2002.

3. World Health Organization. Global Tuberculosis Report 2016. WHO 2016

4. Perez-Porcuna TM, Ascaso C, Malheiro A, Abellana R, Martins M, et al. Mycobacterium tuberculosis Infection in Young Children: Analyzing the Performance of the Diagnostic Tests. PLoS ONE. 2014; 9(5):e97992. https://doi.org/10.1371/journal.pone.0097992 PMid:24879374 PMCid:PMC4039466

5. Starke JR. Tuberculosis in children. Semin Respir Crit Care Med. 2004; 25:353-64. https://doi.org/10.1055/s-2004-829507 PMid:16088476

6. Marais BJ, Gie RP, Hesseling AC, et all. A refined symptom-based approach to diagnose pulmonary tuberculosis in children. Pediatrics. 2006; 118:1350-9. https://doi.org/10.1542/peds.2006-0519 PMid:17079536

7. Gatahun H,Mateelli A, Chaisson RE, Raviglione M. Latent Mycobacterium tuberculosis infection. Engl J Med. 2015; 372(22):2127-35. https://doi.org/10.1056/NEJMra1405427 PMid:26017823

8. Cardona PJ. A dynamic reinfection hypothesis of latent tuberculosis infection. Infection. 2009; 37:80-6. https://doi.org/10.1007/s15010-008-8087-y PMid:19308318

9. Wiker HG, Mustafa T, Bjune GA, Harboe M. Evidence for waning of latency in a cohort study of tuberculosis. BMC Infect Dis. 2010; 10: 1-10. https://doi.org/10.1186/1471-2334-10-37 PMid:20178619 PMCid:PMC2843612

10. Shingadia D, Novelli V. Diagnosis and treatment of tuberculosis in children. Lancet Infect Dis. 2003; 3: 624-32. https://doi.org/10.1016/S1473-3099(03)00771-0

11. Marais BJ, Pai M. Recent advances in the diagnosis of childhood tuberculosis. Arch Dis Child. 2007; 92:446-52. https://doi.org/10.1136/adc.2006.104976 PMid:17449528 PMCid:PMC2083717

12. Uplekar M, Weil D, Lonnroth K, Jaramillo E, Lienhardt C, Dias HM, et al.WHO's new end TB strategy. Lancet. 2015; 385(9979):1799-801. https://doi.org/10.1016/S0140-6736(15)60570-0

13. Review of the national tuberculosis programme on former Yugoslav Republic of Macedonia, 2016: 24-25.

14. Beyers N, Gie RP, Schaaf HS, Van Zyl S, Talent JM, Nel ED, Donald PR. A prospective evaluation of children under the age of 5 years living in the same household as adults with recently diagnosed pulmonary tuberculosis. The International Journal of Tuberculosis and Lung Disease. 1997; 1(1):38-43. PMid:9441057

15. Wang L, Turner MO, Elwood RK, et al. A meta-analysis of the effect of Bacille Calmette Guerin vaccination on tuberculin skin test measurements. Thorax. 2002; 57: 804–809. https://doi.org/10.1136/thorax.57.9.804 PMid:12200526 PMCid:PMC1746436

16. American Thoracic Society. Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Care Med. 2000; 161:1376-95. https://doi.org/10.1164/ajrccm.161.4.16141 PMid:10764337

17. Jakjovski Lj. Belodrobna tuberkuloza vo detska vozrast. Nova Makedoniaj: Skopje, 1996.

18. Diel R, Loddenkemper R, Meywald-Walter K, Gottschalk R, Nienhaus A. Comparative performance of tuberculin skin test, QuantiFERON-TB-Gold InTube assay, and T-Spot. TB test in contact investigations for tuberculosis. Chest. 2009; 135:1010-8. https://doi.org/10.1378/chest.08-2048 PMid:19017873

19. Lalvani A, Thillai M. Diagnosis of tuberculosis: principles and practice of using interferon-γ release assays (IGRAs). Breathe. 2009; 5:303-9. https://doi.org/10.1183/18106838.0504.302

20. Andersen P, Munk ME, Doherty TM, et all. Specific immune-based diagnosis of tuberculosis. Lancet. 2000; 356:1099-104. https://doi.org/10.1016/S0140-6736(00)02742-2

21. Chun JK, Kim CK, Kim HS, i sur. The role of whole blood interferon gamma assay for the detection of latent tuberculosis infection in bacillus Calmette-Guerin vaccinated children. Diagn Microbiol Infect Dis. 2008; 62:389-94. https://doi.org/10.1016/j.diagmicrobio.2008.08.022 PMid:18990532

22. Pai M, Zwerlig A, Menzies D. Systematic review: T-cell based assays for the diagnosis of latent tuberculosis infection – an update. Ann Intern Med. 2008;149:177-84. https://doi.org/10.7326/0003-4819-149-3-200808050-00241 PMid:18593687 PMCid:PMC2951987

23. Sun L, Xiao J, Miao Q, et al. Interferon gamma release assay in diagnosis of pediatric tuberculosis: a meta-analysis. FEMS Immunol Med Microbiol. 2011; 63(2):165–173. https://doi.org/10.1111/j.1574-695X.2011.00838.x PMid:22077219

24. Whittaker E, Gordon A, Kampmann B. Is IP-10 a better biomarker for active and latent tuberculosis in children than IFN gamma? PLoS One. 2008; 3:e3901. https://doi.org/10.1371/journal.pone.0003901 PMid:19065267 PMCid:PMC2588495

25. Latorre I, De Souza-Galvao M, Ruiz-Manzano J, et al. Quantitative evaluation of T-cell response after specific antigen stimulation in active and latent tuberculosis infection in adults and children. Diagn Microbiol Infect Dis. 2009; 65:236-46. https://doi.org/10.1016/j.diagmicrobio.2009.07.015 PMid:19822269

26. Connell TG, Curtis N, Ranganathan SC, et al. Performance of a whole blood interferon gamma assay for detecting latent infection with Mycobacterium tuberculosis in children. Thorax. 2006; 61:616–620. https://doi.org/10.1136/thx.2005.048033 PMid:16601088 PMCid:PMC2104654

27. Connel TG, Ritz N, Paxton GA, et al. A three-way comparison of tuberculin skin testing, quantiFERON-TB gold and T-SPOT. TB in children. PLoS ONE. 2008; 3:e2624. https://doi.org/10.1371/journal.pone.0002624 PMid:18612425 PMCid:PMC2440545

28. Nakaoka H, Lawson L, Squire SB, Coulter B, Ravn P, Brock I, Hart CA, Cuevas LE. Risk for tuberculosis among children. Emerging infectious diseases. 2006; 12(9):1383. https://doi.org/10.3201/eid1209.051606 PMid:17073087 PMCid:PMC3294731

29. Onur H, Hatipoğlu S, Arıca V, Hatipoğlu N, Arıca SG. Comparison of quantiferon test with tuberculin skin test for the detection of tuberculosis infection in children. Inflammation. 2012; 35(4):1518-24. https://doi.org/10.1007/s10753-012-9466-1 PMid:22535495 PMCid:PMC3397234

30. Kang YA, Lee HW, Yoon HI, Cho B, Han SK, Shim YS, Yim JJ. Discrepancy between the tuberculin skin test and the whole-blood interferon γ assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. Jama. 2005; 293(22):2756-61. https://doi.org/10.1001/jama.293.22.2756 PMid:15941805

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Clinical Science

Influence of Combined Therapy on Generation of Neutrophil Extracellular Traps in Patients with Cervical Cancer

Yuriy Fomenko[1], Yevgeniya Kolesnikova[2], Irina Beynikova[2], Larissa Muravlyova[2*], Valentina Sirota[1], Ryszhan Bakirova[3]

[1]Department of Oncology, Karaganda State Medical University, Karaganda, Kazakhstan; [2]Department of the Biological Chemistry, Karaganda State Medical University, Karaganda, Kazakhstan; [3]Department of Internal Diseases, Karaganda State Medical University, Karaganda, Kazakhstan

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***Correspondence:** Larissa Muravlyova. Department of the Biological Chemistry, Karaganda State Medical University, Karaganda, Kazakhstan. E-mail: lem2403@mail.ru

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Abstract

BACKGROUND: According to several authors, neutrophil extracellular traps (NETs) play an important role in the mechanisms of cancer development and metastatic processes, which allows them to be considered as a potential new target for the treatment of cancer.

AIM: To investigate the presence of extracellular neutrophil traps in the blood of patients with cervical cancer on the background of the combined treatment.

MATERIALS AND METHODS: The study was conducted in 28 patients with cervical cancer. Group 1 received only radiation therapy; Groups 2-radiation therapy with ftorafur; Group 3-radiation therapy with cisplatin. To determine the number of spontaneous extracellular neutrophilic traps in the blood of the examined individuals, we used a technique of I.I. Dolgushin and Yu.S. Andreeva.

RESULTS: Peripheral blood neutrophils in 53.57% (33.87; 72.49) of cervical cancer patients showed the ability to generate NETs before treatment. The ability to form NETs was observed in neutrophils isolated from 66.67% (9.43; 99.16) patients of the Group 1. After radiation therapy with ftorafur, the ability of blood neutrophils to form NETs was observed in 50% (1.26; 98.74) of cervical cancer patients. After radiotherapy with cisplatin, 37.50% (15.20; 64.57) of patients were found to have NETs formation

CONCLUSION: The ability to form NETs varied greatly after radiotherapy. The addition of chemotherapy drugs to radiation therapy did not increase the percentage of NETs in the blood of patients with cervical cancer but stimulated the appearance of basophil extracellular traps.

Introduction

The issue of the role of neutrophil extracellular traps (NETs) in oncology is at the stage of the study. An in vitro study of the formation of NETs after stimulation of neutrophils obtained from patients with colorectal cancer and healthy donors of the same age group was carried out. It was found that neutrophils of patients' blood formed spontaneous NETs. A significant increase in the number of NETs in response to the stimulus was recorded in patients with postoperative complications. There was no relationship between the number of NETs, the stage and the localisation of a tumour. According to the authors of the study, these results allow us to consider the formation of NETs as a potential target for therapy [1].

At the same time, according to other researchers, neutrophils isolated from the blood of patients with uterine cancer (stage Ia) had a reduced ability to form traps in response to stimulation by yeast cells [2].

It was suggested that the formation of NETs could be one of the mechanisms of tumour development. The basis for this assumption was the evidence that the granulocyte colony-stimulating factor, which is produced by many tumours and is determined in the blood of cancer patients, triggers the formation of NETs [3].

It has been shown by Garley M. et al., that tumour-associated neutrophil can form and release NETs, but their effects are controversial. There is evidence that NETs have anticarcinogenic properties, which associated with the direct destruction of tumour cells and stimulation of the immune system. In contrast, some authors point to the ability of traps to facilitate the migration of tumour cells or create a physical barrier between tumour cells and immunocompetent cells [4].

NETs are believed to be involved in the pathogenesis of the tumour process through the mechanism of tumour-associated thrombosis. Several models of triggering thrombosis with the participation of NETs have been proposed. Thus, it is shown that neutrophils can produce tissue factor (TF), which is released into the bloodstream during the formation of the trap. The tissue factor forms a complex with factor VII. Complex TF-VIIa can activate the coagulation cascade, leading to the formation of a thrombus. According to another model, factor XII can bind to DNA and histones in the composition of NETs and stimulates the formation of fibrin in the internal pathway [5], [6]. Another way of participation of NETs in the mechanism of thrombosis is the capture and activation of platelets. NETs threads bind platelets and promote their aggregation [7]. [8]. It was also suggested that adhesion molecules, including von Willebrand factor, fibronectin or fibrinogen, are involved in the interaction between NETs and platelets [9], [10].

According to several authors, NETs play an important role in the mechanisms of cancer development and metastatic processes, which allows them to be considered as a potential new target for the treatment of cancer [11], [12]. At the same time, they also express the opposite point of view on the role of NETs in oncopathology. Myeloperoxidase, proteinases and histones can be cytotoxic concerning tumour cells. Moreover, NETs itself is considered as a kind of skeleton for the capture of tumour cells, which prevents their further spread [13].

Purpose of the study: to investigate the presence of extracellular neutrophil traps in the blood of patients with cervical cancer on the background of the combined treatment.

Material and Methods

The study was conducted in 28 patients with cervical cancer in the Karaganda Regional Oncology Centre in 2018. The average age of patients was 48.3 ± 1.9 years, of which 22 patients were diagnosed with stage IIb and 6 patients with stage III (FIGO 2009). The study was approved by the local Ethical Committee at the Medical University of Karaganda. Informed consent was obtained from the patients before they were recruited into the study.

All patients underwent radiation therapy (RT): external beam radiation (EBRT) was performed at the Clinac 600 C (3D CRT), brachytherapy was implemented on the “Agat VU” device. The patients were divided into 3 study groups. Patients of the 1st Group received only RT, 2nd group-RT with ftorafur oral at a dose of 1200 mg on the days of external beam radiation (800 mg in the morning and 400 mg in the evening), the total dose of 27,600 mg; 3 the group-RT with cisplatin at the rate of 40 mg/m^2 7 days during the entire course of EBRT.

To determine the number of spontaneous extracellular neutrophilic traps in the blood of the examined individuals, we used a technique of I.I. Dolgushin and Yu.S. Andreeva [14]. The samples were stained with Hematoxylin-eosin and were examined microscopically under magnification of 1400. The results were expressed in the number of neutrophils that generated NETs in 100 neutrophils. Photos of NETs were obtained using the ToupView 3.7. We counted the percentage of patients whose smears contained NETs. Confidence intervals of percentages were determined by Clopper-Pearson.

Results

As a result of the study, it was shown that in practically healthy people, the ability of neutrophils to form NETs was not found, which corresponds with data from other researchers [15] [16]. Peripheral blood neutrophils in 53.57% (33.87; 72.49) of cervical cancer patients showed the ability to generate NETs before treatment. The number of NETs ranged from 1 to 23 in a smear (Figure 1).

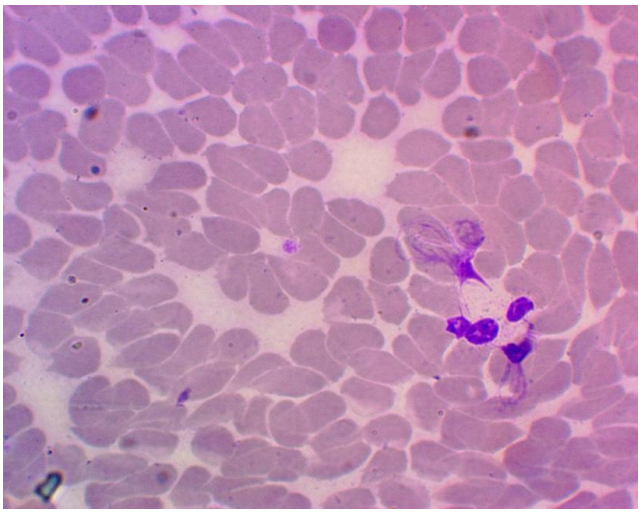

Figure 1: Neutrophil extracellular traps in the blood of a patient with cervical cancer (Hematoxylin-eosin staining, magnification 1400 x)

The dependence of NETs formation on the stage of cancer (IIb or III) was not found. NETs were not observed in 46.43% (27.51; 63.13) of the patients. In 3.57% (0.09; 18.35) of patients with cervical cancer, the formation of extracellular traps was detected not only by neutrophils but also by basophils.

The ability to form NETs was observed in neutrophils isolated from 66.67% (9.43; 99.16) patients of the 1st group. The number of NETs varied from 7 to 21 in a smear (Figure 2).

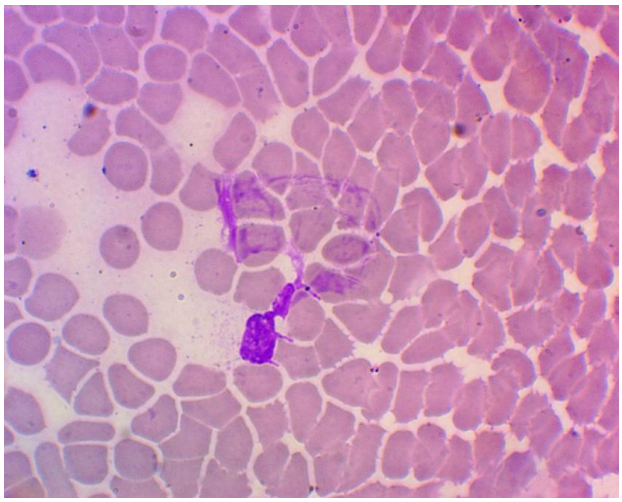

Figure 2: Neutrophil extracellular trap in the blood of a patient with cervical cancer after radiation therapy (hematoxylin-eosin staining, magnification 1400 x)

After radiation therapy with ftorafur, the ability of blood neutrophils to form NETs was observed in 50% (1.26; 98.74) of cervical cancer patients, but in a small amount - 1-2 in a smear (Figure 3). A feature of this group of patients should be considered the appearance in the blood of these patient's basophil extracellular traps (from 1 to 7 in a smear).

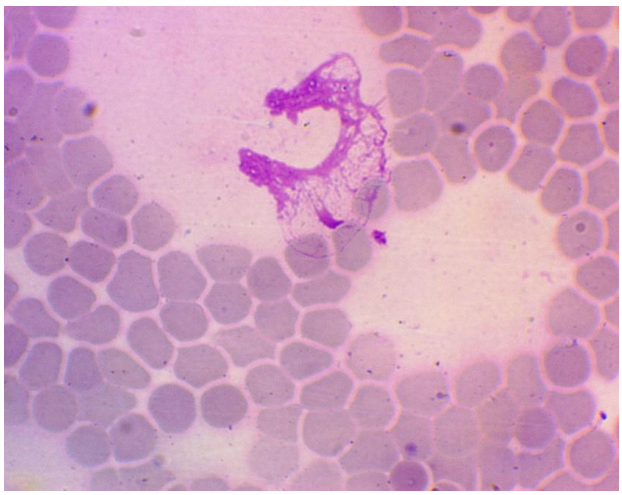

Figure 3: Neutrophil extracellular trap in the blood of a patient with cervical cancer after radiation therapy with ftorafur (Hematoxylin-eosin staining, 1400 x magnification)

After radiotherapy with cisplatin, 37.50% (15.20; 64.57) of patients were found to have NETs formation, and their number varied from 2 to 15 in a smear. In 12.50% (1.55; 38.35) of patients with cervical cancer, basophil extracellular traps were found in a number of 1 to 9 in a smear (Figure 4). It should be noted that neutrophil and basophil extracellular traps were found in the same patients.

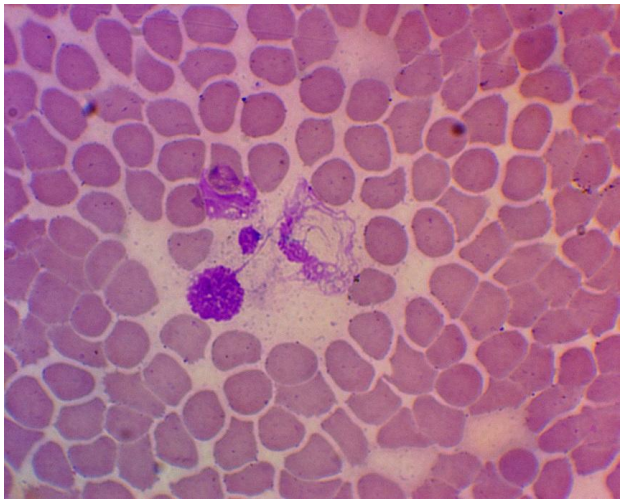

Figure 4: Neutrophil extracellular trap in the blood of a patient with cervical cancer after radiation therapy with cisplatin (Hematoxylin-eosin staining, 1400 x magnification)

Discussion

In all patients, the formation of NETs proceeds via suicidal NETosis which includes chromatin decondensation and releasing of network-like DNA after the rupture of the plasma membrane, i.e. according to the classical way [17]. It is noteworthy that not all neutrophils isolated from patients with cervical cancer have demonstrated the ability to form NETs. In our opinion, this is due to several reasons. First of all, neutrophils in the blood may be present in several subpopulations [18]. In some pathological states, including oncology, along with high-density neutrophils, the appearance of low-density neutrophils has been described [19]. It has been established that in cancer, low-density neutrophils exhibit a reduced ability to explode [20] oxidatively, but they are more susceptible to the formation of NETs [21]. According to other researchers, in the circulation, there are subpopulations of immature, mature and old neutrophils. The older the neutrophil, the easier it forms the NETs [22]. Trellakis S. et al. established that the neutrophils of the peripheral blood of patients with certain types of cancer had a prolonged period of life [23], which made it possible to assume that tumour cells probably produce factors that modulate the life of neutrophils [24]. The main cause of pre-activation of neutrophils is the excessive generation of reactive oxygen species as a result of ionising radiation. This

assumption is based on literature data showing that hydrogen peroxide can be an inducer of NETs [25]. Addition of ftorafur and cisplatin to the radiotherapy did not lead to a significant increase in the number of NETs. In case of a combination of RT with cisplatin, the percentage of neutrophils capable of forming NETs was even lower than that before treatment. It is due to the decreased level of neutrophils under the influence of chemotherapy.

In conclusion, peripheral blood neutrophils generate NETs in 53.57% (33.87; 72.49) of cervical cancer patients before treatment. There are no NETs in healthy people.

There was no dependence of the formation of NETs on the stage of cervical cancer (IIb or III).

The ability to form NETs varied greatly after Radiotherapy. The addition of chemotherapy drugs to RT did not increase the percentage of NETs in the blood of patients with cervical cancer but stimulated the appearance of basophil extracellular traps.

References

1. Richardson JJR, Hendrickse C, Gao-Smith F, Thickett DR. Neutrophil Extracellular Trap Production in Patients with Colorectal Cancer In Vitro. Int. J. Inflam. 2017; 2017:4915062.

2. Abakumova TV, Antoneeva II, Gening TP, Dolgova DR, Gening SO. Phenotype of peripheral blood neutrophils in the initial stage of endometrial cancer. 2016; 58(1):23-29.

3. Demers M, Krause DS, Schatzberg D, et al. Cancers predispose neutrophils to release extracellular DNA traps that contribute to cancer-associated thrombosis. Proc Natl Acad Sci USA. 2012; 109(32):13076–13081. https://doi.org/10.1073/pnas.1200419109 PMid:22826226 PMCid:PMC3420209

4. Garley M, Jabłońska E, Dąbrowska D. NETs in cancer. Tumour Biol. 2016; 37(11):14355-14361. https://doi.org/10.1007/s13277-016-5328-z PMid:27614687

5. Von Brühl ML, Stark K, Steinhart A, et al. Monocytes neutrophils and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. J Exp Med. 2012; 209(4):819-35. https://doi.org/10.1084/jem.20112322 PMid:22451716 PMCid:PMC3328366

6. Wolberg AS, Aleman MM, Leiderman K, Machlus KR. Procoagulant activity in hemostasis and thrombosis: Virchow's triad revisited. Anesth Analg. 2012; 114(2):275-85. https://doi.org/10.1213/ANE.0b013e31823a088c PMid:22104070 PMCid:PMC3264782

7. Ma A, C, Kubes P. Platelets neutrophils and neutrophil extracellular traps (NETs) in sepsis. J Thromb Haemost. 2008; 6(3):415–20. https://doi.org/10.1111/j.1538-7836.2007.02865.x PMid:18088344

8. Fuchs TA, Bhandari AA, Wagner DD Histones induce rapid and profound thrombocytopenia in mice. Blood. 2011; 118(13):3708-14. https://doi.org/10.1182/blood-2011-01-332676 PMid:21700775 PMCid:PMC3186342

9. Fuchs TA, Brill A, Duerschmied D, et al. Extracellular DNA traps promote thrombosis. Proc Natl Acad Sci USA. 2010; 107(36):15880-5. https://doi.org/10.1073/pnas.1005743107 PMid:20798043 PMCid:PMC2936604

10. Fuchs TA, Brill A, Wagner DD. Neutrophil extracellular trap (NET) impact on deep vein thrombosis. Arterioscler Thromb Vasc Biol. 2012; 32(8):1777-83. https://doi.org/10.1161/ATVBAHA.111.242859 PMid:22652600 PMCid:PMC3495595

11. Cools-Lartigue J, Spicer J, Najmeh S, Ferri L. Neutrophil extracellular traps in cancer progression. Cell Mol Life Sci. 2014; 71(21):4179-94. https://doi.org/10.1007/s00018-014-1683-3 PMid:25070012

12. Erpenbeck L, Schön MP. Neutrophil extracellular traps: protagonists of cancer progression? Oncogene. 2017; 36(18):2483-90. https://doi.org/10.1038/onc.2016.406 PMid:27941879

13. Berger-Achituv S, Brinkmann V, Abed UA, et al. A proposed role for neutrophil extracellular traps in cancer immunoediting. Frontiers Immunol. 2013; 4:48. https://doi.org/10.3389/fimmu.2013.00048 PMid:23508552 PMCid:PMC3589747

14. Dolgushin II, Andreeva YuS. Method for detection of extracellular neutrophilic traps: Russian Federation patent for invention. № 2384844; publ. 04.01.2008. [In Russian]

15. Korotina OL, Generalov II. Neutrophil extracellular traps: mechanisms of formation, functions. Immunopathology, allergology, infectology. 2012; 4:23-32.

16. Brinkmann V, Zychlinsky A. Beneficial suicide: why neutrophils die to, make NETs. Nature Rev. 2007; 5:577-82. https://doi.org/10.1038/nrmicro1710

17. Phillipson M., Kubes P. The neutrophil in vascular inflammation. Nature medicine. 2011; 17(11):1381-90. https://doi.org/10.1038/nm.2514 PMid:22064428

18. Gerasimov IG. Neutrophilic functional heterogeneity. Russian clinical laboratory diagnostics. 2006; 2:34-6.

19. Avila J, Adrover JM, Hidalgo A. Neutrophils in Homeostasis, Immunity, and Cancer. Immunity. 2017; 46(17):15-28. https://doi.org/10.1016/j.immuni.2016.12.012 PMid:28099862

20. Sagiv JY, Michaeli J, Assi S, et al. Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. Cell Rep. 2015; 10:562–573. https://doi.org/10.1016/j.celrep.2014.12.039 PMid:25620698

21. Villanueva E, Yalavarthi S, Berthier CC, et al. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. J Immunol. 2011; 187:538–52. https://doi.org/10.4049/jimmunol.1100450 PMid:21613614 PMCid:PMC3119769

22. Ortmann W, Kolaczkowska E. Age is the work of art? Impact of neutrophil and organism age on neutrophil extracellular trap formation. Cell Tissue Res. 2018; 371(3):473–88. https://doi.org/10.1007/s00441-017-2751-4 PMid:29250748 PMCid:PMC5820386

23. Trellakis S, Bruderek K, Dumitru CA, et al. Polymorphonuclear granulocytes in human head and neck cancer: enhanced inflammatory activity, modulation by cancer cells and expansion in advanced disease. Int J Cancer. 2011; 129:2183-93. https://doi.org/10.1002/ijc.25892 PMid:21190185

24. Danilova AB, Baldueva IA. Neutrophils as tumor microenviroment member. Problems of oncology. 2016; 62(1):35-44.

25. Savochkina AYu. Neutrophil extracellular traps: mechanisms of formation, detection methods, biological role: dissertation abstract. Dr. Med. Sciences. Chelyabinsk, 2012:48.

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Clinical Science

The Relationship between Clinical Findings of Shoulder Joint with Bone Damage of Shoulder Joint in Patients with Isolated Shoulder Blunt Trauma

Amin Zamani, Mohammad Davood Sharifi, Roohie Farzaneh, Hamideh Feiz Disfani, Behrang Rezvani Kakhki, Amir Masoud Hashemian*

Department of Emergency Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

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***Correspondence:** Amir Masoud Hashemian. Department of Emergency Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. E-mail: hashemianam@mums.ac.ir

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Abstract

BACKGROUND: Due to the prevalence of shoulder injuries among athletes and other people and the prevalence of radiography for these injuries, there are still no valid criteria for indication of doing shoulder radiography.

AIM: This study aimed to examine the relationship between some signs and clinical examinations of the shoulder with shoulder bone injuries and the need for radiography.

METHODS: This is a cross-sectional study. All patients aged 18-70 years who referred to the emergency ward of Imam Reza and Hasheminejad Hospital in the year 2014 due to blunt trauma and had criteria for entering the study and lacking exclusion criteria were included in the study process. Data on clinical symptoms, radiographic results, and final diagnosis were extracted from the patients' records through a questionnaire and analysed statistically.

RESULTS: There was a significant relationship between the clinical signs of patients Existence of ecchymosis in the shoulder fractures with glenoid and humerus fractures ($p = 0.029$, $p = 0.004$ respectively). There was also a significant relationship between clavicle fracture and limitation in shoulder rotation and abduction ($p = 0.000$ and $p = 0.001$ respectively). Other clinical symptoms did not show any significant relationship with radiographs indicative of the problem requiring specific treatment.

CONCLUSION: Although it is possible to define critters based on clinical symptoms that reduce the need for unnecessary radiographs that the does not reliably help inpatient treatment, but finding these critters to indicate the performance of the graphs in shoulder injuries requires further studies with the higher population and more clinical variables.

Introduction

The shoulder joint is a complex and intricate joint collection [1]. This joint has the greatest range of motion in the joints of the body and moves in an area more than one hemisphere [2]. In Emergency medicine, we commonly encountered with shoulder injuries [3]. The statistics show that 8-13% of all athletes injuries are related to shoulder and shoulder dislocation accounts for 50% of total dislocation in the emergency room [4]. Damage to the shoulder can be caused by a hit (direct or indirect) or excessive use. Shoulder injuries are common in most sports because almost every major sport uses a shoulder joint in some way [5].

Most shoulder injuries are evaluated based on bone damage with simple radiography, and in a few cases, CT scan, MRI, or shoulder ultrasound are needed [6]. Shoulder injuries consist of a large percentage of athlete's shoulder injuries, and they are a common cause for emergency attendance [7]. Timely diagnosis, treatment, and management of these patients are among the important tasks of the emergency department [8]. Getting accurate biography and physical examination in the first place

is the most important and most complete work to be done, including physical examination of the clavicle, shoulder, arm including precise inspection, touch, examination of active and passive motion of the joint, neurovascular evaluation, muscular strength tests and diagnostic tests [9].

Deformities due to glenohumeral dislocation, clavicle fracture, acromioclavicular joint separation are usually clinically apparent [10]. Effusion, ecchymosis, and erythema should be taken into account at first glance [11]. Sternoclavicular joint, clavicle, acromioclavicular joint and proximal humerus should be investigated in line with tenderness [12]. The lack of early diagnosis of shoulder bone damage results in undesirable long-term outcomes and, in some cases, permanent in the shoulder joint, some of which include chronic dislocation, degenerative injuries, and major vascular injuries [13].

The method for detecting the type and extent of injury to the shoulder joint is completed at first in the clinic, by taking into account the patient's precise history and physical examination, and it is used in the post-imaging phase to help diagnose that the simplest of which is standardised radiographs of the shoulder. Performing radiography involves spending time and expenses and exposing the patient to radiation [14]. Also, researches have shown that in many cases these graphs do not show any particular problem and are not a guide to treatment; therefore, researchers have always sought to find criteria for the diagnosis of suitable part of the body for the radiography of the damaged part of the body. In this regard, Ottawa and Nexus Critters have been developed as criteria for knee and neck radiography in trauma. In this regard, due to the prevalence of shoulder injuries among athletes and other people and the prevalence of radiography for these injuries, there is still no valid indication for shoulder radiography.

Therefore, in this study, we aimed to investigate the relationship between some signs and clinical examinations of the shoulder with shoulder bone injuries and the need for radiography.

Material and Methods

This study was a cross-sectional study. All patients aged 18-70 years who referred to Emam Reza and Hasheminejad Hospitals due to blunt shoulder trauma and had criteria for entering the study and did not have exclusion criteria were studied. On admission, the clinical criteria were evaluated with an initial examination including abduction, rotation, and examination of the localised tenderness of the acromioclavicular joint, clavicle, humerus, to examine shoulder joint ecchymosis. Then, the results of the examination were recorded in pre-prepared forms, followed by standard shoulder radiography (three posterior-anterior, axillary, Y-view views). Then the results of the graphs were examined and recorded. Then the findings of clinical examination were compared with radiographic findings.

Inclusion criteria:

1. Patients with shoulder blunt trauma.

2. Patients aged 18-70 years.

Exclusion criteria:

1. Previous history of shoulder bone injury.

2. Dissatisfaction for participating in the study.

3. Patients with previous shoulder deformity.

4. Patients with previous shoulder surgery.

5. Patients with inflammatory and degenerative diseases of the shoulder.

Based on $n = z^2pq/d^2$ with a confidence coefficient of 95% and $p = q = 0.5$, in the most conservative mode, the sample size of the sample can be calculated for a high value and finally 100 cases were considered.

The results after being recorded were analysed by SPSS software, MacAdam test, and Kappa coefficient, the agreement between clinical examinations and radiographic findings was assessed.

Results

In this study, of 104 patients referred to Emam Reza and Hasheminejad Hospital during the 2014 and 2015 due to blunt shoulder trauma, 67 were males (64%), and 37 were female (34%). This number of patients was divided by age into four groups: in the first group, that was the age range of 18 to 30 years, 59 patients with a frequency of 56.7%, in the second group who were between the ages of 31 and 43 years, 20 patients with a frequency of 19.2%, in the third group, in the age range of 44 to 56 years old, 14 patients with a frequency of 13.5% and the fourth group, aged between 57 and 70, there were 11 patients with a frequency of 10.6 % that the highest frequency was in the age group of 18-30 years and the lowest was in the age group of 57 and above.

These 104 patients were also categorised about damage in five groups: falling with 19 participants (18.3%), direct hit with 29 (27.9%), pedestrian and vehicle collisions with 16 (15.4%), bikers with 25 (24%) and car drivers with 15 (14.4%)

In the clinical examination for each patient,

the prevalence of clinical signs before radiography showed that 18 (17.3%) of patients were affected by joint ecchymosis, 92 (88.5%) had shoulder joint tenderness, 47 (45.2%) had limitations Shoulder joint rotation and 41 (39.4%) had had shoulder joint abduction limitation.

According to radiographic images, it was shown that 2 (1.9%) patients had combined fracture of shoulder bones, 2 (1.9%) had glenoid fracture, 2 patients (1.9%) had acromion fracture, 10 patients (9.6 %) had a clavicle fracture, 4 (3.8%) had scapular fracture, and 3 (2.9%) had a proximal humerus fracture.

Data analysis showed that there was no significant relationship between the four diagnostic variables in clinical examinations including joint ecchymosis, shoulder joint spotted tenderness, shoulder joint rotation limitation, shoulder joint abduction limitation with a combined fracture of shoulder bones (P > 0.05) (Table 1).

Table 1: Relationship between combined fracture of shoulder bones and clinical examination findings

		Combination fracture of shoulder bones		
		Has	Does not have	
Joint ecchymosis	Has	0	18	P-value
	Does not have	2	84	0.381
Shoulder joint spotted tenderness	Has	2	90	
	Does not have	0	12	0.481
Shoulder joint rotation limitation	Has	2	45	
	Does not have	0	57	0.202
Shoulder joint rotation abduction	Has	2	39	
	Does not have	0	63	0.153

Data analysis also showed that there is a significant relationship between joint ecchymosis and glenoid fracture (P < 0.05), but there is no meaningful relationship with other variables of clinical symptoms including shoulder joint spotted tenderness, shoulder joint rotation limitation and shoulder joint rotation abduction with glenoid fracture (P > 0.05) (Table 2).

Table 2: Relationship between glenoid fracture and Clinical examination findings

		Glenoid fracture		
		Has	Does not have	
Joint ecchymosis	Has	2	16	P-value
	Does not have	0	86	0.029
Shoulder joint spotted tenderness	Has	2	90	
	Does not have	0	12	0.481
Shoulder joint rotation limitation	Has	1	46	
	Does not have	1	56	0.891
Shoulder joint rotation abduction	Has	0	41	
	Does not have	2	61	0.518

Data analysis showed that there is no significant relationship between any of the diagnosed clinical symptoms with acromion fracture (P > 0.05) (Table 3).

Table 3: Relationship between acromion fracture and clinical examination findings

		Acromion fracture		
		Has	Does not have	
Joint ecchymosis	Has	3	15	P-value
	Does not have	6	80	0.186
Shoulder joint spotted tenderness	Has	9	83	
	Does not have	0	12	0.593
Shoulder joint rotation limitation	Has	4	43	
	Does not have	5	52	0.962
Shoulder joint rotation abduction	Has	5	36	
	Does not have	4	59	0.518

Also, data analysis showed that there is a significant relationship between the rotation limitation and shoulder joint abduction with clavicle fracture (P < 0.05) (Table 4).

Table 4: Relationship between Clavicle Fracture and Clinical Findings

		Clavicle fracture		
		Has	Does not have	
Joint ecchymosis	Has	2	16	P-value
	Does not have	8	78	0.683
Shoulder joint spotted tenderness	Has	8	84	
	Does not have	2	10	0.324
Shoulder joint rotation limitation	Has	9	37	
	Does not have	0	57	0.000
Shoulder joint rotation abduction	Has	9	32	
	Does not have	1	62	0.001

According to (Table 5) and P value, it was found that there was no significant relationship between any of the findings of clinical symptoms with Scapular fracture (P > 0.05).

Table 5: Relationship between scapular fracture and clinical examination findings

		Scapular fracture		
		Has	Does not have	
Joint ecchymosis	Has	2	16	P-value
	Does not have	2	84	0.683
Shoulder joint spotted tenderness	Has	3	89	
	Does not have	1	11	0.324
Shoulder joint rotation limitation	Has	1	46	
	Does not have	3	54	0.000
Shoulder joint rotation abduction	Has	2	39	
	Does not have	2	61	0.001

Also according to (Table 6) and P value, it was determined that there was a significant relationship between shoulder joint ecchymosis and humerus fracture (P < 0.05). However, in other clinical symptoms, there was no significant relation with the fracture of the humerus.

Data analysis showed that none of the trauma mechanisms included falling, direct hit, pedestrian, and collisions with the vehicle, bikers, and car drivers have no statistical relationship with clinical diagnostic symptoms including shoulder joint ecchymosis (P-value = 0.231), the shoulder joint spotted tenderness (P-value = 0.136), shoulder joint rotation limitation (P-

value = 0.603), and shoulder joint abduction limitation (P-value = 0.967).

Table 6: Relationship between Humerus Fracture and Clinical Findings

		Humerus Fracture		
		Has	Does not have	
Joint ecchymosis	Has	3	15	P-value
	Does not have	0	86	0.004
Shoulder joint spotted tenderness	Has	3	89	
	Does not have	0	12	0.387
Shoulder joint rotation limitation	Has	1	46	
	Does not have	2	55	0.672
Shoulder joint rotation abduction	Has	0	41	
	Does not have	3	60	0.277

Also, the findings of the relationship between gender and clinical signs of diagnosis including ecchymosis, tenderness, rotation limitation, and limitation of shoulder abduction showed that gender was significantly correlated with shoulder joint ecchymosis (P-value = 0.017), so that among women is common to be affected by ecchymosis, gender had no significant relationship with other clinical diagnostic symptoms.

Finally, the findings of the relationship between age and clinical symptoms showed that the age of patients had statistically significant relationship with shoulder joint ecchymosis (P-value = 0.001) and shoulder joint rotation limitation (P-value = 0.002) and did not have a statistically significant relationship with shoulder joint spotted tenderness (P-value = 0.131) and shoulder joint abduction limitation (P-value = 0.313).

The frequency of clinical signs of patients is shown in (Table 7).

Table 7: Table of Clinical Symptoms Frequency

	Frequency	Percentage
Ecchymosis	2	1.9
Ecchymosis and tenderness	10	9.6
Ecchymosis and tenderness and abduction	1	1
Ecchymosis and Tenderness and Rotation	2	1.9
Ecchymosis and tenderness and routine and abduction	3	2.9
Rotation	2	1.9
Rotation and abduction	8	7.7
Tenderness	31	29.8
Tenderness and abduction	13	12.5
Tenderness and rotation	16	15.4
Tenderness and rotation and abduction	16	15.4

To describe or find a pattern between clinical signs and radiographic findings and the occurrence of these symptoms, the Associate rules algorithms are used that is one of the data mining algorithms; these algorithms are variable in line with the coordinated occurrence of events in variables.

The meaningful and interesting rules are extracted as follows.

1. In 42% of cases, there was no ecchymosis and rotation, but tenderness was positive.

2. In 30% of the cases, there was no ecchymosis and no rotation and abduction, but tenderness was positive.

3. In 96% of cases, those who did not have rotation had a positive tenderness.

4. In 94% of the cases, those who did not have an addiction had positive tenderness.

Discussion

The shoulder is the most mobile joint that performs a vast range of actions, but on the other hand, it can be unstable and can, therefore, be at increased risk of injury [15]. In emergency medicine, we commonly encountered with shoulder injuries [16]. The statistics show that shoulder joint dislocation accounts for half of the total dislocation in the emergency room [4]. Timely diagnosis, treatment, and management of these patients are among the important tasks of the emergency department. Getting a precise biography and physical examination is what should be done first [17]. Failure to diagnose shoulder bone injuries leads to long-term adverse effects of the shoulder while paying attention to signs and symptoms in the doctor's examination leads to timely diagnosis, even in rare cases [18].

Various studies have been conducted to assess the value of clinical signs and different physical tests of shoulder to distinguish between types of shoulder injuries. Litaker and colleagues conducted a study to determine the value of biographies and physical examination in predicting the results of arthrography in older patients with the suspicion of Rotator Cuff Tear. This study aimed to reduce the need for other diagnostic measures, taking into account the age of patients and the value of correct diagnosis along the patient's bed. In their study, shoulder pain in 87.7% of cases was associated with Rotator Cuff Tear. They conclude that physical examinations can effectively show the rupture of Rotator Cuff, with important symptoms including the presence or absence of specific symptoms, the duration of symptoms, and the mechanism of injury [19].

Hedges and colleagues also conducted a systematic study of the diagnostic value of physical examination tests, and they concluded that it was not clear at the time of examination that the usual physical examination tests were useful in differentiating shoulder injuries [20]. In another study, they updated their previous study. Hedges has stated in this article that, based on the results of the previous study and his new study, he does not recommend using any shoulder physical examination (SHPE) alone for diagnosis. Of course, there are some tests that look like these, but they should be evaluated in more than

one study. Also, the use of several physical examinations together improves the accuracy of the diagnosis. The findings of this study appear to suggest that more emphasis should be placed on a comprehensive clinical evaluation, including biographies and physical examination [21].

After the biography and physical examination, the next diagnostic procedure is usually radiography to assess the type of shoulder injury. Most of the shoulders injuries in bone damage are examined with simple radiography, and in rare cases, CT scan, MRI, or shoulder ultrasound are needed. In this regard, some studies have shown that the use of shoulder radiography in the emergency department is excessive, which imposes cost and exposure to unnecessary radiation and time spent [22] [23]. In a study published by Fraenkel and colleagues in 1998, the results showed that only 20% of patients with shoulder radiography showed a special problem that needed special treatment and helped treat it [22]. Another study by Fraenkel et al., (2000) found that 88% of the patients with shoulder pain who received radiography in the emergency ward, radiography was not helpful therapeutically and did not provide any particular information to the therapist [23]. With regard to the research that has been made and the similar studies that have been carried out with regard to the use of radiography in knee trauma (Ottawa knee rule) and neck trauma (NEXUS Low-risk Criteria), it seems that Criteria can be defined according to the clinical symptoms of the patient with shoulder pain, which reduces the unnecessary use of radiography [24] [25].

This study aimed to investigate the relationship between some clinical signs and symptoms of the patient with the shoulder with the type of shoulder injury and the usefulness of shoulder radiography in the next therapeutic intervention.

The results of this study showed that 28 patients (27%) out of 104 patients had fractures, and therefore their radiography was helpful in treatment, which included 2% glenoid fracture (2 patients), 9% acromion fracture (9 patients), 10% clavicle fracture (10 cases), 4% Scapular fracture (4%), 3% proximal Humerus fracture (3 patients) and 2 patients with combined fractures. These results are roughly the same and close to the results of Fraenkel's study, which showed that about 20% of the combs' radiographs are medically informative and show a fracture or dislocation.

Among the clinical symptoms of patients, there was a significant relationship between ecchymosis in the shoulder and the glenoid and humerus fracture ($p = 0.029$ and $p = 0.004$, respectively). All cases of Humerus (3) and Glenoid fractures (2) were associated with ecchymosis, but the total number of cases was 18. In total, the clinical symptom of ecchymosis was useful in fractures and radiography in 27% of cases.

In the present study, there was a significant relationship between Clavicle fracture and limitation in shoulder rotation and abduction ($p = 0.001$ and $p = 0.001$ respectively). Of the 10 cases, the fracture of the clavicle of each 10 cases was associated with restriction of the shoulder joint rotation and 9 cases with the limitation of abduction. 45% of the subjects had had shoulder joint rotation limitation before radiography, and 39% had shoulder joint abduction limitation before the radiography.

In Fraenkel's study, the deformity was the most important variable in shoulder examination with radiography, so that among 23 patients diagnosed with deformity, 21 cases had suitable radiographs and indicating specific damage. Among the other 162 patients, only people over 43.5 years of age with a history of falls (40) had a great chance to have radiographs. No illness without deformity and a history of the crash (90) did not provide radiographs [22].

In our study, the relationship between ecchymosis and fracture of glenoid and humerus was significant, but in general, ecchymosis was useful only in 27% of cases with radiography. Also, in the present study, there was a significant relationship between Clavicle bone fracture with limitation of rotation and abdominal aberration, however, with 45% of subjects having had shoulder rotation limitation and the total fracture with limitation was 15 (including 10 clavicle fracture, 4 Acromion, and a scapula) and 39% had shoulder joint abduction, while the total fractures with it were 13 (including 9 cases of clavicle and 4 acromion fractures). Therefore, it can be said that the limitation of joints rotation and joint abduction in approximately 1/3 of the cases with is associated with fractures and, consequently, radiographs have been helpful. In Fraenkel study, the deformity was found in 91% of cases with fractures and helping factors in radiography, while in our study, the association between abduction and rotation and radiotherapy was 33% and ecchymosis was 27%. Regarding these results, it can be said that although the abnormalities and limitation of abduction and rotation have a significant relationship with radiography, this association is not so strong that it can be used as a guide critter to perform shoulder radiography and in case of using them as radiographic criterion, again in 66% of cases, unnecessary radiographies have been done.

In conclusion, the results of our study, along with the results of Fraenkel's studies [22], show that, although based on critters clinical symptoms, we can define that the need for unnecessary radiology, which does not help the patient treatment, is reduced, but finding these critters and generalizing the using them like the Ottawa and Nexus Critters require more studies with higher population and more clinical variables.

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References

1. McKoy BE, Bensen CV, Hartsock LA. Fractures about the shoulder: conservative management. Orthop Clin North Am. 2000; 31(2):205-16. https://doi.org/10.1016/S0030-5898(05)70141-3

2. Morey VM, Chua KH, Ng ZD, Tan HM, Kumar VP. Management of the floating shoulder: does the glenopolar angle influence outcomes? A systematic review. Orthopaedics & Traumatology: Surgery & Research. 2017. PMid:29246483

3. Strudwick K, McPhee M, Bell A, Martin-Khan M, Russell T. Methodology for the 'rapid review'series on musculoskeletal injuries in the emergency department. Emergency Medicine Australasia. 2018; 30(1):13-7. https://doi.org/10.1111/1742-6723.12906 PMid:29224233

4. Heyward OW, Vegter RJK, de Groot S, et al. Shoulder complaints in wheelchair athletes: A systematic review. PLoS One. 2017; 12(11):e0188410. https://doi.org/10.1371/journal.pone.0188410 PMid:29161335 PMCid:PMC5697842

5. Sekiguchi T, Hagiwara Y, Momma H, et al. Coexistence of Trunk or Lower Extremity Pain with Elbow and/or Shoulder Pain among Young Overhead Athletes: A Cross-Sectional Study. Tohoku J Exp Med. 2017; 243(3):173-178. https://doi.org/10.1620/tjem.243.173 PMid:29162768

6. Pezeshki Rad M, Mohammadifard M, Ravari H, et al. Comparing color Doppler ultrasonography and angiography to assess traumatic arterial injuries of the extremities. Iran J Radiol. 2015; 12(1):e14258. PMid:25785180 PMCid:PMC4347799

7. Yard EE, Comstock RD. Injuries sustained by pediatric ice hockey, lacrosse, and field hockey athletes presenting to United States emergency departments, 1990-2003. J Athl Train. 2006; 41(4):441-9. PMid:17273471 PMCid:PMC1748420

8. Gibbons LJ. Managing acute shoulder injuries in the emergency department. Emerg Nurse. 2014; 22(6):20-9. https://doi.org/10.7748/en.22.6.20.e1350 PMid:25270818

9. Beck S, Chilstrom M. Point-of-care ultrasound diagnosis and treatment of posterior shoulder dislocation. Am J Emerg Med. 2013; 31(2):449.e3-5. https://doi.org/10.1016/j.ajem.2012.06.017 PMid:22944540

10. Hootman JM. Acromioclavicular Dislocation: Conservative or Surgical Therapy. J Athl Train. 2004; 39(1):10-11. PMid:15085205 PMCid:PMC385255

11. Brun S. Initial assessment of the injured shoulder. Aust Fam Physician. 2012; 41(4):217-20. PMid:22472683

12. Lancaster ST, HOROwITz MA, Alonso JO. Complete acromioclavicular separations. A comparison of operative methods. Clinical orthopaedics and related research. 1987; (216):80-8. PMid:3815974

13. Razmjou H, Lincoln S, Geddes C, et al. Management of Acute Work-Related Shoulder Injuries by an Early Shoulder Assessment Program: Efficiency of Imaging Investigations. Physiother Can. 2016; 68(4):357-366. https://doi.org/10.3138/ptc.2015-49 PMid:27904235 PMCid:PMC5125498

14. McFarland E, Bernard J, Dein E, et al. Diagnostic Injections About the Shoulder. J Am Acad Orthop Surg. 2017; 25(12):799-807. https://doi.org/10.5435/JAAOS-D-16-00076 PMid:29176503

15. O'Kane JW, Toresdahl BG. The evidenced-based shoulder evaluation. Curr Sports Med Rep. 2014; 13(5):307-13. https://doi.org/10.1249/JSR.0000000000000090 PMid:25211618

16. Callaghan MJ, Baombe JP, Horner D, et al. A prospective, observational cohort study of patients presenting to an emergency department with acute shoulder trauma: the Manchester emergency shoulder (MESH) project. BMC Emerg Med. 2017; 17(1):40. https://doi.org/10.1186/s12873-017-0149-y PMid:29273012 PMCid:PMC5741868

17. Helfen T, Ockert B, Pozder P, et al. Management of prehospital shoulder dislocation: feasibility and need of reduction. Eur J Trauma Emerg Surg. 2016; 42(3):357-62. https://doi.org/10.1007/s00068-015-0545-5 PMid:26156391

18. Asker M, Waldén M, Källberg H, et al. A prospective cohort study identifying risk factors for shoulder injuries in adolescent elite handball players: the Karolinska Handball Study (KHAST) study protocol. BMC Musculoskelet Disord. 2017; 18(1):485. https://doi.org/10.1186/s12891-017-1852-2 PMid:29166930 PMCid:PMC5700469

19. Litaker D PM, El Bilbeisi H, Brems J. Returning to the bedside: using the history and physical examination to identify rotator cuff tears. J Am Geriatr Soc. 2000; 48(12):1633-7. https://doi.org/10.1111/j.1532-5415.2000.tb03875.x PMid:11129754

20. Hegedus EJ GA, Campbell S, Morin A, et al. Physical examination tests of the shoulder: a systematic review with meta-analysis of individual tests. Br J Sports Med. 2008; 42(2):80-92. https://doi.org/10.1136/bjsm.2007.038406 PMid:17720798

21. Hegedus EJ1 GA, Cook CE, Michener L, et al. Which physical examination tests provide clinicians with the most value when examining the shoulder? Update of a systematic review with meta-analysis of individual tests. Br J Sports Med. 2012; 46(14):964-78. https://doi.org/10.1136/bjsports-2012-091066 PMid:22773322

22. Fraenkel L LM, Felson D. The use of radiographs to evaluate shoulder pain in the ED. Am J Emerg Med. 1998; 16(6):560-3. https://doi.org/10.1016/S0735-6757(98)90218-2

23. Fraenkel L, Shearer P, Mitchell P, et al. Improving the selective use of plain radiographs in the initial evaluation of shoulder pain. J Rheumatol. 2000; 27(1):200-4. PMid:10648039

24. Panacek EA, Mower WR, Holmes JF, et al. Test performance of the individual NEXUS low-risk clinical screening criteria for cervical spine injury. Ann Emerg Med. 2001; 38:22-2. https://doi.org/10.1067/mem.2001.116499 PMid:11423807

25. Stiell IG, Wells GA, Hoag RH, et al. Implementation of the Ottawa Knee Rule for the use of radiography in acute knee injuries. JAMA. 1997; 278(27):2075–9. https://doi.org/10.1001/jama.1997.03550230051036 PMid:9403421

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Elevated High-Sensitivity C-Reactive Protein And Interleukin-6 Plasma As Risk Factors For Symptomatic Lumbar Osteoarthritis In Postmenopausal Women

I Ketut Suyasa[1], Anak Agung Wiradewi Lestari[2], I Gusti Ngurah Yudhi Setiawan[3], Tjokorda Gde Bagus Mahadewa[3*], I Putu Eka Widyadharma[4]

[1]Department of Orthopaedic and Traumatology, Faculty of Medicine Udayana University, Sanglah General Hospital, Bali, Indonesia; [2]Clinical Pathology, Faculty of Medicine Udayana University, Sanglah General Hospital, Bali, Indonesia; [3]Department of Neurosurgery, Faculty of Medicine Udayana University, Sanglah General Hospital, Bali, Indonesia; [4]Department of Neurology, Faculty of Medicine Udayana University, Sanglah General Hospital, Bali, Indonesia

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Keywords: Low back pain; hs-CRP; ESR; IL-6

***Correspondence:** Tjokorda Gde Bagus Mahadewa. Department of Neurosurgery, Faculty of Medicine Udayana University/Sanglah General Hospital, Bali, Indonesia. E-mail: tjokmahadewa@unud.ac.id

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Abstract

AIM: To determine whether elevated high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and erythrocyte sedimentation rate (ESR), as risk factors of symptomatic lumbar osteoarthritis (OA) in estrogen deficiency postmenopausal women.

METHODS: A case-control study was conducted between January and June 2017. The inclusion criteria include post-menopausal women with estrogen deficiency with low back pain. Exclusion criteria were: patients with a history of undergoing bilateral oophorectomy, taking hormonal replacement therapy or corticosteroid, malignancies, and lumbosacral spine trauma. The blood examinations were taken to measure IL-6 level by ELISA, hs-CRP level by spectrophotometry and ESR by modified Westergren method.

RESULTS: A group of 44 pairs of subjects were divided equally into case and control groups showed that in estrogen deficiency postmenopausal women, an increased level of hs-CRP increased the risk for symptomatic lumbar OA (OR = 2.83, CI95% = 1.065-8.776, p = 0.034). Also, a high level of IL-6 increased the risk of symptomatic lumbar OA (OR = 2.7, CI95% = 0.991-8.320, p = 0.033). No such significant findings were found for an increased ESR level.

CONCLUSION: Elevated level of plasma hs-CRP and IL-6 were concluded as risk factors for symptomatic lumbar OA in post-menopausal women.

Introduction

Low back pain (LBP) is often as a chief complaint of the degenerative spine lesion. The prevalence of osteoarthritis at the age of 50 years both in men and women is relatively similar and increasing in women of over 50 years old. Numerous causes have been proposed, including estrogen alterations that frequently arise in post menopausal women in post-menopausal age [1], [2].

Lumbar osteoarthritis (OA) is a cartilage degeneration involving the narrowing of the intervertebral discs, lumbar vertebral osteophyte, and osteoarthritis of the facet joints [2], [3], [4]. Some suspected causes of lumbar OA include hormonal changes in post menopausal women, including changes in the hormone estrogen, mechanical stress caused by weight gain and ageing process, and inflammatory process.

Chronic inflammation that occurs in lumbar OA involved the role of cytokines, like interleukin 6 (IL-6), IL-10 and IL-1ra [5]. According to another study, IL-6 level and IL-6/IL-10 ratio were predictive as the risk of lumbar OA [2]. An increased level of inflammatory mediators will cause a systemic

inflammatory reaction. It is now believed that patients with LBP have increased levels of hs-CRP and ESR [6] [7]. C-reactive protein is well established as a systemic marker for inflammation and tissue injury [7]. Commonly, can be detected within 6-8 hours after the injury. Meanwhile, ESR is well-known as a non-specific marker of inflammation.

Currently, limited studies have been reported regarding hsCRP and ESR levels in LBP patients, especially in hormonal-related OA [6] [7] [8] [9]. The association between increased hsCRP and low back pain is controversial.

The goal of this study is to elucidate elevated hs-CRP, IL-6, and ESR, as risk factors for symptomatic lumbar OA in estrogen deficiency postmenopausal women.

Patients and Methods

This was a case-control study that conducted between January and June 2017 at Sanglah Hospital. The ethical clearance was certified by the Committee of Ethical Research of Udayana University/Sanglah Hospital. All patients have signed an informed consent paper to be included in this study.

The total subjects were 88 patients, distributed equally into two groups. The case group consists of 44 post-menopausal women with symptomatic lumbar OA and estrogen deficiency. Subjects whose history of bilateral oophorectomy, taking hormonal replacement therapy or corticosteroid, malignancies, lumbosacral spine trauma, or other large joint arthritis, were excluded from the study. The control group consists of equally 44 postmenopausal women, with asymptomatic lumbar OA. They were matched individually by body mass index (BMI) and age to the case group.

Plasma IL-6, hs-CRP, and ESR were analysed using enzyme-linked immunosorbent assay (ELISA), spectrophotometry, and modified Westergren method, respectively. The descriptive analysis described subjects characteristics. McNemar's chi-square was used for bivariate analysis. The odds ratio was used for risk estimation. The correlation between hs-CRP and IL-6 was analysed using Pearson's Correlation test.

Results

The total number of subjects in this study was 88. They were paired and distributed equally to the case and control groups. The median age of the subjects was 58 years old. Subject characteristics are shown in Table 1.

We used the cut-off point of IL-6 was 2.264 pg/L and hs-CRP level of 5.00 mg/L. The odds ratio (OR) was 2.7 (95% CI = 0.991-8.320, p = 0.033); 2.83 (95% CI = 1.065-8.776, p = 0.034) respectively, as shown in Table 2.

Table 1: Subject characteristic of each study group

Characteristic	Case group	Control group
Age (years), median (IQR[1])	58 (54-61)	58 (53-60)
Duration of menopause (years), median (IQR)	7 (4-10)	8 (3-10)
Blood estrogen level (pg/mL), median (IQR)	12.7 (9.00-20.87)	14.16 (9.51-19.23)
BMI (kg/m^2), median (IQR)	25.92 (23.27-28.06)	25.28 (22.86-27.37)
IL-6 (pg/L), mean ± SD[2]	76.358 ± 152.798	6.966 ± 12.244
hs-CRP (mg/L), mean ± SD	5.795 ± 4.137	3.568 ± 3.303
ESR (mm/hour), mean ± SD	13.831 ± 14.798	16.730 ± 12.517

[1]Interquartile range; [2]Standard deviation.

Discussion

Osteoarthritis is a complicated process of joint degeneration. Inflammation maybe plays a critical role as interleukin upregulation, which arises following the ageing of the immune system or obesity [10].

Table 2: McNemar's test for IL-6, hs-CRP, and ESR in symptomatic lumbar osteoarthritis

Variables	OR[1]	p	95% CI
IL-6 (pg/L)	2.7	0.033	0.991-8.320
hs-CRP (mg/L)	2.8	0.034	1.065-8.776
ESR (mm/hour)	0.5	0.179	0.538-0.181

[1]McNemar's test, p < 0.05 as significant.

Despite being a degeneration process, inflammation also plays a role in OA. The inflammatory development that happens in lumbar osteoarthritis involves the role of proinflammatory cytokines, or anti-inflammatory cytokines [2], [4].

In this study, IL-6 was found to be statistically significant with (OR = 2.7; p = 0.033). Both Weber et al., and Valdes also reported that IL-6 was higher in LBP patients [11], [12]. Both IL-1 and IL-10 were also reported to correlate with the risk of osteoarthritis [11], [12]. Chingford reported that circulating IL-6 was related to OA development in women.[13] Postmenopausal female with knee osteoarthritis also showed higher IL-6 levels (p < 0.001) as compared to healthy female subjects [14], [17].

Ershler et al. also found an increase of IL-6 in postmenopausal patients [18], [19]. In postmenopausal patients, as the estrogen levels decline, the osteoclast formation increased and leading to bone resorption. The cartilage regulation also affected by estrogen levels because the isomer of the estrogen receptor (ER-α) is expressed in joint and growth plate cartilage in humans and other

species [1], [20].

We also found that hs-CRP levels significantly acted as a risk factor (OR = 9.0, p = 0.011) similar to reports by Rannou et al., [21]. Reports suggested that hs-CRP was elevated in the acute phase of inflammation [21], [22], [23]. Gebhardt found that hs-CRP level was not associated with either somatic function or pain level in LBP patients [24].

Both ESR and hs-CRP tests are used to detect inflammation. In inflammation, fibrinogen will enter the blood and cause raise the ESR [25]. The hs-CRP is synthesised in the liver and is often called an acute-phase protein [25]. The elevated hs-CRP level is also linked with the manifestation of synovial inflammatory infiltrates, and it is also correlated with proportions of T-cells in the synovial membrane. These findings were independent of the stage of OA disease [25]. Stümer reporteda correlation between pain scale and hs-CRP in patients with acute sciatic pain, where a higher hs-CRP level was associated with a higher pain level [26].

Kim found that IL-6 levels were elevated in postmenopausal women who healthy, nonobese, and elderly. The IL-6 may be a better marker of constant mild inflammatory activity [27]. In this study, as the age and BMI were controlled and adjusted, the hs-CRP and IL-6 level in symptomatic lumbar osteoarthritis in postmenopausal women were significantly elevated.

Mild systemic inflammation may have a more significant role in symptomatic lumbar OA rather than radiographic findings [28]. Symptomatic lumbar OA was a degenerative spine disorder in which the inflammation also plays a role in the symptom manifestation. In this study, the ESR was not significant statistically as a risk factor (OR = 0.53, p = 0.179). This possibly caused by the fact that ESR is less specific for inflammation marker compared than hs-CRP.

In conclusion, a high level of plasma IL-6 and hs-CRP are risk factors for symptomatic lumbar OA in estrogen deficiency postmenopausal women, indicating the role of the inflammation process in this lumbar OA.

References

1. Richette P, Corvol M, Bardin T. Estrogen, cartilage, and osteoarthritis. Joint Bone Spine. 2003; 70(4):257-62. https://doi.org/10.1016/S1297-319X(03)00067-8

2. Suyasa IK, Kawiyana IKS, Bakta IM, Widiana IGR. Interleukin-6 and ratio of plasma interleukin-6/interleukin-10 as risk factors of symptomatic lumbar osteoarthritis. World Journal of Orthopedics. 2017; 8(2):149-155. https://doi.org/10.5312/wjo.v8.i2.149 PMid:28251065 PMCid:PMC5314144

3. Fujiwara A, Lim T-H, An HS, et al. The Effect of Disc Degeneration and Facet Joint Osteoarthritis on the Segmental Flexibility of the Lumbar Spine. Spine. 2000; 25(23):3036-3044. https://doi.org/10.1097/00007632-200012010-00011 PMid:11145815

4. Sniekers YH, Weinans H, van Osch GJ, van Leeuwen JP. Oestrogen is important for maintenance of cartilage and subchondral bone in a murine model of knee osteoarthritis. Arthritis Research & Therapy. 2010; 12(5): R182. https://doi.org/10.1186/ar3148 PMid:20923566 PMCid:PMC2991014

5. Wluka AE, Cicuttini FM, Spector TD. Menopause, estrogen, and arthritis. Maturitas. 2000; 30:183-99. https://doi.org/10.1016/S0378-5122(00)00118-3

6. Park CH, Lee SH. Investigation of High-Sensitivity C-reactive Protein and Erythrocyte Sedimentation Rate in Low Back Pain Patients. The Korean Journal of Pain. 2010; 23(2):147. https://doi.org/10.3344/kjp.2010.23.2.147 PMid:20556218 PMCid:PMC2886244

7. Macphail K. C-Reactive Protein, Chronic low back pain, diet, and lifestyle. Journal of Pain & Relief. 2014; 03(05). https://doi.org/10.4172/2167-0846.1000160

8. Zhang Y, Guo TM, Guo X, Wu S. Clinical diagnosis for discogenic low back pain. Int JBiol Sci. 2009; 5(7):647-58. https://doi.org/10.7150/ijbs.5.647

9. Xiao Y, Haynes WL, Michalek JE, Russell IJ. Elevated serum high-sensitivity C-reactive protein levels in fibromyalgia syndrome patients correlate with body mass index, interleukin-6, interleukin-8, erythrocyte sedimentation rate. Rheumatology International. 2012; 33(5):1259-1264. https://doi.org/10.1007/s00296-012-2538-6 PMid:23124693

10. Livshits G, Zhai G, Hart DJ, et al. Interleukin-6 is a significant predictor of radiographic knee osteoarthritis. Arthritis Rheum. 2009; 60(7):2037-45. https://doi.org/10.1002/art.24598 PMid:19565477 PMCid:PMC2841820

11. Suyasa I, Setiawan I. The role of ageing, body mass index and estrogen on symptomatic lumbar osteoarthritis in post-menopausal women. International Journal of Research in Medical Sciences. 2016:1325-1328.

12. Conrozier T, Saxne T, Fan CSS, et al. Serum concentrations of cartilage oligomeric matrix protein and bone sialoprotein in hip osteoarthritis: A one-year prospective study. Annals of the Rheumatic Diseases. 1998; 57(9):527-532. https://doi.org/10.1136/ard.57.9.527 PMid:9849311 PMCid:PMC1752738

13. Stannus OP, Jones G, Quinn SJ, Cicuttini FM, Dore D, Ding C. The association between leptin, interleukin-6, and hip radiographic osteoarthritis in older people: a cross-sectional study. Arthritis Research & Therapy. 2010; 12(3). https://doi.org/10.1186/ar3022 PMid:20482813 PMCid:PMC2911879

14. Sharma P, Rahman A, Mahmood T, Singh N. Role of tumour necrosis factor alpha (TNF-α) and interleukin-6 (il-6) in postmenopausal osteoarthritic female patients. Journal of Evidence-Based Medicine and Healthcare. 2016; 3(27):1242-1244. https://doi.org/10.18410/jebmh/2016/285

15. Wojdasiewicz P, Poniatowski ŁA, Szukiewicz D. The Role of Inflammatory and Anti-Inflammatory Cytokines in the Pathogenesis of Osteoarthritis. Mediators of Inflammation. 2014; 2014:1-19. https://doi.org/10.1155/2014/561459 PMid:24876674 PMCid:PMC4021678

16. Lotz M, Guerne PA. Interleukin-6 induces the synthesis of tissue inhibitor of metalloproteinases-1/erythroid potentiating activity (TIMP-1/EPA). J Biol Chem. 1991; 266(4):2017-20. PMid:1846608

17. Giuliani N, Sansoni P, Girasole G, et al. Serum interleukin-6, soluble interleukin-6 receptor and soluble gp130 exhibit different patterns of age- and menopause-related changes. Experimental Gerontology. 2001; 36(3):547-557. https://doi.org/10.1016/S0531-5565(00)00220-5

18. Holm S, Mackiewicz Z, Holm AK, et al. Pro-inflammatory,

Pleiotropic, and Anti-inflammatory TNF-α, IL-6, and IL-10 in Experimental Porcine Intervertebral Disk Degeneration. Veterinary Pathology. 2009; 46(6):1292-1300. https://doi.org/10.1354/vp.07-VP-0179-K-FL PMid:19605905

19. Keller ET. Molecular and cellular biology of interleukin-6 and its receptor. Frontiers in Bioscience. 1996; 1(4):d340-357. https://doi.org/10.2741/A136 PMid:9159238

20. Svensson CI. Interleukin-6: a local pain trigger? Arthritis Research & Therapy. 2010; 12(5):145. https://doi.org/10.1186/ar3138 PMid:21067533 PMCid:PMC2991005

21. Rannou F, Ouanes W, Boutron I, et al. High-sensitivity C-reactive protein in chronic low back pain with vertebral end-platemodec signal changes. Arthritis & Rheumatism. 2007; 57(7):1311-1315. https://doi.org/10.1002/art.22985 PMid:17907216

22. Sugimori K, Kawaguchi Y, Morita M, Kitajima I, Kimura T. High-sensitivity analysis of serum C-reactive protein in young patients with lumbar disc herniation. The Journal of Bone and Joint Surgery British volume. 2003; 85-B(8):1151-1154. https://doi.org/10.1302/0301-620X.85B8.14538

23. Shimura Y, Kurosawa H, Sugawara Y, et al. The factors associated with pain severity in patients with knee osteoarthritis vary according to the radiographic disease severity: a cross-sectional study. Osteoarthritis and Cartilage. 2013; 21(9):1179-1184. https://doi.org/10.1016/j.joca.2013.05.014 PMid:23973128

24. Gebhardt K, Brenner H, Stürmer T, et al. The course of high-sensitive C-reactive protein in correlation with pain and clinical function in patients with acute lumbosciatic pain and chronic low back pain-Asix6 months prospective longitudinal study. European Journal of Pain. 2006; 10(8):711-711. https://doi.org/10.1016/j.ejpain.2005.11.005 PMid:16403662

25. Pearle A, Scanzello C, George S, et al. Elevated high-sensitivity C-reactive protein levels are associated with local inflammatory findings in patients with osteoarthritis. Osteoarthritis and Cartilage. 2007; 15(5):516-523. https://doi.org/10.1016/j.joca.2006.10.010 PMid:17157039

26. Sturmer T. Pain and high sensitivity C reactive protein in patients with chronic low back pain and acute sciatic pain. Annals of the Rheumatic Diseases. 2005; 64(6):921-925. https://doi.org/10.1136/ard.2004.027045 PMid:15897311 PMCid:PMC1755532

27. Kim OY, Chae JS, Paik JK, et al. Effects of aging and menopause on serum interleukin-6 levels and peripheral blood mononuclear cell cytokine production in healthy nonobese women. Age. 2011; 34(2):415-425. https://doi.org/10.1007/s11357-011-9244-2 PMid:21487705 PMCid:PMC3312621

28. Jin X, Beguerie JR, Zhang W, Blizzard L, Otahal P, Jones G, Ding C. Circulating C reactive protein in osteoarthritis: a systematic review and meta-analysis. Annals of the rheumatic diseases. 2015; 74(4):703-10. https://doi.org/10.1136/annrheumdis-2013-204494 PMid:24363360

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The Levels of Hepcidin and Erythropoietin in Pregnant Women with Anemia of Various Geneses

Dmitriy Vazenmiller*, Olga Ponamaryova, Larisa Muravlyova, Vilen Molotov-Luchanskiy, Dmitriy Klyuyev, Riszhan Bakirova, Zhanna Amirbekova

Karaganda State Medical University, Karaganda, Kazakhstan

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***Correspondence:** Dmitriy Vazenmiller. Karaganda State Medical University, Karaganda, Kazakhstan. E-mail: stop_@mail.ru

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Abstract

AIM: The purpose of the present research was to study the content of erythropoietin and hepcidin in serum in pregnant women with iron deficiency anaemia and anaemia of chronic inflammation.

METHODS: The authors examined 98 pregnant women who were observed in LLP (Regional obstetric-gynaecological centre) in Karaganda. The including criteria for pregnant women in the study was the informed consent of the woman to participate in the study. Exclusion criteria were oncological diseases, HIV-infection, tuberculosis, severe somatic pathology, mental illness, drug addiction. The design of the study was by the legislation of the Republic of Kazakhstan, international ethical norms and normative documents of research organisations, approved by the ethics committee of the Karaganda State Medical University.

RESULTS: As a result of the study, it was determined that the content of erythropoietin and hepcidin in pregnant women with anemias of different genesis varies ambiguously. In the main group of pregnant women with IDA, the erythropoietin content rises, and the hepcidin level decreases. In pregnant women with ACI, on the contrary, the level of hepcidin increases, and in one subgroup it is significant. However, in pregnant women and with IDA and anemia of chronic inflammation, there is a subgroup of women in whom erythropoietin is either comparable with hepcidin, or their changes are of opposite nature.

CONCLUSION: The authors concluded that the obtained data indicate ambiguous changes in the level of erythropoietin and hepcidin in pregnant women with anaemias of various origins. In all likelihood, there are still unaccounted factors affecting the content of these protein-regulators of iron metabolism, which require further definition and interpretation in anaemia of pregnant women.

Introduction

Anaemia of pregnant women continues to be one of the main problems of obstetrics and gynaecology. The multifactorial nature of the development of anaemia in pregnant women (AP), the complexity of pathogenesis creates problems for determining the criteria for diagnosing the aetiology of anaemia and, subsequently, for adequate therapeutic correction. Traditional anaemia biomarkers are not always sufficiently informative to determine the aetiology of anaemia in pregnant women. This significantly complicates the implementation of preventive or therapeutic interventions to correct the violation of iron metabolism. A convincing opinion was expressed that in some cases, the appointment of iron preparations to pregnant women with anaemia of chronic inflammation can induce oxidative stress and promote the growth of bacteria, thereby exacerbating the complications of pregnancy, i.e. anaemia of medium degree is a protective mechanism against bacterial and fungal pathogens [1] [2].

Currently, the main emphasis was made on the study of erythropoietin and hepcidin to deepen the understanding of the state of iron metabolism in particular and erythron in general in the body of pregnant women (including with AP).

Erythropoietin is a hormone, synthesised in the kidneys and perisinusoidalliver cells (mainly in the embryonic and perinatal periods). It is a glycoprotein with a molecular weight of 34 kDa. The secretion of erythropoietin in the blood is regulated by the body oxygen regime [3]. Synthesis of erythropoietin has no humoral or neural regulation. The production of erythropoietin depends only on the oxygen content and is controlled by the feedback principle.

The main function of erythropoietin is the regulation of erythropoiesis [4]. The intensity of the formation of new erythrocytes in the bone marrow depends on the level of endogenous erythropoietin in the plasma. Inadequate to hypoxia levels, the development of own erythropoietin can lead to the occurrence of anaemia. Under normal conditions, in response to a decrease in oxygenation of tissues and cells, there is an increase in the synthesis of erythropoietin by the kidneys. The isolated hormone interacts in the bone marrow with specific receptors on the surface of the progenitor cells of erythrocytes, which stimulates their proliferation and differentiation and, as a result, increases the concentration of haemoglobin.

Also, erythropoietin induces other effects, including increases systemic blood pressure, increases iron absorption by inhibiting hepcidin activity [5]. Erythropoietin also has a cytoprotective effect on the brain, kidney and heart cells by limiting apoptosis, oxidative stress, and has anti-inflammatory activity [6].

The erythropoietin concentration increases in 2-4 times at the developing physiological anaemia of pregnant women [7]. According to Erdem et al., [8], a low concentration of haemoglobin and ferritin with an increase in serum erythropoietin content was noted in pregnant women with anaemia. A similar view that anaemia during pregnancy induces the secretion of erythropoietin in response to a low content of haemoglobin and ferritin is expressed in a later study of Ervasti M. et al., [9]. It should also be noted that studies have appeared on the use of erythropoietin as a therapeutic agent in anaemia of pregnant women [10].

At the moment about 20 regulatory molecules are known to control this complex process. Over the past few years, the role of hepcidin as a key regulator of iron metabolism has been actively discussed.

Hepcidin, a peptide hormone consisting of 25 amino acid residues, is synthesised in the liver. For the first time, hepcidin was detected in urine and described by S.N. Park et al., Later, this peptide was also isolated from the plasma. A distinctive feature of the hepcidin molecule is the presence in it of disulfide bonds between two neighbouring molecules of the amino acid cysteine, which can determine the high reactivity of the molecule. With the development of systemic infection, hepcidin rises more than 100 times. However, as has been shown in recent years, the role of hepcidin is associated with clinical abnormalities in the parameters of iron metabolism, and some cases – with the development of anaemia.

In addition to hepatocytes, hepcidin mRNA is also expressed in the cells of the heart, lungs and placenta. Hepcidin regulates the intestinal absorption of iron, iron release by macrophages and placental passage of iron. Also, this hormone inhibits the release of iron from cells by binding and enhancing the degradation of the protein-iron exporter ferroportin [11]. Hepcidin inhibits ferroportin, a specific carrier protein that carries iron to the interior of the cell, thereby impairing the absorption of iron in the small intestine. Another mechanism of action: hepcidin blocks the release of iron from macrophages, (locking) it inside the cell. Both these mechanisms lead to a violation of the homeostasis of iron, actually to iron deficiency and the development of anaemic syndrome, including in pregnant women. Analysis of literature data showed a limited number of studies about hepcidin in pregnant women. It was shown by Amat Bah et al. studies that the hepcidin concentration decreased by the 20th week of gestation, while the iron stores in the body of pregnant women decreased by the 30th gestation week [12]. The decrease in hepcidin level in pregnant women is suggested to be considered as a potential criterion for determining individual needs for intervention with iron preparations [13] [14]. At the same time, other data have been obtained that the concentration of hepcidin does not change depending on the trimester of pregnancy; there is also no correlation between hepcidin level and iron status [15].

Investigations of hepcidin level in pregnancy complications have been carried out. A study by M. Koenig et al., [16] showed an increase in serum hepcidin level in women with a high risk of pregnancy complications in comparison with healthy pregnant women. The hepcidin level was higher in pregnant women with pre-eclampsia than in the control group and positively correlated with the C-reactive protein. It is suggested that inflammation is a regulator of hepcidin production [11]. In inflammation, an increase of hepcidin level leads to a limitation of iron and a decrease in its availability for placental transfer.

Consequently, analysis of literature data has shown a limited number of studies of erythropoietin and hepcidin anaemia in pregnant women. Because, according to some authors, erythropoietin and hepcidin are in reciprocal relationships, their joint determination has a great interest AP.

In this regard, the purpose of our study was to study the content of erythropoietin and hepcidin in serum in pregnant women with iron deficiency anaemia and anaemia of chronic inflammation.

Methods

We examined 98 pregnant women who were observed in LLP (Regional obstetric-gynaecological centre) in Karaganda. The including criteria for pregnant women in the study was the informed consent of the woman to participate in the study. Exclusion criteria were oncological diseases, HIV-infection, tuberculosis, severe somatic pathology, mental illness, drug addiction. The design of the study

was by the legislation of the Republic of Kazakhstan, international ethical norms and normative documents of research organisations, approved by the ethics committee of the Karaganda State Medical University.

The object of the study was blood. The level of erythropoietin and hepcidin in serum was determined by the method of enzyme immunoassay. A set of Vector Best production (Russian Federation) was used to determine erythropoietin; a Cloud Cloncorp set was used to determine hepcidin (Houston, USA). Also in the blood of patients, the level of haemoglobin and serum iron was determined spectrophotometrically using Sysmex KX-21N and A-15 analysers (BioSystems, Japan, Spain)

Pregnant women were divided into 3 groups: 22 women entered the group with the physiological course of pregnancy (1st group), 19 pregnant women with (iron deficiency anaemia) IDA (2nd group), 57 pregnant women with anaemia of chronic inflammation (ACI) (3rd group).

Table 1: Characteristics of study groups

Groups	Average age (years)	Average gestation (weeks)	Second trimester (persons)	Third trimester (persons)	Parity: The first pregnancy	Parity: The second pregnancy	Parity: The third and more pregnancy
Physiologicalpregnancy(22 persons)	33.13	31.81	2	20	10	6	6
IDA (19 persons)	30.38	32.77	1	18	3	6	10
ACI (57 persons)	29.51	36.11	2	55	12	29	16

The diagnosis of iron deficiency anaemia was established on the basis of the lowered norm of the level of iron in the blood (less than 8 mmol/l), the diagnosis of ACI was established on the basis of anamnestic data on the presence of chronic pyelonephritis, arterial hypertension and edema during a previous or existing pregnancy, normal serum iron level, the presence of proteinuria and/or leukocyturia.

The results were processed by statistical methods.

Results

After determining the hepcidin and erythropoietin content of, a multidirectional change in these parameters in the serum of pregnant women of the 2nd and 3rd groups was observed. By the obtained data, 2 subgroups were singled out within the 2nd group and 4 subgroups within the 3rd group. The results of the study are presented in Table 1. It follows from the data in Table 1 that in pregnant women with IDA, a significant decrease in erythropoietin content is observed against a background of a decrease in haemoglobin and serum iron against a background of a clear trend towards an increase in hepcidin (subgroup 1). In pregnant women with IDA of subgroup 2 erythropoietin level exceeds control indices, whereas the content of hepcidin was not different from the control.

In pregnant women with ACI of subgroup 1 the erythropoietin and haemoglobin content is significantly lower control indices, while the hepcidin level shows a distinct tendency to increase.

Table 2: The level of haemoglobin, erythropoietin, hepcidin and iron in the investigated groups

Group	Statistic value	Haemoglobin, g/l	Erythropoietin, miu/ml	Hepcidin, ng/ml	Iron, mmole/l
Control, N=22	Median	121.5	17.24	13.14	11.1
	Lower quartile	90	12.04	11.26	10.5
	Upper quartile	127.5	22.54	19.6	12.12
IDA, subgroup 1, n=12	Median	85.5*	7.20*	26.03	5.6*
	Lowerquartile	79.5	5.98	13.05	3.65
	Upperquartile	87.25	10.85	34.38	8.6
IDA, subgroup2, n=7	Median	88	23.19^	12	8.11*
	Lowerquartile	85.5	18.47	9.65	6
	Upperquartile	125.5	24.88	22.34	9.5
ACI Subgroup1, n=43	Median	87*	9.30*	24.9	10.5
	Lowerquartile	82.5	5.61	13.78	8.9
	Upperquartile	89	11.57	35.56	10.85
ACI Subgroup2, n=3	Median	89	7.64*	199.62*!	11.25
	Lowerquartile	79.5	6.81	192.35	10.42
	Upperquartile	108	8.15	250.75	11.52
ACI Subgroup3, n=7	Median	124#;	25.23*#;	12;	10.75
	Lowerquartile	118.5	23.51	10	10.1
	Upperquartile	129	26.43	21.39	12.1
ACI Subgroup4, n=4	Median	83.5?	14.60:?	89.9*:?	10.05*:
	Lowerquartile	81	11.57	85.37	9.82
	Upperquartile	95.75	17.33	93.86	10.27

*-reliability of differences with the control, p = 0.005; ^-reliability of differences in individuals with IDA between subgroups 1 and 3, p = 0.005; #-reliability of differences in persons with ACI between subgroups 1 and 4, p = 0.005; !!-reliability of differences in persons with ACI between subgroups 1 and 2+3, p = 0.005; ;-reliability of differences in individuals with ACI between clusters 2+3 and 4, p = 0.005; :-reliability of differences in individuals with ACI between clusters 2+3 and 5, p = 0.005; ?-the reliability of differences in individuals with ACI between clusters 4 and 5, p = 0.005.

Against a background of a significant decrease in the level of erythropoietin, a sharp, significant increase in hepcidin level was registered in pregnant women with ACI, included in the subgroup 2. There was a significant increase in erythropoietin, while serum hepcidin concentration did not differ from control, in pregnant women with ACI, included in subgroup the 3. There was a significant decrease in erythropoietin level of relative to the subgroups 3 and 2, with a significant increase in the content of hepcidin in pregnant women with ACI, included in the subgroup 4. Also in patients in this group, the serum iron level was reliably reduced. It should be noted that multidirectional changes in the concentrations of erythropoietin and hepcidin in the blood serum were recorded in a small proportion of patients with ACI.

Discussion

It is known that an increased level of estrogen, accompanying pregnancy, causes a decrease in the production of erythropoietin. ACI is the result of inhibition of the synthesis of erythropoietin in the juxtaglomerular apparatus of the kidneys. Simultaneously, cytokines produced by macrophages in the presence of foci of chronic latent

inflammation, both in the organs of the female reproductive system and in other organs, in particular, the kidneys, contribute. An increase in the level of erythropoietin in the blood of pregnant women may reflect a high risk of preeclampsia [17].

As a result of the study, it was determined that the content of erythropoietin and hepcidin in pregnant women with anaemias of different genesis varies ambiguously. In the main group of pregnant women with IDA, the erythropoietin content rises, and the hepcidin level decreases. In pregnant women with ACI, on the contrary, the level of hepcidin increases, and in one subgroup it is significant. However, in pregnant women and with IDA and anaemia of chronic inflammation, there is a subgroup of women in whom erythropoietin is either comparable with hepcidin, or their changes are of opposite nature.

Thus, our data indicate ambiguous changes in the level of erythropoietin and hepcidin in pregnant women with anaemias of various origins. In all likelihood, there are still unaccounted factors affecting the content of these protein-regulators of iron metabolism, which require further definition and interpretation in anaemia of pregnant women.

References

1. Drakesmith H, Prentice AM, Hepcidin and the iron-infection axis. Science. 2012; 338:768–72. https://doi.org/10.1126/science.1224577 PMid:23139325

2. Prentice AM. Clinical Implications of New Insights into Hepcidin-Mediated Regulation of Iron Absorption and Metabolism Ann Nutr Metab. 2017; 71(suppl 3):40–48. PMid:29268258

3. Jelkmann W. Erythropoietin after a century of research: younger than ever. European journal of haematology. 2007; 78(3):183-205. https://doi.org/10.1111/j.1600-0609.2007.00818.x PMid:17253966

4. Obeagu EI, Ezimah AC, Obeagu GU. Erythropoietin in the Anaemias of Pregnancy: A Review. Int J Curr Res Chem Pharm Sci. 2016; 3(3):10-8.

5. Ashby DR, Gale DP, Busbridge M, Murphy KG, Duncan ND, Cairns TD, Taube DH, Bloom SR, Tam FW, Chapman R, Maxwell PH. Erythropoietin administration in humans causes a marked and prolonged reduction in circulating hepcidin. haematologica. 2010; 95(3):505-8. https://doi.org/10.3324/haematol.2009.013136 PMid:19833632 PMCid:PMC2833083

6. Kowalska-Kańka A, Maciejewski T, Niemiec KT. The role and regulation of secretion of erythropoietin in pregnancy. Medycyna wieku rozwojowego. 2013; 3:270-5.

7. McMullin MF, White R, Lappin T, Reeves J, MacKenzie G. Haemoglobin during pregnancy: relationship to erythropoietin and haematinic status. European journal of haematology. 2003; 71(1):44-50. https://doi.org/10.1034/j.1600-0609.2003.00085.x PMid:12801298

8. Erdem A, Erdem M, Arslan M, Yazici G, Eskandari R, Himmetoglu Ö. The effect of maternal anemia and iron deficiency on fetal erythropoiesis: comparison between serum erythropoietin, hemoglobin and ferritin levels in mothers and newborns. The Journal of Maternal-Fetal & Neonatal Medicine. 2002; 11(5):329-32. https://doi.org/10.1080/jmf.11.5.329.332 PMid:12389675

9. Ervasti M, Kotisaari S, Heinonen S, Punnonen K. Elevated serum erythropoietin concentration is associated with coordinated changes in red blood cell and reticulocyte indices of pregnant women at term. Scandinavian journal of clinical and laboratory investigation. 2008; 68(2):160-5. https://doi.org/10.1080/00365510701550102 PMid:17852830

10. Sienas L, Wong T, Collins R, Smith J. Contemporary uses of erythropoietin in pregnancy: a literature review. Obstetrical & gynecological survey. 2013; 68(8):594-602. https://doi.org/10.1097/OGX.0b013e3182a2d51c PMid:23921673

11. Ganz T, Nemeth E. Iron sequestration and anemia of inflammation. Semin Hematol. 2009; 46:387–393. https://doi.org/10.1053/j.seminhematol.2009.06.001 PMid:19786207 PMCid:PMC2755591

12. Bah A, Pasricha SR, Jallow MW, Sise EA, Wegmuller R, Armitage AE, Drakesmith H, Moore SE, Prentice AM. Serum Hepcidin Concentrations Decline during Pregnancy and May Identify Iron Deficiency: Analysis of a Longitudinal Pregnancy Cohort in The Gambia–3. The Journal of nutrition. 2017; 147(6):1131-7. https://doi.org/10.3945/jn.116.245373 PMid:28424258 PMCid:PMC5443464

13. van Santen S, Kroot JJ, Zijderveld G, Wiegerinck ET, Spaanderman ME, Swinkels DW. The iron regulatory hormone hepcidin is decreased in pregnancy: a prospective longitudinal study. Clinical chemistry and laboratory medicine. 2013; 51(7):1395-401. https://doi.org/10.1515/cclm-2012-0576 PMid:23241678

14. Hedengran KK, Nelson D, Andersen MR, Stender S, Szecsi PB. Hepcidin levels are low during pregnancy and increase around delivery in women without iron deficiency–a prospective cohort study. The Journal of Maternal-Fetal & Neonatal Medicine. 2016; 29(9):1506-8. PMid:26212583

15. Simavli S, Derbent AU, Uysal S, Turhan NÖ. Hepcidin, iron status, and inflammation variables among healthy pregnant women in the Turkish population. The Journal of Maternal-Fetal & Neonatal Medicine. 2014; 27(1):75-9. https://doi.org/10.3109/14767058.2013.804054 PMid:23662610

16. Koenig M, Tussing-Humphreys L, Day J, Cadwell B, Nemeth E. Hepcidin and iron homeostasis during pregnancy. Nutrients. 2014; 6(8):3062-83. https://doi.org/10.3390/nu6083062 PMid:25093277 PMCid:PMC4145295

17. Medvedev BI, Syundyukova EG, Sashenkov SL. Placental expression of erythropoietin in preeclampsia. Rossiiskii vestnik akushera-ginekologa. 2015; 15(1):4-8. https://doi.org/10.17116/rosakush20151514-8

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Clinical Science

CD4 and Its Relevance to Advanced Glycation End Products in Tuberculosis Patients with Co-morbidity Diabetes

Sry Suryani Widjaja[1*], Rusdiana[1], Maya Savira[2]

[1]Biochemistry Department, Medical Faculty, Universitas Sumatera Utara, Medan, Indonesia; [2]Physiology Department, Medical Faculty, Universitas Sumatera Utara, Medan, Indonesia

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***Correspondence:** Sry Suryani Widjaja. Biochemistry Department, Medical Faculty, Universitas Sumatera Utara, Medan, Indonesia. E-mail: srysuryani@gmail.com

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Abstract

BACKGROUND: Tuberculosis (TB) is one of the most common infectious diseases found in developing countries. One of the risk factors for TB is diabetes, a chronic metabolic disorder characterised by hyperglycemia. The altered in glucose metabolism will cause dysfunction of phagocyte and antibacterial that furthermore impaired activation of natural killer cells, dendritic cells. These together will alter the balance of T-cell immunity. Under hyperglycemic conditions, AGEs (advanced glycation end products) was increasingly formed and was believed to play a role in cell dysfunctions and diabetic complications. The CD4 deficiency will alter the immunity status in diabetes and TB with co-morbidity diabetes.

AIM: This aim of this study was to evaluate CD4, and it's relevant to Advanced Glycation End Products (AGEs) in TB with co-morbidity diabetes.

METHODS: This is a case-control study with a total of 80 patients (40 diabetes and 40 TB with co-morbidity diabetes were recruited from Murni Teguh memorial Hospital Medan after ethical approval from Health Research Ethical Committee. The CD4, AGEs, Blood glucose and HbA1C were measured.

RESULTS: There was no statistical difference of CD4, HbA1C and blood glucose within diabetes and TB with co-morbidity diabetes but BMI ($p = 0.009$) and AGEs ($p = 0.001$) did. The CD4 below 500 were seen in 15% diabetes and 25% in TB with co-morbidity diabetes but did not show statistical significance difference ($p = 0.07$). No correlation was found between CD4 and AGEs in TB with co-morbidity diabetes ($p = 0.44$).

CONCLUSION: The CD4 was not correlated significantly with AGEs.

Introduction

Tuberculosis (TB) is one of the most common infectious diseases found in developing countries, in Indonesia the prevalence remains very high despite the decrease [1]. Study about TB including the risk factors, multidrug-resistant play an important part in the prevention and treatment of the disease. One of the risk factors of TB is diabetes, a chronic metabolic disorder characterised by hyperglycemia resulting from a defect in insulin secretion or resistance [2]. Diabetes Mellitus (DM) is considered one of the largest non-infectious health problems in the world; it is estimated there will be 380 million of people with diabetes in 2025 with the most in developing countries [3]. The chronic state of low-grade inflammation due to increased formation of advanced glycation end products, activation of proinflammatory mediators, and the increase of oxidative stress will impair the phagocytic and antibacterial activity of neutrophils and macrophages, that can further impaired activation of natural killer cells, dendritic cells, that can impair T Cell-mediated immune responses, to promote the establishment of acute intracellular bacterial infections in diabetic patients. The increased incidence of intracellular bacterial infections is one of many diabetic's complications, one of the most common bacterial infection is TB [4] [5] [6] [7].

TBs is one of the most significant causes of death from intracellular bacterial infection especially in developing countries [8], data from a recent prospective study showed that diabetic patients have a threefold higher risk of developing TB, and at least

10-35% of patients with TB have co-morbidity diabetes [9].

The altered in host immune systems will increase the mortality of patients with TB and co-morbidity diabetes and are more likely to have cavitary lung lesions, increased relapsed rate and most likely to develop multidrug resistance TB [10] [11]. Also, the treatment of TB with Rifampicin, one of the major TB drug will increase the metabolism of oral antidiabetic drugs thus may complicate the glycemic control [12] [13] [14].

The altered in glucose metabolism will cause dysfunction of phagocyte and antibacterial that furthermore impaired activation of natural killer cells, dendritic cells. These together will alter the balance of T-cell immunity [15]. Under hyperglycemic conditions, AGEs (Advanced Glycation End Products) a group of heterogeneous compounds were increasingly formed and was believed to play a role in cell dysfunctions and diabetic complications [16]. Recent studies suggested that CD4 T cells are associated with control of chronic bacterial and viral infections [17] [18] [19], this also has been reported to play an important role in protective immunity against TB [20] [21]. The altered in the immune system and CD4 deficiency will alter the immunity status that leads to death in diabetes and TBs with co-morbidity diabetes [22].

This study aimed to evaluate CD4 and its relevant with AGEs in TB patients with co-morbidity diabetes.

Material and Methods

This is a case-control study to evaluate CD4 and its relevant with Advanced Glycation End Products (AGEs) in TBs patients with co-morbidity diabetes. The study received ethical approval from the Health Research Ethical Committee Medical Faculty Universitas Sumatera Utara/H.Adam Malik General Hospital Medan Indonesia (No.444/TGL/KEPK FK USU-RSUP HAM/2017). Only patients diagnosed with diabetes and who gave signed informed consent were admitted to the study. Patients were recruited between March 2017 and September 2017 from Murni Teguh Memorial Hospitals in Medan, Indonesia. A total of 80 patients (40 diabetes and 40 TB with co-morbidity diabetes were recruited and was admitted into the study. Their age ranged between 36 years and 86 years (mean 60.0 ± 10.0 years) with the Body mass index ranged between 16.4 and 32.1 (mean 23.8 ± 3.0). Their characteristics are shown in Table 1.

Blood sampling was performed from a clean venepuncture using the Vacutainer system (Beckton Dickenson, New Jersey, USA). About 6 mL of blood was collected into EDTA and heparin tubes. Both tubes spun for 15 min at 2000g within an hour of blood collection. Plasma EDTA and heparin samples were aliquoted and kept at -70°C until assayed.

The following assays were performed: blood glucose, HbA1C, CD4, and AGEs was performed at the Integrated Laboratory Medical faculty University Sumatera Utara, Medan Indonesia. Blood glucose was measured with spectrophotometry, HbA1C with HPLC method, CD4 with flow cytometry, and AGEs with ELISA method.

Body mass index was determined by using the BMI calculator with body weight and height.

The Statistical Package for Social Sciences (SPSS22; SPSS Inc, Chicago, Il, USA) was used to perform the statistical analysis. Descriptive statistic and Non-parametric Mann Whitney U test were performed to evaluate the blood glucose, HbA1C, CD4, BMI and AGEs level in diabetes and TBs patients with co-morbidity diabetes. Spearman's Rho correlation analysis was used to determine the correlation between CD4 against AGEs seen in diabetes and TB patients with co-morbidity diabetes. A *P* value of less than 0.05 was considered statistically significant.

Results

In total, 80 patients were studied, (40 diabetes patients, 40 TB with co-morbidity diabetes). Their characteristics and Laboratory assays are shown in table 1. The parameter statistic was shown in table 2. The Box plots of CD4 and AGEs levels between diabetes and TB with co-morbidity diabetes are shown in Figure1 and 2.

Table 1: Characteristic of the samples

Group	Mean Age (range) years	Mean BMI (range)	Mean Blood Glucose (range) mg/dl	Mean HbA1C (range)%	Mean CD4 (range)	Mean AGEs (range) µg/ml
Diabetes	60 (44-73)	24.6 (19.9-32.1)	223 (85-568)	7.8 (5-12)	780 (198-1347)	7.4 (1.24-53.32)
TB +diabetes	60 (36-86)	23 (16.4 - 31.1)	214 (90-351)	8.8 (4-15)	687 (142-1800)	4.2 (1.28-21.5)

Most of the patients were overweights in both groups, in the diabetes group there were 55% normoweight and 45% overweight and in TB with co-morbidity diabetes, there were 60% normoweight, 30% overweight and 10% underweight. The blood glucose levels were not well controlled either in diabetes or TBs with co-morbidity diabetes group. In the diabetes group there were 45% well controlled and 55% bad controlled. Meanwhile, in the TB with

co-morbidity diabetes there were 35% well controlled and 65 % bad controlled. The immunity status in diabetes group was 85% good (CD4 > 500) and 15% bad (CD4 < 500). In the TB with co-morbidity diabetes 75% good and 25 % bad.

Table 2: Statistic of parameters studied in the diabetic group compared to those in TBs with comorbid diabetic

	Diabetic	TB +diabetic	p
Blood glucose mean (SD) mg/dl	223.4 ± 117.4	214.1 ± 78.6	0.75
HbA1c mean(SD) %	7.82 ± 1.75	8.78 ± 2.85	0.19
CD4 mean(SD)	779.8 ± 299.9	687.2 ± 380.5	0.074
AGEs mean(SD) µg/ml	7.4 ± 10.7	4.16 ± 5.9	0.001
BMI mean(SD)	24.6 ± 2.48	23.1 ± 3.33	0.009

Statistical significant p < 0.05.

The mean level of the AGEs was 5.78 with the range 1.24-53.32 in diabetes group and 1.3-19.38 in the TB with co-morbidity diabetes group. No statistically significant differences were seen in blood glucose level (p = 0.75, HbA1C, p = 0.19 and CD4, p = 0.074) between the diabetes group and TB with co-morbidity diabetes, but BMI did with p = 0.009 and so did AGEs with p = 0.001.

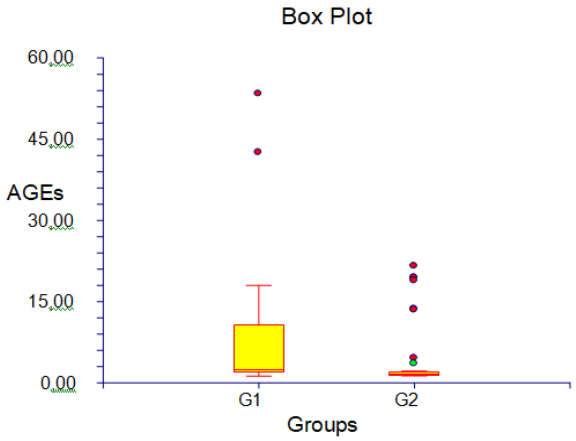

Figure 1: CD4 in diabetes and TB with co-morbidity diabetes; G1: diabetes; G2: TB with co-morbidity diabetes

In diabetes group there was no Statistical significant correlation found between CD4 and AGEs (p = 0.11), CD4 and blood glucose (p = 0.19), CD4 and HbA1C (p = 0.09), CD4 and BMI (p = 0.48). Statistical significant correlation was found between blood glucose and HbA1C (p = 0.013, r = 0.35), blood glucose and AGEs (p = 0.013, r = 0.35).

No statistical significant correlation was found between CD4 and AGEs (p = 0.44), CD4 and blood glucose (p = 0.08), CD4 and HbA1C (p = 0.45), CD4 and BMI (p = 0.44), but between blood glucose and HbA1C (p = 0.003, r = 0.43), HbA1C and AGEs (p = 0.003, r = 0.43).

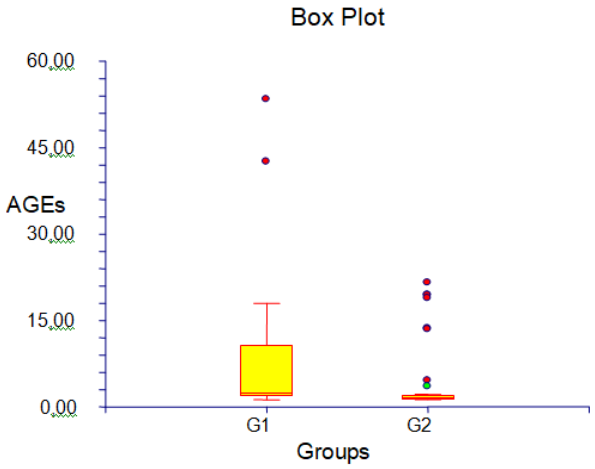

Figure 2: AGEs in diabetes and TB with co-morbidity diabetes; G1: diabetes; G2: TB with co-morbidity diabetes

Discussion

The chronic hyperglycemia will cause various complications; this was supported by the significant correlation between HbA1C and AGEs (p = 0.003) in the TB with co-morbidity diabetes group and significant correlation between blood glucose and AGEs (p = 0.013) in the diabetes group. This has been suggested by Helen et al. that AGEs play a rule in chronic inflammation [23].

The body mass index of the patients in the diabetes group was more with normoweight (22%) than overweight (18%), also in the TB with co-morbidity diabetes group (normoweight 24%, overweight 12%, underweight 4%). Obesity plays a role in metabolic syndrome that will worsen the chronic hyperglycemic effect. This was described by Ann Smith in The Epidemic of Obesity and Diabetes Trends and Treatments 2011 [24]. Underweight was found in the TB with co-morbidity diabetes group.

The CD4 ranged between 142 and 1800 with the mean 733. There were more cases (25%) with CD4 below 500 in the TB with co-morbidity diabetes group than in the diabetes group (15%), assumed that the immunity in the diabetes group is better than the TB with co-morbidity diabetes group, but the data did not show statistical significance difference (p = 0.07). Advanced glycation end products that play a role in cell dysfunctions and diabetic complications did not show the correlation with CD4 (p = 0.44) in the TB with co-morbidity diabetes group and p = 0.114 in the diabetes group. In conclusion, the CD4 below 500 were seen in 15% diabetes and 25% in TB with co-morbidity diabetes groups, but did not show statistical significance difference (p = 0.07). The AGEs was not correlated significantly with the CD4 in both groups (p = 0.44, p = 0.114). The HbA1C correlated significantly

with AGEs (p = 0.003) in the TB with co-morbidity diabetes group and blood glucose with AGEs (p = 0.013) in the diabetes group. No statistically significant differences were seen in blood glucose level (p = 0.75, HbA1C, p = 0.19 and CD4, p = 0.074) between the diabetes group and TB with co-morbidity diabetes. But BMI did with p = 0.009 and so did AGEs with p = 0.001.

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Ethical approval

This study was done after the ethical approval under the Health Research Ethical Committee Medical Faculty of USU/HAM General Hospital Medan - Indonesia No. 44/TGL/KEPK FK-USU-RSUP HAM/2017.

References

1. Atkins RC, Zimmet P. Diabetic kidney disease: Act now or pay later. Saudi J Kidney Dus Transpl 2012; 21:217-21.

2. Mark A, Joshua A, Thomas FL, Francesco C. Diabetes and vascular disease: pathophysiology clinical consequences, and medical therapy: part I. Circulation. 2003. PMid:14517147

3. Alberti KG, Zimmet PF. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabetic medicine. 1998; 15(7):539-53. https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S

4. Dobler CC, Flack JR, Marks GB. Risk of Tuberculosis among people with diabetes mellitus: an Australian nationwide cohort study. BMJ Open. 2012; 2:e000666. https://doi.org/10.1136/bmjopen-2011-000666 PMid:22331390 PMCid:PMC3282295

5. Young F, Wotton CJ, Critchley JA et al. Increased risk of Tuberculosis disease in people with diabetes mellitus: record-linkage study in a UK population. J Epidemiol Community Health. 2012; 66:519–23. https://doi.org/10.1136/jech.2010.114595 PMid:21109542

6. Leung CC, Lam TH, Chan WM et al. Diabetic control and risk of Tuberculosis: a cohort study. Am J Epidemiol. 2008; 167:1486–94. https://doi.org/10.1093/aje/kwn075 PMid:18400769

7. Perez A, Brown HS III, Restrepo BI. Association between Tuberculosis and diabetes in the Mexican border and non-border regions of Texas. Am J Trop Med Hyg. 2006; 74:604–11. https://doi.org/10.4269/ajtmh.2006.74.604 PMid:16606993 PMCid:PMC1464139

8. WHO. Global TBs Report 2013. Geneva: World Health Organisation, 2013. URL http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf

9. Restrepo BI, Camerlin AJ, Rahbar MH et al. Cross-sectional assessment reveals high diabetes prevalence among newly-diagnosed Tuberculosis cases. Bull World Health Organ. 2011; 89:352–9. https://doi.org/10.2471/BLT.10.085738 PMid:21556303 PMCid:PMC3089389

10. Peleg AY, Weerarathna T, McCarthy JS, Davis TM. Common infections in diabetes: Pathogenesis, management, and relationship to glycaemic control. Diabetes Metab Res Rev. 2007; 23:3–13. https://doi.org/10.1002/dmrr.682 PMid:16960917

11. Dooley KE, Chaisson RE. Tuberculosis and diabetes mellitus: Convergence of two epidemics. Lancet Infect Dis. 2009; 9:737–46. https://doi.org/10.1016/S1473-3099(09)70282-8

12. Ruslami R, Aarniutse RE, Alisjahbana B, van der Ven AJ, van Crevel R. Implications of the global increase of diabetes for Tuberculosis control and patient care. Trop Med Int Health. 2010; 15:1289–99. https://doi.org/10.1111/j.1365-3156.2010.02625.x PMid:20955495

13. Baker MA, Harries AD, Jeon CY et al. The impact of diabetes on Tuberculosis treatment outcomes: a systematic review. BMC Med. 2011; 9:81. https://doi.org/10.1186/1741-7015-9-81 PMid:21722362 PMCid:PMC3155828

14. Magee MJ, Foote M, Maggio DM et al. Diabetes mellitus and risk of all-cause mortality among patients with Tuberculosis in the state of Georgia, 2009–2012. Ann Epidemiol. 2014; 24:369–75. https://doi.org/10.1016/j.annepidem.2014.01.012 PMid:24613196 PMCid:PMC4011933

15. Kelly H, Jodie M, Tahnee Br, Brenda G, Rush C and Natkunam K. Immunological mechanisms contributing to the double burden of diabetes and intracellular bacterial infections. Immunology. 2014; 144:171-185.

16. Kerstin Nowotny, Tobias Jung, Annika Höhn, Daniela Weber and Tilman Grune. Advanced Glycation End Products and Oxidative Stress in Type 2 Diabetes Mellitus. Biomolecules. 2015; 5:194-222. https://doi.org/10.3390/biom5010194 PMid:25786107 PMCid:PMC4384119

17. Darrah PA, Patel DT, De Luca PM, Lindsay RW, Davey DF, Flynn BJ, Hoff ST, Andersen P, Reed SG, Morris SL, Roederer M. Multifunctional T H 1 cells define a correlate of vaccine-mediated protection against Leishmania major. Nature medicine. 2007; 13(7):843. https://doi.org/10.1038/nm1592 PMid:17558415

18. Ciuffreda, D. et al. Polyfunctional HCV-specific T-cell responses are associated with effective control of HCV replication. Eur J Immunol. 2008; 38:2665–77. https://doi.org/10.1002/eji.200838336 PMid:18958874

19. Kannanganat S, Kapogiannis BG, Ibegbu C, Chennareddi L, Goepfert P, Robinson HL, Lennox J, Amara RR. Human immunodeficiency virus type 1 controllers but not noncontrollers maintain CD4 T cells coexpressing three cytokines. Journal of virology. 2007; 81(21):12071-6. https://doi.org/10.1128/JVI.01261-07 PMid:17728221 PMCid:PMC2168799

20. Kalsdorf B, Scriba TJ, Wood K, Day CL, Dheda K, Dawson R, Hanekom WA, Lange C, Wilkinson RJ. HIV-1 infection impairs the bronchoalveolar T-cell response to mycobacteria. American journal of respiratory and critical care medicine. 2009; 180(12):1262-70. https://doi.org/10.1164/rccm.200907-1011OC PMid:19797156 PMCid:PMC2796736

21. Day CL, Abrahams DA, Lerumo L, van Rensburg EJ, Stone L, O'rie T, Pienaar B, de Kock M, Kaplan G, Mahomed H, Dheda K. Functional capacity of Mycobacterium tuberculosis-specific T cell responses in humans is associated with mycobacterial load. The Journal of Immunology. 2011:1101122. https://doi.org/10.4049/jimmunol.1101122

22. Hull MW, Phillips P, Montaner JS. Changing global epidemiology of pulmonary manifestations of HIV/AIDS. Chest. 2008; 134(6):1287-98. https://doi.org/10.1378/chest.08-0364 PMid:19059959

23. Helen V, Uribarri. Advanced Glycation End Products (AGE) and Diabetes: Cause, Effect, or Both? Curr Diab Rep. 2014; 14(1):453. https://doi.org/10.1007/s11892-013-0453-1 PMid:24292971 PMCid:PMC3903318

24. Ann SB. The Epidemic of Obesity and Diabetes Trends and Treatments. Tex Heart Inst J. 2011; 38(2):142–144.

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Epidemiological Characteristics of Work-Related Ocular Trauma among the Carpenters in Medan, Indonesia

Rodiah Rahmawaty Lubis[1*], Vera Limanto[2], Ruri Putri[2], Arlina Nurbaity Lubis[3], Nurfida Khairina Arrasyid[4]

[1]Department of Ophthalmology, Faculty of Medicine, Universitas Sumatera Utara, Dr. Mansyur Road 5, Medan, Indonesia; [2]Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia; [3]Faculty of Economic and Business, Universitas Sumatera Utara, Indonesia; [4]Department of Parasitology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

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Keywords: Work-related ocular trauma; Carpenters; Age; Educational level; Working hours; Eye protection

***Correspondence:** Rodiah Rahmawaty Lubis. Department of Ophthalmology, Faculty of Medicine, Universitas Sumatera Utara, Dr Mansyur Road 5, Medan, Indonesia. E-mail: rahma.lubis@yahoo.com

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Abstract

BACKGROUND: Medan is the capital of North Sumatera Province and the most industrialised area of North Sumatera. One of the largest industries in Medan is the wooden industry. Ocular trauma is often happened in Medan and causes a serious problem.

AIM: This study aimed to analyse the correlation between ocular trauma among the carpenters and the using of eye protection during work and educational level.

METHODS: This study is conducted among the carpenters that work in the wooden industry. There were 30 carpenters that being observed about age, educational level and working hours and the using of eye protection during work that might be related to ocular trauma. All carpenters completed a comprehensive examination and interview.

RESULTS: The most common age range of ocular trauma was between 26-45 years (56.7%), and all were male. Most of the traumatised carpenters educational level had a higher educational level (50%), and workers that had traumatised works more than 8 hours a day (66.7%). From this study, there was a significant correlation between ocular trauma among the carpenters and age ($p = 0.047$), and working hours ($p = 0.039$).

CONCLUSION: No significant correlation between ocular trauma among the carpenters and the using of eye protection during work ($p = 0.464$), and educational level (0.925) was found. Furthermore to anticipate the high rate of worked-related ocular trauma required labour regulations that cover the age of recruitment workers and working hours a day. Work safety regulation protects the workers from work-related ocular trauma.

Introduction

Eye injuries represent a frequent cause of emergency ophthalmic visits and have a significant impact regarding suffering, impairment of life quality, and the reduction of workability. Furthermore, eye injuries pose a relevant cost for the health system and result in a relevant loss of working days worldwide [1].

Injuries to the eyes accounted for 37 per cent of all head injuries involving days away from work in 2008 and 62 per cent of all. Face injuries involving days away from work. Men experienced far more eye injuries than a woman, and men age 25 to 44 suffered more eye injuries. Workers who were most at risk of incurring an eye included those in the manufacturing, construction, and trade industries, and those in the production, installation, maintenance and repair, construction and extraction, and service occupations [2]

Occupational ocular trauma is an important cause of preventable vision loss worldwide, with significant socioeconomic impact. It is a major cause of emergency ophthalmic visits and accounts for a substantial proportion of eye injuries, many of which occur in the workplace [3], [4].

More than 65,000 work-related eye injuries and illness are reported in the United States annually [5]. More than 2000 eye injuries occur at work daily, and approximately 10%-20% of eye injuries result in

temporary or permanent vision loss [5], [6]. Work-related injuries were noted to have a much higher incidence of intraocular foreign bodies and cataracts compared to non-work-related open globe injuries [7].

Ocular trauma is related to gender, age and educational level. In Northern Italy, the frequency of ocular trauma in younger workers (16-24 years old) is about double that of the oldest age class (55-64 years old) [8]. In the US population, men had a more than four times higher rate of an eye injury at work than women. Workers with less than a high-school education, non-Hispanic whites, self-employed, and in the Midwest region were more likely to experience ocular trauma with the highest rate in the age of 45-54 years [3].

Indonesia is an agrarian country. According to Indonesia's central statistics agency in February 2017, most Indonesian (31.86%) work in agriculture, as a farmer [9]. Only a few Indonesians are working as a carpenter. Moreover, being a farmer, carpenters or fishermen, having low educational attainment, and being men are positively associated with the frequency of injuries [10].

In Indonesia, the provision of working time has been established in Law no.13/2003 fourth Paragraph, article 77 verse 2, where entrepreneurs are required to the determine the working time of 7 hours a day and 40 hours a week in 6 working days [9].

We present the results of the study, an epidemiological characteristic of work-related ocular trauma among the carpenters in Medan the capital of Sumatera Utara province, one of the most densely populated and industrialised areas of Sumatera Utara, with a high number of active workers.

Material and Methods

This is a cross-sectional analysis study to describe epidemiological characteristics of work-related ocular trauma among the carpenters. The population of this study is all the carpenters that are working on the wooden industry in Medan, the North Sumatera capital, on January-December 2017. A total of 30 carpenters who worked in 6 working days. Data was directly obtained from all the carpenters that got an ocular trauma by using questionnaire and interview. The selected variables are the following:

1. Age: age of injured worker, distributed into 3 groups: (≤ 25), (26-45), (≥ 46).

2. Educational level, distributed into 2 groups: low (no school, primary school, junior high school), high (senior high school, bachelor).

3. Working time, distributed into 2 groups: (≤ 7 hours a day), (more than 7 hours a day).

4. Obedience using of eye protection devices during work into 2 groups: obey, not obey.

All samples in this study received a comprehensive ocular examination at H. Adam Malik Hospital Medan and a face-to-face interview. H. Adam Malik General Hospital is a government hospital, teaching hospital grade A in Medan North Sumatera. The samples were the carpenters who got an eye injury or ocular trauma at work or during working time. The ocular trauma includes penetrating injury, laceration, intraocular foreign bodies, etc., that caused by blunt or sharp trauma, and chemical trauma. The characteristics data were analysed by using univariate analysis and the correlation between all the variables, and worked-related ocular trauma was analysed by using bivariate analysis with Spearman correlation. Confidence intervals of 95% were employed. All significance values (ρ) were two-sided. Significance values (ρ) less than 0.05 were considered statistically significant.

Results

From 1 January to 31 December 2017 there were 28work-related ocular trauma among the carpenters. All carpenters that were observed during the study were male.

1. Work-related ocular trauma among the carpenters based on the age

From this study, the age of 26-45 years was the most common age of group (56.7%) followed by ≤ 25 years and ≥ 46 years.

Table 1: Distribution of Work-related ocular trauma among the carpenters based on the age

Variable (age)	Total (n)	Percentage (%)	Correlation Coef.	Sig. (2-tailed)
≤ 25	7	23.3		
26 – 45	17	56.7	0.365*	0.047
≥ 46	4	13.3		
Total	**28**	**93.3**		

More than 50% (56.,7%) carpenters were in the age between 26-45. There was a statistically significant correlation between age and work-related ocular trauma among the carpenters with significance values was 0.047 ($\rho < 0.05$).

2. Work-related ocular trauma among the carpenters based on the educational level

Table 2: Distribution of Work-related ocular trauma among the carpenters based on the educational level

Educational Level	Total (n)	Percentage (%)	Correlation Coef.	Sig. (2-tailed)
High	13	43.3	0.018	0.925
Low	15	50		
Total	**28**	**93.3**		

From Table 2 the Carpenters with low educational level most often experience ocular trauma (50%), but there was no statistically significant correlation between educational level and work-related ocular trauma among the carpenters with significance values was 0.925 ($\rho > 0.05$).

3. Work-related ocular trauma among the carpenters based on the working time

Table 3: Distribution of Carpenters-related trauma based on working time

Working time (hours)	Total (n)	Percentage (%)	Correlation Coef.	Sig. (2-tailed)
< 7	8	26.7	0.378*	0.039
≥ 7	20	66.7		
Total	**28**	**93.3**		

From Table 3 the Carpenters who work more than 7 hours a day tend to have an ocular trauma (66.7%) than those who work less than 7 hours a day. There was a statistically significant correlation between working time and work-related ocular trauma among the carpenters with significance values was 0.039 ($\rho < 0.05$).

4. Work-related ocular trauma among the carpenters based on the using of eye protection

Table 4: Distribution of carpenters-related trauma based on the obedience using of eye protection devices

Obedience	Work-related Ocular Injury		p-value
	Yes, n (%)	No, n (%)	0.464
Obey	6 (100%)	0 (0%)	
Not obey	22 (91.7%)	2 (8.3%)	

The carpenters that didn't obey of using eye protection devices more likely suffer from ocular injury 22 (91.7%). There are 2 carpenters that did not obey using eye protection devices, but they didn't get an ocular injury (8.3%). However, the chi-squared test data shows there was no statistically significant correlation between obedience using of eye protection devices and work-related ocular trauma among the carpenters($\rho > 0.05$).

Discussion

Indonesia is a developing country which most of the population live from agricultural products. This study to find out workers other than farmers that associated with the occurrence of ocular trauma in Medan. Limited study about the work-related ocular trauma among the carpenters Indonesia. In previous studies reported, male workers are most frequently involved [12], [13], [14]. From this study, we found that all subjects are male (100%), because wooden workers are usually male, and most of the Indonesian woman work as housewives or indoor workers. The highest frequency of ocular trauma among the carpenters in Medan is in younger workers (26-45 years old) 56.7%, and the lowest frequency is in oldest workers (≥ 46 years old) 13.3%. A study in Australia about 80% of worked-related ocular injuries occurred to persons aged between 20 and 44 years [11]. In the Southwest region of China, the highest proportion of occupational eye trauma was observed in the group between 36 and 45 years of age [12]. A similar study in Turkey, males was significantly greater than that for females, and males between 25 and 34 years of age had the highest eye injury rate [13]. This study also indicated that the risk of exposure to ocular trauma is inversely proportional to the job experience and young workers with little experience had a higher risk of exposure.

The educational level also plays an important rule in work-related ocular trauma. This study showed that Carpenters with low educational level most often experience ocular trauma (46.7%), but there was no statistically significant correlation between educational level and work-related ocular trauma — the workers with low educational level less understanding of the importance of using safety goggle during work.

Another variable that been examined in this study is working hours. Carpenters who work more than 7 hours a day tend to have an ocular trauma (66.7%) than those who work less than 7 hours a day. There was a correlation between working time and wok-ocular trauma in this study ($\rho < 0.05$). The Indonesian government issued a regulation the provision of working time in Law no.13/2003 fourth Paragraph, article 77, verse 2, that every entrepreneur is under an obligation to observe the ruling concerning working hours and required to determine the working time 7 (seven) hours a day and 40 (forty) hours a week for 6 (six) workdays in a week; or 8 (eight) hours a day, 40 (forty) hours a week for 5 (five) workdays in a week [9]. This regulation protects the workers from overtime working, so the incidence of work-related trauma in Indonesia may be reduced significantly. Another study that is held by the U. S. Department of Health and Human Services, overtime work was also associated with increased morbidity and mortality [15]. A study in Taiwan indicated that eye protection devices could reduce the risk of work-related eye injury by up to 60%, but only 18.4% of workers were wearing eye protection devices when injured [16]. In this study, we found that most of the carpenters who did not obey using eye protection devices likely suffer from ocular injury (91.7%) than others who obey (8.3%). A study in Iran, work-related eye injury is the major cause of eye injury and most often occurs as a result of the lack of proper eye protection [17]. A study in Scotland, UK, only 10 out 12 (83%) of workers were documented as wearing eye protection at the time of injury [18]. A study in Taiwan indicated that eye protection devices could reduce the risk of work-related eye injury by up to 60%, but only 18.4% of workers were wearing eye protection devices when injured [16].

In conclusion, this study provides insight into the epidemiological characteristics of occupational ocular trauma in Haji Adam Malik General Hospital, North Sumatera, Indonesia. Our research indicates that male worker, age and overtime working were a significant risk factor for work-related ocular trauma among the carpenters in Medan, North Sumatera.

These findings suggest that extensive occupational eye safety programs could be arranged and these should focus on the specific tasks or kinds of work with a high risk of ocular trauma, regardless of sex, age, educational level, working time, safety goggle and safety regulation. The incidence of work-related ocular trauma can be avoided through proper regulations, an improvement of work safety climate, rules and the entrepreneur under strict supervision by the government, so that the workers can work well and more prosperous.

Not only Safety regulation for workers such as using eye protection devices during work is needed, but also safety training and compliance within the workplace should be provided.

Author Contributions

Rodiah Rahmawaty Lubis and Arlina Nurbaity Lubis conceived, designed the study and also revised the final manuscript, analysed the clinical and occupational data and wrote the paper. Rodiah Rahmawaty Lubis, Ruri Putri and Vera made the ophthalmic examinations of the patients and recorded the data.

References

1. Sahraravand A, Haavisto AK, Holopainen JM, Leivo T. Ocular traumas in working age adults in Finland—Helsinki Ocular Trauma Study. Acta Ophthalmol. 2017; 95:288–294. https://doi.org/10.1111/aos.13313 PMid:27935236

2. Harris MP. Workplace Injuries Involving the Eyes. U.S. Bureau of labor Statistics, 2008:1-7. Available at:https://www.bls.gov/opub/mlr/cwc/workplace-injuries-involving-the-eyes-2008.pdf (accessed on 17 January 2017)

3. Forrest KY, Cali JM. Epidemiology of lifetime work-related eye injuries in the US population associated with one or more lost days of work. Ophthalmic epidemiology. 2009; 16(3):156-62. https://doi.org/10.1080/09286580902738175 PMid:19437310

4. McCall BP, Horwitz IB, Taylor OA. Occupational eye injury and risk reduction: Kentucky workers' compensation claim analysis 1994–2003. Injury prevention. 2009; 15(3):176-82. https://doi.org/10.1136/ip.2008.020024 PMid:19494097

5. Peate W.F. Work-related eye injuries and illness. Am. Fam. Physician. 2007; 75:1017–1022. PMid:17427615

6. Xiang H, Stallones L, Chen G, Smith GA. Work-related eye injuries treated in hospital emergency departments in the US. Am J Ind Med. 2005; 48:57–62. https://doi.org/10.1002/ajim.20179 PMid:15940717

7. Bauza AM, Emami P, Son JH, Langer P, Zarbin M, Bhagat N. Work-related open-globe injuries: demographics and clinical characteristics. European journal of ophthalmology. 2013; 23(2):242-8. https://doi.org/10.5301/ejo.5000209 PMid:23112040

8. Gobba F, Dall'Olio E, Modenese A, De Maria M, Campi L, Cavallini GM. Work-related eye injuries: A relevant health problem. Main epidemiological data from a highly-industrialized area of Northern Italy. International journal of environmental research and public health. 2017; 14(6):604. https://doi.org/10.3390/ijerph14060604 PMid:28587288 PMCid:PMC5486290

9. Indonesian Labour Law No.13. Working Hours. Fourth Paragraph, article 77 verse 2, 2003. Available online: http://www.ilo.org/dyn/travail/docs/760/Indonesian%20Labour%20Law%20-%20Act%2013%20of%202003.pdf (accessed on 17 January 2017)

10. Irianti S, Prasetyoputra P. Environmental, spatial, and sociodemographic factors associated with nonfatal injuries in Indonesia. Journal of environmental and public health. 2017; 2017.

11. Safety A, Council C. Type of occurrence classification system. Canberra: Australian Safety and Compensation Council, 2008.

12. Cai M, Zhang J. Epidemiological characteristics of work-related ocular trauma in southwest region of China. International journal of environmental research and public health. 2015; 12(8):9864-75. https://doi.org/10.3390/ijerph120809864 PMid:26295403 PMCid:PMC4555316

13. Serinken M, Turkcuer I, Cetin EN, Yilmaz A, Elicabuk H, Karcioglu O. Causes and characteristics of work-related eye injuries in western Turkey. Indian J Ophthalmol. 2013; 61(9):497-501. https://doi.org/10.4103/0301-4738.119435 PMid:24104708 PMCid:PMC3831765

14. Pandita A, Merriman M. Ocular trauma epidemiology: 10-year retrospective study. Work (professional & DIY). 2012; 203(24.7):48-1.

15. Caruso CC, Hitchcock EM, Dick RB, Russo JM, Schmit JM. Overtime and Extended Work Shifts: Recent Findings on Illnesses, Injuries, and Health Behaviours. USA: National Institute for Occupational Safety and Health, 2004. Available online: http://www,cdc.gov/niosh/docs/2004-143/pdfs/2004-143.pdf (accessed on 8 December 2016)

16. Chen SY, Fong PC, Lin SF, Chang CH, Chan CC. A case-crossover study on transient risk factors of work-related eye injuries. Occupational and environmental medicine. 2009. https://doi.org/10.1136/oem.2008.042325

17. Mansouri MR, Hosseini M, Mohebi M, Alipour F, Mehrdad R. Work-Related Eye Injury: The Main Cause Ocular TraumainIran. Eur J Ophthalmol. 2010; 20(4):770-775. https://doi.org/10.1177/112067211002000420 PMid:19967674

18. Thompson GJ, Mollan SP. Occupational eye injuries: a continuing problem. Occupational medicine. 2009; 59(2):123-5. https://doi.org/10.1093/occmed/kqn168 PMid:19129239

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Procalcitonin Level in Non-Small Cell Lung Cancer Patients among Indonesian Population

Noni Novisari Soeroso[1*], Muhammad Faiz Tanjung[1], Dina Afiani[1], Andika Pradana[1], Setia Putra Tarigan[1], Arlinda Sari Wahyuni[2]

[1]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, University of Sumatera Utara, University of Sumatera Utara Hospital, Jl. Dr. Mansyur No.66 Medan 20154, Sumatera Utara, Indonesia; [2]Department of Public Health, Faculty of Medicine, University of Sumatera Utara, Jl. Dr. Mansyur No.66 Medan 20154, Sumatera Utara, Indonesia

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***Correspondence:** Noni Novisari Soeroso. Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, University of Sumatera Utara, University of Sumatera Utara Hospital, Jl. Dr Mansyur No.66 Medan 20154, Sumatera Utara, Indonesia. E-mail: noni@usu.ac.id

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Abstract

BACKGROUND: Serum Procalcitonin (PCT) is a biomarker that is frequently used to diagnose an infection. In some cases of thoracic malignancy, procalcitonin level appears to increase. However, the role of procalcitonin to diagnose malignancy is not certain yet, and the causes have not been known.

AIM: This study aimed to investigate procalcitonin levels in non-small cell lung cancer patients.

METHODS: This was an observational study with a cross-sectional design. All lung cancer patients did not diagnose based on cytology/histopathology results with no evidence nor were signs and symptoms of infection recruited through consecutive sampling. The subtypes of lung cancer include adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, staged III and IV. The procalcitonin levels were analysed from blood using immunofluorescent assay. Data were then analysed with the Chi-Square test by Epi Info™ 7 programs in which p-value < 0.05 was considered statistically significant.

RESULTS: A total of 68 lung cancer patients fulfilled the criteria of this study, 55 men (80.9%) and 13 women (19.1%). The highest percentage of cytology/histopathology type found was adenocarcinoma (80.9%), and 60.3% of those were diagnosed in stage IV. An increased procalcitonin level (greater than 0.01 ng/mL) occurred in 80.9% of Non-Small Cell Lung Cancer (NSCLC) patients. It appears that the higher the stage of lung cancer, the lower procalcitonin levels would be, although it was not statistically significant. There was no association between lung cancer subtype with procalcitonin levels.

CONCLUSION: An increased level of procalcitonin may be an indication not only for infection but also for Non-Small Cell Lung Cancer.

Introduction

Procalcitonin (PCT) is a derivate of calcitonin hormones. It is produced primarily in the liver by macrophages (Kupffer cells) or neuroendocrine cells which are involved in systemic reactions in response to endotoxin circulation and inflammatory cytokine produced during bacterial or fungal infections [1], [2] [3]. The plasma level is associated with the severity of the infection [4]. In healthy individuals, PCT levels are very low (< 0.1 ng/ml). Procalcitonin has shown the importance of distinguishing the diagnosis of cancer patients with clinical symptoms of fever and increased levels of CRP [5], [6]. On the other hand, recent studies showed PCT concentrations at the right level with sepsis in patients with an advanced stage of cancer [7]. This raises doubts about the role of procalcitonin in diagnosing infections in cancer patients.

Patients with malignant diagnoses have a high risk of developing infections which are non-specific clinical signs and the sign of several different clinical outcomes, such as drug reactions, actual

infections, or paraneoplastic syndromes commonly known as 'neoplastic fever' [7]. The PCT values rise rapidly at 2-4 hours from the bacterial infection onset. PCT has a half-life of 22-24 hours; therefore, this concentration can be halved when the infection is cured [8]. Although PCT in patients with oncology neutropenia fever has been investigated in several studies, recent meta-analysis studies failed to define its role. Also, Shomalli et al., [9] studied the role of PCT as a biomarker to distinguish fever due to infection and non-infectious fever in non-neutropenia patients with solid tumours and hematologic malignancy. They also argued that the increase in PCT levels and C-Reactive Protein could also increase in malignancy. Several malignancies usually show false positive for PCT.

Information about the values and characteristics of PCT are limited to lung cancer incidence [10]. Based on the above description, this study aimed to investigate the procalcitonin levels in non-small cell lung cancer patients.

Material and Methods

This was an observational study with a cross-sectional design to investigate the characteristics of procalcitonin levels in non-small cell lung cancer patients among Indonesia population. The study was conducted in Adam Malik General Hospital for 3 months. Data were collected through the medical records of Adam Malik General Hospital from November 2016 to June 2017.

Subjects were recruited through consecutive sampling based on cytology/histopathology result. Patients diagnosed with tumour mediastinum and lung tumour metastasis were excluded from this study. Smoking status was also recorded. Patients categorised as active smokers if they have a smoking history of ≥ 100 cigarettes throughout their lives [11], passive smoker (a person who inhales cigarette smoke from a smoker). Type of cigarettes includes clove cigarettes (kretek) and filter (white) cigarettes. The Brinkman Index value was obtained from the multiplication of the average number of cigarettes smoked a day and multiplied by the duration of smoking (years). The value of Brinkman Index (IB) is mild if 0-199, moderate if 200-599, and severe if > 600 [12].

First, 4 ml of peripheral venous blood were taken and then inserted into the EDTA tube. Next, the serum was isolated. The procalcitonin values were determined by immunofluorescence using BRAHMS procalcitonin sensitive Kryptor automated system (Thermo Scientific, Brahms, Henningsdorf, Germany) [10]. The values of serum procalcitonin were determined, in which levels above 0.01 ng/ml was considered high [10].

To assess the relationship between gender, age, and the Brinkman index with procalcitonin levels in non-small cell lung cancer patients, the Chi-Square test was performed. While the procalcitonin levels based on the cytologic/histopathologic subtypes cancer stage were analysed with Mann-Whitney and Kruskal Wallis test, data were analysed using Epi Info™ 7 programs in which p-value of < 0.05 was considered statistically significant.

Results

Based on the characteristics of the subjects, it was found that the highest number of gender in lung cancer incidence was male (80.9%). The average age dominant with lung cancer was 40–60 years (51.5%). All subjects were smokers and mostly categorised as heavy smoker based on Brinkman index (75%). The most common subtype of lung cancer was adenocarcinoma type (80.9%). The data can be seen in Table 1.

Table 1: Demographic characteristics of the study subjects

Characteristics		n	%
Gender	Male	55	80.9
	Female	13	19.1
Age	Under 40 years-old	3	4.4
	40-60 years-old	35	51.5
	Over 60 years old	30	44.1
Smoking status	Active smoker	68	100.0
	Non smoker	0	0.0
Type of cigarettes	Clove cigarettes (kretek)	44	64.7
	Filter cigarettes (white)	24	35.3
Brinkman Index	Severe	51	75.0
	Moderate	14	20.6
	Mild	3	4.4
Cytology/histopathology	Adenocarcinoma	55	80.9
	Squamous Cell Carcinoma	12	17.6
	Large Cell Carcinoma	1	1.5
pTNM	I	8	11.8
	II	10	14.7
	III	9	13.2
	IV	41	60.3
Procalcitonin levels	Increased (> 0.01 ng/ml)	55	80.9
	Normal (≤ 0.01 ng/ml)	13	19.1

The association between procalcitonin levels and gender, age and Brinkman index on Non-Small Cell Lung Cancer patients were displayed in table 2.

Table 2: The association between demographic factors and the levels of procalcitonin

Variables		Procalcitonin level				*p-value*
		Increased		Normal		
		n	%	n	%	
Sex	Male	44	80.0	11	84.6	0.702[a]
	Female	11	20.0	2	15.4	
Age	Under 40 years-old	2	3.6	1	7.7	0.53[a]
	40-60 years-old	30	54.5	5	38.5	
	Over 60 years old	23	41.8	7	53.8	
Brinkman Index	Severe	40	72.7	11	84.6	0.38[a]
	Moderate	13	23.6	1	7.7	
	Mild	2	3.6	1	7.7	
Total		55	100.0	13	100.0	

a) Chi-Square test.

Table 3 showed that there was no difference in the levels of procalcitonin between adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. It appears that the higher the stage of lung cancer, the lower procalcitonin levels would be. However, this correlation was not observed in stage IV cancer patients, and thus it was not statistically significant.

Table 3: The comparison of procalcitonin levels based on tumour diagnosis

Variables		n	Procalcitonin levels		*p value*
			mean ± SD	median (min-max)	
Cytology/ histopathology	Adenocarcinoma	(n = 55)	1.02 ± 2.06	0.34 (0.02 - 11.9)	0.206 [a]
	Squamous Cell Carcinoma and large cell	(n = 13)	0.91 ± 1.24	0.48 (0.14 - 4.2)	
Stage	I	(n = 8)	1.21 ± 2.23	0.44 (0.02 - 6.71)	0.12 [b]
	II	(n = 10)	0.84 ± 0.45	0.90 (0.14 - 1.47)	
	III	(n = 9)	0.63 ± 1.04	0.17 (0.04 - 2.86)	
	IV	(n = 41)	1.07 ± 2.24	0.25 (0.02 - 11.9)	

a) Mann-Whitney test; b) Kruskal Wallis test.

Generally, it can be concluded that there was no effect of the cancer cell types nor cancer stage on serum procalcitonin levels.

Discussion

The sample of this research was 68 cases of lung cancer patients who have been diagnosed definitively (cytology/histopathology). Based on the gender of the subjects, there were 55 male patients (80.9%) whereas there were 13 female patients (19.1%). The same situation was also reflected in other parts of the world. Aareleid conducted epidemiological studies of lung cancer from 1985 to 2014. According to that study, 18,399 cases (80.3%) were male patients whereas 4491 cases (19.7%) were female patients [13]. In Indonesia, specifically in Adam Malik General Hospital, Medan, the highest number of patients based on gender was male with 85.62% compared to a female with 24 patients (24.37%) [3].

In this study, the number of patients between the age of 40-60 years were predominant with 36 patients (51.4%), followed by the age group of over 60 years with 30 patients (42.9%). An epidemiological study conducted by Ridge in 2015 also suggested that about 60% of lung cancer cases were suffered by patients aged 50-60 years old [14]. In his study, he also stated that the prevention of cigarettes was a top priority for public health. Globally, cigarettes are one of the biggest death factors with a 1:10 ratio in adults and a mortality rate of about 5 million people per year [14]. Similarly, a study conducted by Soeroso in 2018 showed that 60% of lung cancer cases suffered by patients aged 55-64 years [15], [16].

Brinkman index is used to assess the severity level of smoking. The Brinkman index in this study found that 75.7% of subjects were heavy smokers, 20% were moderate smokers, and the other 4.3% were light smokers. A similar result was also found by Soeroso et al., [15], [16] in which 52.9% of their research participants were categorised as heavy smokers. Also, this study was to evaluated nicotine dependence based on the Fagerstrom Tolerance Questionnaire (mFTQ), and the results showed that 65% of Fagerstrom scores were very high. Epidemiologic studies suggest that smoking is the leading cause of lung cancer. Smokers are 22 times more likely to die of lung cancer than nonsmokers [1]. Smoking has a role in lung cancer at various levels. Smoking can cause mutations of genes that leads cell to be oncogenic. One of the mutations is a p53 mutation found in more than 53% in smokers [2]. Furthermore, smoking can also influence adverse effects during therapy, either chemotherapy or radiotherapy [2].

Lung cancer is one of the leading causes of death around the world, particularly in Indonesia. In 2014, WHO stated that the mortality rate due to lung cancer reached 21.8% for men and 9.1% for a woman in Indonesia [17]**.** The most common type of cancer found in this study was adenocarcinoma (80.9%). Although previous studies found that squamous cell carcinoma as the most common type found, new paradigm depicted that adenocarcinoma was the most common type of cancer. This is probably because the type of cigarettes favoured by Indonesians is clove cigarettes. This study found that the most common type of lung cancer found in Batak tribe in Indonesia was adenocarcinoma (92.9%), and the most consumed cigarette type was clove cigarettes. Clove cigarettes contain clove that will make smokers suck in more deeply; thus, smokes containing carcinogenic substances eventually enter the peripheral respiratory tract [16], [18]. Syahruddin et al., [19] found that the EGFR mutations rate among Indonesian population reached 57.1% common mutations (exon 19 ins/dels, L858r) and about 29% uncommon mutations (G719X, exon 20 ins, T790M, L861Q).

In this study, most of the patients have entered the stage IV of cancer (60%), and the patients in stage III were 14.3%. This is slightly different from the results found by Soeroso in 2010-2012 in which most of the cases were in stage III with 56 cases (33.54%) from a total of 167 patients [3]. However, there is a trend found that lung cancer is generally diagnosed when it has entered an advanced stage. There are some factors that may cause late diagnosis. One of them is the absence of accurate screening to date [4].

The results showed that serum procalcitonin based on histopathology (adenocarcinoma, squamous cell, and large cell) in this study did not show a significant difference in the Kruskal-Wallis test ($p > 0.05$). This study also showed that the values of procalcitonin increased in 55 samples (80.9%). This contrasts with Avrillon's findings that 42% of 89 samples experienced an increase in procalcitonin

levels [10]. Procalcitonin is not a substance commonly used as a diagnostic tool for cancer, but recent research conducted by Vincenzi et al., [5] showed an increased in procalcitonin levels with cancerous conditions. The study also stated that during an advanced stage of cancer, the production of inflammatory cytokines is more active than usual leading to procalcitoninemia conditions [5].

Ghillani et al., [20] found an increased level of procalcitonin compared with healthy subjects by 17.5%, 53%, and 29% respectively in patients with squamous cell cancer, large cell, and adenocarcinoma. Non-cancer patients had low procalcitonin levels in average compared with patients with stage I-III cancer (0.029 ng/mL vs 0.127 ng/mL, $p < 0.0001$) or the stage IV disease (0.029 ng/mL vs 0.190 ng/mL, $p < 0.0001$). Cancer patients who have accelerated developmental stage have a higher mean of PCT than those in the low stages (0.190 ng/mL vs 0.127 ng/mL, $p = .004$) [21]. However, the patients in severe stages had an increase in the PCT values baseline compared with the patients in low stages (0.47 vs 0.27 ng/mL), $p = 0.017$) [9]. The PCT values were higher in the patients with small cell lung cancer than adenocarcinoma (0.33 ng/mL vs 0.07 ng/mL, $p < 0.001$). Furthermore, the PCT levels were significantly higher in the patients with liver metastasis (0.37 vs 0.09 ng/mL, $p < 0.001$) [22]. Another study showed an increase in PCT associated with metastatic stage cancer in 43 patients with solid tumour and 15 healthy control subjects; the highest level was found in metastatic cancer [23]. Also, it was found that procalcitonin levels were high in colorectal cancer. Keramidaris et al., [6] studied the relationship between bacterial translocation and cancer condition and found PCT positive in the majority of their samples (55.3%), higher procalcitonin levels were also found in metastatic patients than no metastasis ($p = 0.01$).

One of the limitations of this study was incomplete medical records data, especially data of lung cancer patients undergoing distant metastasis. Therefore, the relationship between procalcitonin levels and lung cancer patients undergoing metastasis cannot be assessed. Also, this study did not conduct C-reactive protein examination in all lung cancer patients due to health insurance limitations in Indonesia.

In conclusion, an increased level of procalcitonin was observed in most patients with Non-Small Cell Lung Cancer. It appears that the higher the stage of lung cancer, the lower procalcitonin levels would be, although it was not statistically significant. There was no association between lung cancer subtype with procalcitonin levels.

An increased level of procalcitonin may raise a suspicion for Non-Small Cell Lung Cancer, particularly if no evidence of infection was found.

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References

1. Soeroso NN, Soeroso L, Syafiuddin T. Kadar Carcinoembryogenic Antigen (CEA) Serum Penderita Kanker Paru Karsinoma Bukan Sel Kecil di RSUP Adam Malik.

2. Furrukh M. Tobacco Smoking and Lung Cancer. Sultan Qaboos Univ Med J. 2013; 13(3):345-358. https://doi.org/10.12816/0003255 PMid:23984018 PMCid:PMC3749017

3. Soeroso N. N. Soeroso L. Syafiuddin L. Level of Serum Carcinoembryogenic Antigen (CEA) In Non Small Cell Lung Cancer (NSCLC) at Adam Malik Hospital Medan. J Respir Indo. 2014; 34:17-25.

4. Molina JR, Yang P, Cassivi SD. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. Mayo Clin Proc. 2008; 83(5):584-594. https://doi.org/10.1016/S0025-6196(11)60735-0

5. Vincenzi B, Fioroni I, Pantano F. Procalcitonin as diagnostic marker of infection in solid tumors patients with fever. Sci Rep. 2016; 6:28090. https://doi.org/10.1038/srep28090 PMid:27312877 PMCid:PMC4911581

6. Keramidaris D, Koronakis N, Lagoudinakis EE. Procalcitonin in patients with colorectal cancer. J BUON. 2013; 623-628. PMid:24065474

7. Zell JA, Chang JC. Neoplastic fever; a neglected paraneoplastic syndrome. Support Care Cancer. 2005; 13(11):870-877. https://doi.org/10.1007/s00520-005-0825-4 PMid:15864658

8. Becker KL, Nylen ES, White JC, Muller B, Snider RH. Procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis:a journey from procalcitonin back to in precursors. J Clin Endocrinol. Metab. 2004; 89(4):1512-1525. https://doi.org/10.1210/jc.2002-021444 PMid:15070906

9. Shomali W, Hachem R, Chaftari AM, Jiang Y, Bahu R et al. Can procalcitonin distinguish infectious fever from tumor-related fever in non neutropenic cancer patients?. Cancer. 2012; 118(23):5823-5812. https://doi.org/10.1002/cncr.27602 PMid:22605389

10. Avrillon V, Locatelli-Sanchez M, Folliet L. Lung Cancer May Increase Serum Procalcitonin Level. Infect Disord Drug Targets. 2015; 15(1):57-64. https://doi.org/10.2174/1871526515666150320162950 PMid:25809625

11. Ryan H, Trosclair A, Gfroerer J. Adult current smoking: differences in definitions and prevalence estimates—NHIS and NSDUH, 2008. Journal of environmental and public health. 2012; 2012.

12. The Indonesia Society of Respirology. Chronic Obstructive Pulmonary Disease (COPD): Diagnosis and Management COPD, 2011.

13. Aareleid T, Zimmermann M, Baburin A. Divergent trends in lung cancer incidence by gender, age and histological type in Estonia: a nationwide population-based study. BMC Cancer. 2017; 17: 596. https://doi.org/10.1186/s12885-017-3605-x PMid:28854969 PMCid:PMC5577806

14. Ridge C, McErlean A, Ginsberg M. Epidemiology of Lung Cancer. Semin Intervent Radiol. 2013; 30(2):93-98. https://doi.org/10.1055/s-0033-1342949 PMid:24436524

PMCid:PMC3709917

15. Soeroso NN, Sinaga BYM, Zain-Hamid R, Sadewa AH, Syahruddin B. The CYP2A13 Arg257cys polymorphism and its relationship to lung cancer. Stem Cell Oncology. 2018; 265-269. https://doi.org/10.1201/9781351190152-57

16. Soeroso NN, Zain-Hamid R, Sinaga BYM, Sadewa AH, et al. The role of CYP2A6 genetic polymorphism in nicotine dependence and tobacco consumption among Bataknese male smokers. Open Access Maced J Med Sci. 2018; 6(5):864-866. https://doi.org/10.3889/oamjms.2018.224 PMid:29875862 PMCid:PMC5985876

17. World Health Organization: Age Adjusted Death Rate Estimates: 2017. Available from: https://www.worldlifeexpectancy.com/indonesia-lung-cancers

18. Soeroso NN, Zain-Hamid R, Sinaga BYM, Sadewa AH, et al. Genetic polymorphism of CYP2A6 and its relationship with nicotine metabolism in male Bataknese smokers suffered from lung cancer in Indonesia. Open Access Maced J Med Sci. 2018; 6(7):1199-1205. https://doi.org/10.3889/oamjms.2018.259 PMid:30087722 PMCid:PMC6062282

19. Syahruddin E, Wulandari L, Muktiati NS, Rima A, Soeroso N, Ermayanti S. Uncommon EGFR mutations in cytological specimens of 1,874 newly diagnosed Indonesian lung cancer patients. Lung Cancer: Targets and Therapy. 2018; 9:25–34. https://doi.org/10.2147/LCTT.S154116 PMid:29615847 PMCid:PMC5870662

20. Ghillani PP, Motte P, Troalen F. 'Identification and measurement of procalcitonin precursors in serum of patients with malignant diseases. Cancer Res. 1989; 49:6845-6851. PMid:2555054

21. Chaftari AM, Hachem R, Reitzel R. Role of procalcitonin and interleukin-6 in predicting cancer, and its progression independent of infection. PLoS One. 2015; 10(7). https://doi.org/10.1371/journal.pone.0130999

22. Patout M, Salaun M, Brunel V. Diagnostic and prognostic value of serum procalcitonin concentrations in primary lung cancers. Clin Biochem. 2014; 47(18):263-267. https://doi.org/10.1016/j.clinbiochem.2014.09.002 PMid:25218831

23. Matzaraki V, Alexandraki KI, Venetsanou K et al. Evaluation of serum procalcitonin and interleukin-6 levels as markers of liver metastasis. Clin Biochem. 2007; 40:336-42. https://doi.org/10.1016/j.clinbiochem.2006.10.027 PMid:17306245

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Clinical Science

Outcomes of Operative Management of 96 Cases with Traumatic Retroperitoneal Hematoma: A Single-Institution Experience

Harth Mohamed Kamber[1], Tawfiq Jasim Mohammed Al-Marzooq[2], Haider Raheem Neamah[3], Qays Ahmed Hassan[4*]

[1]Division of Urology, Department of Surgery, Al-Kindy College of Medicine, University of Baghdad, Baghdad, Iraq; [2]Department of Surgery, Al-Kindy College of Medicine, University of Baghdad, Baghdad, Iraq; [3]Division of Cardiovascular Surgery, Department of Surgery, Al-Kindy College of Medicine, University of Baghdad, Baghdad, Iraq; [4]Division of Radiology, Department of Surgery, Al-Kindy College of Medicine, University of Baghdad, Baghdad, Iraq

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***Correspondence:** Qays Ahmed Hassan. Division of Radiology, Department of Surgery, Al-Kindy College of Medicine, University of Baghdad, Baghdad, Iraq. E-mail: qtimeme@yahoo.com

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Abstract

AIM: To analyse our experiences in the management of traumatic retroperitoneal hematoma (RPH), highlighting the various challenges faced and to report on the outcome of these patients.

METHODS: From May 2014 to May 2017, all patients with traumatic RPH who underwent surgical treatment were retrospectively analysed. The kind of injury, intraoperative findings, sites of hematoma, postoperative morbidity and the overall outcomes were recorded.

RESULTS: Ninety-six patients; 53 with blunt trauma and 43 with penetrating injury, were included in this study. The centre-medial hematoma was observed in 24 (25%) patients, lateral hematoma in 46 (47.9%) patients, pelvic hematoma in 19 (19.8%) patients, and multiple zone hematomas in 7 (7.3%) patients. All cases were managed surgically. Exploration of the retroperitoneal space was done in 72 cases. Thirty-three patients died, and the overall death rate was 34.4%.

CONCLUSION: Surgical exploration should be done in RPH caused by penetrating injury, but the need for urgent exploration in blunt injury is not so high, and it depends on the anatomical site of hematoma, concomitant organ injury and the hemodynamic status of patients.

Introduction

Retroperitoneal hematoma (RPH) assigns to an aggregation of blood in the retroperitoneal space [1]. It is a life-threatening condition since they may cause a serious hypovolemic shock or severe ischemia in their end organ and require prompt diagnosis and treatment [2]. Traumatic RPH may be caused by blunt or penetrating injury to the abdomen or pelvis [3]. Injury to the bone, vascular structures, hollow viscera or solid organs may be responsible for the occurrence of RPH making the diagnosis and management of this serious condition more difficult [1] [4]. Vascular injury accounts for more than 90% of RPH presentation [5], [6]. The aorta, superior mesenteric artery (SMA), iliac arteries, inferior vena cava (IVC), portal vein (PV), and iliac veins are the most frequently injured abdominal vessels. Though many advances have been made in overall management and care of the injured patient, traumatic RPH continue to result in significant morbidity and mortality. The mortality from traumatic RPH is reported in different modern series remains high at 14-50% [4], [7] with early deaths due to exsanguination and late deaths due to multisystem organ failure [8]. The surgeons involved in the care of these patients need to gain and maintain skills and knowledge related to these injuries if outcomes are going to be improved. Therefore, we reviewed retrospectively 96 patients with traumatic RPH in our institution.

This study aimed to analyse our experiences in the management of patients with traumatic RPH, highlighting the various challenges faced while managing this life-threatening injury and to specifically report on the peri-operative management and outcome of these patients.

Patients and Methods

From May 2014 to May 2017, all patients with RPH due to blunt or penetrating trauma who underwent surgical treatment were included in this retrospective study. Exclusion criteria included all cases of RPH resulting from reasons other than trauma such as complications of anticoagulant therapy, those with clotting disorders or those with aortic aneurysm rupture. Also, patients with extra-abdominal injury, head injury, major thoracic injury, peripheral orthopaedic injury were excluded from the study. The study was conducted in a tertiary hospital, and it was approved by the local Institutional Review Board with a protocol no.116/2014.

Data regarding patient's age, sex, mechanism of injury, type of injury, ultrasound (US) and computed tomography (CT) findings, intraoperative findings, and classification of the zone of the hematoma, type of operative procedure done, postoperative hospital stay, mortality, and morbidity were collected.

Diagnosis of RPH was made using US and CT. But on many occasions, no imaging procedures were done because of unstable hemodynamic state so that diagnosis was made clinically and confirmed in exploratory laparotomy. Surgical management was applied in all cases. The RPH were classified into three zones: centre-medial (zone I), lateral (zone II) and pelvic (zone III) hematomas according to Selivanov et al., classification [9].

Results

Ninety-six patients enrolled in this study composed of 82 (85.42%) men and 14 (14.58%) women with a mean age of 38.3 years (range from 16 to 62 years). Fifty-three (55.21%) patients sustained blunt trauma and 43 (44.79%) patients sustained penetrating injury. The cause of trauma was an automobile (Motorcycle road traffic accident and vehicular road traffic accident) in 38 (39.58%) cases, fall from height in 11 (11.46%) cases, strikes injuries in 4 (4.17%) cases, stab wound to the abdomen in 6 (6.25%) cases, gunshot injury in 4 (4.17%) cases and shell injury in 23 (23.96%) cases (Table 1).

Table 1: Characteristics of the study sample

Character	Number (%)
Age group	
< 20 years	3 (3.13)
20-29 yrs	38 (39.58)
30-39 yrs	31 (32.29)
40-49 yrs	17 (17.71)
50-59 yrs	5 (5.21)
> 60 yrs	2 (2.08)
Total	96 (100)
Sex	
Male	82 (85.42)
Female	14 (14.58)
Mechanism of injury	
Blunt injury	53 (55.21)
RTA	38 (39.58)
Fall from height	11 (11.46)
Strike	4 (4.17)
Penetrating injury	43(44.79)
Stab Wound	6 (6.25)
Gunshot	14 (14.58)
Shells (blast)	23 (23.96)

The initial US was performed in 36 patients; 24 (66%) of them were diagnosed with RPH. CT was done in 32 patients and the diagnosis of RPH was established in 28 (87.5%) of them. For 44 patients, neither initial preoperative US nor CT scanning was done because of severing hemodynamic instability they presented with and the urgent surgical intervention which was undertaken.

For 96 cases, all were managed surgically. Ten (10.42%) patients were found to had single site injury (pure RPH) while 86 (89.58%) patients were had multiple injuries. Features of intraabdominal organ damage linked to RPH with subsequent mortality were exhibited in Table 2.

Table 2: Associated intra-abdominal organ injury

Associated intra-abdominal injury	Number of patients (%)	Number of death (%)
Splenic rupture	5 (5.21)	1 (1.04)
Duodenal injury	5 (5.21)	2 (2.08)
Gastric& Small bowel injury	12 (12.50)	2 (2.08)
Large bowel injury	8 (8.33)	1 (1.04)
Bladder rupture and posterior urethral disruption	3 (3.13)	1 (1.04)
Hepatic laceration	7 (7.29)	2 (2.08)
Diaphragmatic laceration	3 (3.13)	2 (2.08)
Pancreatic injury	4 (4.17)	2 (2.08)
Renal injury	10 (10.42)	2 (2.08)
Multiple organ injury	29 (30.21)	16 (16.67)
Pure RPH	10 (10.42)	2 (2.08)
Total	96 (100)	33 (34.38)

Different procedures were performed for the management of RPH included ligation or repair of major blood vessels and repairing or resection of retroperitoneal organs (duodenum, colon, kidney, pancreas, e.t.c.). Exploration of the retroperitoneal space was made in 72 (75%) cases. The kind of surgical work in 96 patients was displayed in Table 3.

The surgical findings were as follow: Centro-medial hematoma (zone I) was detected in 24 (25%) patients, lateral hematoma (zone II) in 46 (47.9%) patients, pelvic hematoma (zone III) in 19 (19.8%), multiple zone RPH in 7 (7.3%).

The overall mortality in this study sample was 33 patients (34.4%). Half of them died during the

operation due to continues uncontrolled bleeding, hypovolemic shock and multi-organ failure. The others died were due to septicemia, respiratory failure, myocardial infarction, pulmonary embolism and acute renal failure few days postoperatively.

Table 3: Operations performed in 96 patients

Operations performed to control RPH	Number of patients (%)
Packing only	16 (16.67)
IVC repair	7 (7.29)
Abdominal aorta repair	2 (2.08)
Ligation or repair of the internal iliac artery	7 (7.29)
Repair of common iliac vessels	2 (2.08)
Repair of lumbar vessels	4 (4.17)
Associated operations performed	
Splenectomy	5 (5.21)
Colostomy with colonic repair	8 (8.33)
Gastric repair	2 (2.08)
Small bowel repair	4 (4.17)
Small bowel resection with end to end anastomosis	3 (3.13)
Drainage or repair of the pancreas	4 (4.17)
Pelvic fixation	2 (2.08)
Repair or partial resection of liver	7 (7.29)
Repair of duodenum	8 (8.33)
Repair of diaphragm	3 (3.13)
Repair of mesentery	1 (1.04)
Repair of kidney or nephrectomy	10 (10.42)
Repair of urinary bladder	3 (3.13)

Postoperatively, wound infection, pancreatic and duodenum fistula, chest infection were noted (Table 4).

Wound infection was treated by daily dressings under broad spectrum antibiotics. Pancreatic or duodenum fistula was treated conservatively; both fistulae were closed within few weeks. The cases with chest infections were treated with a suitable antibiotic combined with chest physiotherapy. The mean hospital stays were 8.4 days (range from 4 to 14 days). The mean follow-up was 2 months, ranging from one month to 3 months.

Table 4: Postoperative morbidity

Postoperative Complication	Number of patients (%)
Surgical site infection	7 (7.29)
Cardiovascular complications: MI, pulmonary embolism, DIC	4 (4.17)
Respiratory complications: Lobar pneumonia, atelectasis, ARDS	5 (5.21)
Re-exploration for continuous bleeding	3 (3.13)
Pancreatic fistula	1 (1.04)
Duodenal fistula	1 (1.04)
Multiple organ dysfunction syndromes	12 (12.50)
Acute renal failure	6 (6.25)
Septic shock	6 (6.25)
Nil postoperative complication	58 (60.42)

Discussion

The traumatic RPH is a common, life-threatening condition resulting from abdominal or pelvic injuries. To decrease the morbidity and mortality of this potentially fatal condition, urgent diagnosis and immediate operational interference are of high significance [10]. In this study, 43 out of 96 RPH arising from penetrating abdominal injury, most of them (23 out of 43) sustained in blast injury. This is in contrast to findings in other studies of RPH [11], [12] and this can be explained by the engagement of our county in sustained terrorist events during the period of study.

Regarding the diagnosis of RPH, the clinical presentation are usually nonspecific with abdominal pain, abdominal distension, severe back pain or abdominal mass making the clinical diagnosis of this serious condition very difficult [13]. Furthermore, large amounts of blood can accumulate in the abdominal cavity without significant changes in physical examination [14]. Trauma patients manifesting with hemorrhagic shock and an unknown origin of bleeding should undergo promote further evaluation according to the European guidelines by the Multidisciplinary Task Force for Advanced Bleeding Care [15].

For patients with abdominal trauma, an imaging study such as CT and US play an important role in the diagnosis of RPH, and to rule out a concomitant occult abdominal injury helping surgeons make treatment decision [16], [17]. Unlike CT, US is not precise and can't certainly identify the correct site or amount of hematoma, furthermore, its sensitivity for direct demonstration of abdominal organ injury is relatively low [18], [19]. In the current study, 24 patients were diagnosed with RPH out of 36 patients who underwent US examination. Although CT scan is a more advanced tool, its diagnostic accuracy may affect by some determinants such as the position and extent of hematoma, expertise of radiologists and resolution of CT machine. To achieve reliable decisions, CT scan needs both oral and IV contrast [19]. In the current study, a native CT examination was done for 32 patients, and 28 were diagnosed with RPH. The CT scan we carried was done without contrast because the patient was in an unstable hemodynamic state and without actually having the time for a complete radiological study. Consequently, the exploratory laparotomy was firmly advised as the secured choice to diagnose this fatal lesion, particularly in a hemodynamically unstable patient.

Many classifications of RPH have been done according to the site of hematomas. In this study, we adapted the Selivanov et al. classification (1984) [9]. In this classification, centromedial localisation was described as zone I, lateral localisation as zone II, and pelvic localisation as zone III.

The RPH in the centre-medial zone is often the result of duodenum, pancreas or great vessels damage. The appearance of rising sign and symptoms, increased serum or urinary amylase, the free gas inside the peritoneal cavity and effusion nearby duodenum or pancreas indicate duodenal or pancreatic injury and exploratory laparotomy must be done. Furthermore, even in the setting of a stable centre-medial hematoma, we suggest exploration of the hematoma to avoid possible fatal sequel of missed pancreatic or duodenal injury. In the present study, pancreas damage was established in four patients, and the pancreatic repair and drainage were performed immediately, two patients died, and the other 2 patients improved and were discharged. Injury to the abdominal aorta is correlated with high morbidity and death rates varying from 50% to 78% in

several studies [20], [21], [22], [23], [24]. We encountered two patients with aortic injury, one of them died from multiple organ failures while the other patient who sustained a stab wound to the back was survived. Seven patients with IVC injury were found, and primary repair of IVC was done for them, 2 of those patients died during the early postoperative period.

The need for urgent surgery is not necessary for all patients with an RPH in the lateral zone. Here, we observed most of the RPH were co-occurred with other organic injuries such as kidney and less commonly colon. The perirenal RPH followed a blunt trauma can be managed conservatively, and most patients improved [3], [4], [5]. But, the decision for emergency laparotomy should be applied when the hematoma is becoming expanded, pulsatile, or ruptured. We encountered 46 cases with zone II hematoma, in those with penetrating injury (most of them was shell injuries from explosive terrorist events), the hematoma was explored and dealt with in the majority of the patients but 5 of them were not explored because it was not expanding, not pulsatile and away from vital structures. Ten hematomas in those with blunt trauma were left undisturbed because it doesn't fulfil the criteria of exploration.

Most of the patients with zone III hematoma were due to blunt injury (14 out of 19), most of them were due to RTA, 4 of them were not explored and only preperitoneal packing was applied because they were of small size, not expanding and not pulsatile after assuring no rectal injury and intact femoral artery pulsation. Those with penetrating injury all are explored. Preperitoneal packing has also shown value in stopping bleeding in blunt pelvic trauma as an optional extra to angioembolization and pelvic fixation [25], [26]. It has been proposed that hematoma in the retroperitoneal space can be stopped by using direct pressure on the bleeding site, while exploration of hematoma may lead to severe bleeding resulting in a patient's death. We support this viewpoint in our study where 4 patients with pelvic zone RPH were not explored. Yet, the surgical exploration becomes necessary when the RPH were associated with a simultaneous injury of the rectum, bladder or other organs.

Although traumatic rupture of a lumbar artery is an unusual complication of a blunt abdominal trauma that can lead to a potentially massive RPH, lateral and pelvic RPH secondary to injury of lumbar and pelvic blood vessels are the most common cause of hemorrhagic shock from vertical deceleration injuries [22]. In this study, RPH secondary to injury of the lumbar vessels was noted in four patients. In two patients, the hematoma was explored on assumption of renal and colonic injury respectively, but no such injuries nor active bleeder was found on exploration and hematomas in both patients were evacuated and packed pressures were applied. In the other two patients, the hematomas were not interfered with.

As an operational plan, exploring an RPH should be the latest option because the opening of this closed space may result in marked and fatal bleeding once the pressure on the bleeding tissues is taken-off. All areas of the retroperitoneal space are supplied by several collateral vessels. This can tell why once a bleeding artery is under control, collateral supply to the same area can produce further bleeding. This is one of the main causes why the surgeons avoid exploration of the retroperitoneum in the context of trauma. As damage control surgery was applied in our cases, we did not risk further exploration of the hematoma as saving the patient was more critical to us than controlling the hematoma.

The overall mortality rate in the current study sample was 34.4%. Several other studies reported a wide range of mortality rate between 18% and 60% [10], [11], [12], [13]. One of the challenges we encountered in the management of those patients that may raise the death rate was the delayed surgery. This can be attributed to deficient sterile operations bundles, limited and busy surgical theatre places, wait in receiving blood for transfusion, and also delay in handing out laboratory results.

The treatment of RPH due to blunt trauma is still difficult for the surgeons as there is invariably a large chance of converting it into an uncontrollable haemorrhage. In general, we propose that the management plan which decided by the surgeon should respect the patient's ages, kind of injury, associated organ damages and the hemodynamic status of the patients.

In conclusion, the traumatic RPH is a serious, life-threatening condition, rapid diagnosis and immediate treatment are of great concern. We suggest that surgical exploration should be performed in RPH caused by penetrating injury, but the need for urgent exploration in blunt injury is not so high and it depends on the anatomical site of hematoma, concomitant organ injury and the hemodynamic status of patients.

References

1. Farouque HM, Tremmel JA, RaissiShabari F, et al. Risk factors for the development of retroperitoneal hematoma after percutaneous coronary intervention in the era of glycoprotein IIb/IIIa inhibitors and vascular closure devices. J Am Coll Cardiol. 2005; 45:363 368. https://doi.org/10.1016/j.jacc.2004.10.042 PMid:15680713

2. Abdulhussein BJ, Hussein YF, Nawar AH. Pattern of presentation of retroperitoneal hematoma among sample of Iraqi patients attending surgery clinic of a teaching hospital. Surgical Science. 2015; 6:208 213. https://doi.org/10.4236/ss.2015.65032

3. Feliciano DV. Management of traumatic retroperitoneal hematoma. Ann Surg. 1990; 211:109-123. https://doi.org/10.1097/00000658-199002000-00001 PMid:2405790 PMCid:PMC1357953

4. Tolga M, Umit T, Ali A, Mehmet AS. The management of retroperitoneal haematomas. Scand J Trauma Resusc Emerg Med. 2004; 12:152-156.

5. Bageacu S, Kaczmarek D, Porcheron J. Management of traumatic retroperitoneal hematoma. J Chir (Paris). 2004; 141:243 249. https://doi.org/10.1016/S0021-7697(04)95603-7

6. Stagnitti F, Toccaceli S, Spaziani E, et al. Traumatic retroperitoneal haematoma. G Chir. 2007; 28:356 362. PMid:17915048

7. Velmahos GC, Toutouzas KG, Vassiliu P, et al. A prospective study on the safety and efficacy of angiographic embolization for pelvic and visceral injuries. J Trauma. 2002; 53:303 308. https://doi.org/10.1097/00005373-200208000-00019 PMid:12169938

8. Kobayashi L, Costantini T, Coimbra R. Mesenteric vascular trauma. In: Dua A, Desai S, Holcomb J, et al, eds. Clinical review of vascular trauma. Springer Medical Publishing, 2014:213–224. https://doi.org/10.1007/978-3-642-39100-2_18

9. Selivanov V, Chi HS, Alverdy JC, Morris JA Jr, Sheldon GF. Mortality in retroperitoneal hematoma. J Trauma. 1984; 24:1022-1027. https://doi.org/10.1097/00005373-198412000-00004 PMid:6512896

10. Wang F, Wang F. The diagnosis and treatment of traumatic retroperitoneal hematoma. Pak J Med Sci. 2013; 29:573 576. https://doi.org/10.12669/pjms.292.3168 PMid:24353579 PMCid:PMC3809226

11. Stagnitti F, Toccaceli S, Spaziani E, et al. Traumatic retroperitoneal haematoma. G Chir. 2007; 28:356 362. PMid:17915048

12. Cardia G, Loverre G, Pomarico N, Nacchiero M. Traumatic retroperitoneal lesions. Ann ItalChir. 2000; 71:457 467. PMid:11109670

13. Murai Y, Adachi K, Yoshida Y, Takei M, Teramoto A. Retroperitoneal hematoma as a serious complication of endovascular aneurysmal coiling. J Korean Neurosurg Soc. 2010; 48:88-90. https://doi.org/10.3340/jkns.2010.48.1.88 PMid:20717521 PMCid:PMC2916158

14. Lalancette M, Scalabrini B, Martinet O. Seat-belt aorta: a rare injury associated with blunt abdominal trauma. Ann Vasc Surg. 2006; 20:681-683. https://doi.org/10.1007/S10016-006-9058-3 PMid:16732445

15. Bland ZM, Dobos N, Gosselin MV: Case of the season: imaging of blunt traumatic injury to the abdominal aorta. Semin Roentgenol. 2006, 41:157-158. https://doi.org/10.1053/j.ro.2006.04.002 PMid:16849046

16. Daly KP, Ho CP, Persson DL, Gay SB. Traumatic Retroperitoneal Injuries: Review of Multidetector CT Findings. Radiographics. 2008; 28:1571-1590. https://doi.org/10.1148/rg.286075141 PMid:18936022

17. Mohammadi A, Ghasemi-Rad M. Evaluation of gastrointestinal injury in blunt abdominal trauma "FAST is not reliable": the role of repeated ultrasonography. World J Emerg Surg. 2012; 7:2. https://doi.org/10.1186/1749-7922-7-2 PMid:22264345 PMCid:PMC3287959

18. Tyburski JG, Wilson RF, Dente C, et al. Factors affecting mortality rates in patients with abdominal vascular injuries. J Trauma. 2001; 50:1020–1026. https://doi.org/10.1097/00005373-200106000-00008 PMid:11426115

19. Van der Vlies CH, Olthof DC, Gaakeer M, Ponsen KJ, van Delden OM, Goslings JC. Changing patterns in diagnostic strategies and the treatment of blunt injury to solid abdominal organs. Int J Emerg Med. 2011; 4:47. https://doi.org/10.1186/1865-1380-4-47 PMid:21794108 PMCid:PMC3170179

20. Deree J, Shenvi E, Fortlage D, et al. Patient factors and operating room resuscitation predict mortality in traumatic abdominal aortic injury: a 20-year analysis. J Vasc Surg. 2007; 45:493–497. https://doi.org/10.1016/j.jvs.2006.11.018 PMid:17254736

21. Junaid S, Badar BB, Haris BB, Azam Y, Kiran. H. Management of retro peritoneal hematoma. Professional Med J. 2005; 12:230-236.

22. Katsoulis E, Tzioupis C, Sparks I, Giannoudis PV. Compressive blunt trauma of the abdomen and pelvis associated with abdominal aortic rupture. Acta orthopaedica belgica. 2006; 72(4):492. PMid:17009833

23. Davis TP, Feliciano DV, Rozycki GS, et al. Results with abdominal vascular trauma in the modern era. Am Surg. 2001; 67:565–570. PMid:11409805

24. Asensio JA, Chahwan S, Hanpeter D, et al. Operative management and outcome of 302 abdominal vascular injuries. Am J Surg. 2000; 180:528–533. https://doi.org/10.1016/S0002-9610(00)00519-5

25. Cothren CC, Osborn PM, Moore EE, et al. Preperitonal pelvic packing for hemodynamically unstable pelvic fractures: a paradigm shift. J Trauma. 2007; 62:834–839. https://doi.org/10.1097/TA.0b013e31803c7632 PMid:17426537

26. Cullinane DC, Schiller HJ, Zielinski MD, et al. Eastern Association for the Surgery of Trauma practice management guidelines for hemorrhage in pelvic fracture—update and systematic review. J Trauma. 2011; 71:1850–1868. https://doi.org/10.1097/TA.0b013e31823dca9a

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Case Report

Acupuncture Treatment after Shoulder Arthroscopy after Recurrent Dislocations

Jihe Zhu[1], Blagica Arsovska[1,2], Kristina Kozovska[1,3*]

[1]Faculty of Medical Sciences, University Goce Delchev, Shtip, Republic of Macedonia; [2]Institute of Biology, Faculty of Natural Sciences and Mathematics, Skopje, Republic of Macedonia; [3]Medicine Faculty, Ss. Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

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Keywords: Treatment; Acupuncture; Traditional Chinese Medicine; Shoulder; Dislocation

***Correspondence:** Kristina Kozovska. Faculty of Medical Sciences, University Goce Delchev, Shtip, Republic of Macedonia; Medicine Faculty, Ss. Cyril and Methodius University of Skopje, Republic of Macedonia. E-mail: tongdatang-tcm@hotmail.com

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Abstract

BACKGROUND: The shoulder has the greatest range of motion of the entire joint in the body. Therefore it accounts for almost 50% of all joint dislocations. Most commonly, the dislocation is anterior and may occur because of trauma. Symptoms are intense pain, inability to move, numbness of the arm, weakness, swelling and visibly out of place shoulder. This condition requires emergency medical assistance to relocate the shoulder and do X = ray and MRI scans.

CASE REPORT: The treated patient is a 20-year-old girl who has undergone shoulder arthroscopy after recurrent dislocations. 2 years after the surgery, another trauma happened, and the shoulder was dislocated again along with a broken piece of the bone. The patient decided instead of open surgery to do acupuncture treatment. 10 treatments were done, 3 with fire needle and 7 with normal needle acupuncture in 5 months. The patient was also taking Glucosamine and Calcitriol and was advised to rest the arm, not to bore it, not to lift weight and not to make sudden movements. Less than a year, the results are amazing, there's no pain in the shoulder, the movement is unlimited and eased. The MRI image is with normal findings with a little piece of 3 mm still to heal on the broken part.

CONCLUSION: For such a serious condition as shoulder dislocation, acupuncture gave amazing and very satisfying results, with no need of open surgery.

Introduction

The shoulder has the greatest range of motion of all the joint in the body, and it accounts for almost 50% of all joint dislocations. Most commonly, the dislocation is anterior and may occur because of trauma. Symptoms are intense pain, inability to move, numbness of the arm, weakness, swelling and visibly out of place shoulder. This condition requires emergency medical assistance to relocate the shoulder and do an X-ray and MRI scans [1] [2]. In cases where is needed cartilage and ligament repair for tears, it is done via arthroscopy. In this case, the surgeon manipulates with instruments through the thin tube inserted through a few small (1 cm) incisions into the patient's skin (shoulder). There's a minimum of pain after surgery, and the patient can get back to work quickly. Recovery is usually rapid, throughout 4 weeks and if the person is an athlete, will need a few more months before starting with the sports activities [3].

When the ligaments in the shoulder are looser, shoulder dislocation can happen relatively easy, and it is harder for the shoulder's rotator cuff muscles to maintain the stability of the shoulder [4]. When the shoulder has been dislocated, a bone structure can be broken as well in the process, and many soft tissue structures may be damaged. After the acute care administration, acupuncture plays an important role in the promotion of soft tissue healing. Acupuncture treatment can restore the normal function of the muscle and muscle tone and alleviate

the pain in the shoulder to a greater degree, effectively treats the pathologies of the soft tissues of the shoulder and improves the quality of the person`s life [5] [6] [7].

Case report

The treated patient is a 20-year-old girl who has undergone shoulder arthroscopy after recurrent dislocations. The recurrent dislocations were happening because of lifting weights. After a sudden movement, the shoulder could not back in the joint position, and urgent intervention was needed with non-bloody reposition to the right humeroscapular joint. After three months of immobilisation, the doctors concluded that it is necessary to make shoulder arthroscopy. The MRI scan before the surgery showed smooth transplantation of bone structures without signs of fresh bleeding. In the projection of the medial side of the rotator cuff supraspinatus was visible a rupture of the same. Signs of complete rupture and contraction were not seen. In the projection of soft tissue structures, there were no signs of the existence of free fluid. Figure 1 is shown the MRI scan of the right shoulder before the surgery.

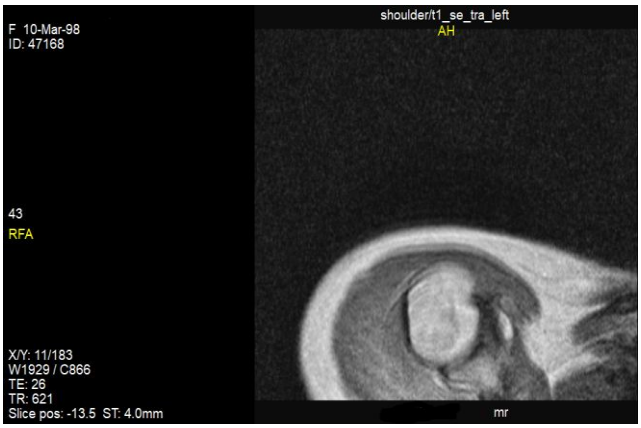

Figure 1: MRI scan of the right shoulder before the surgery

Two years after the surgery, another trauma happened after falling off a scooter in water, and the shoulder was dislocated again along with a broken piece of the bone. The damage was greater, and the doctors suggested to do an open stem cell transplant operation or by installing a specially designed titanium plate in the shoulder. The doctors said the surgery is inevitable and there is no other way to treat the shoulder. The patient decided instead of open surgery to do acupuncture treatment. On Figure 2 is shown an MRI scan right after the second trauma and before starting the acupuncture treatment.

The treatment was done in a clinic for Traditional Chinese Medicine and acupuncture in Skopje, Macedonia by a doctor specialist in acupuncture. Treatments were done indoors, at room temperature, once weekly. The fire needle treatments were with duration of 5-10 minutes and the treatments with a normal needle were with duration of 30-45 minutes.

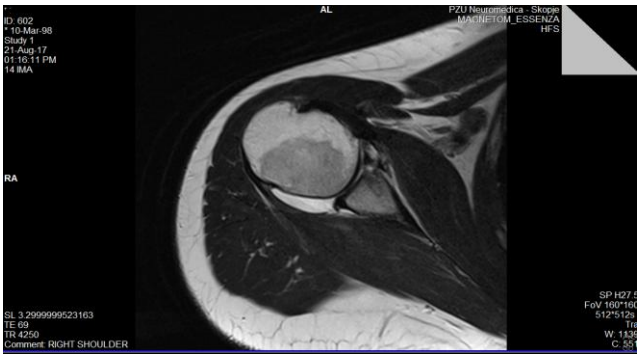

Figure 2: MRI scan done right after the second trauma and before starting the acupuncture treatment

Ten treatments were done, 3 with fire needle and 7 with normal needle acupuncture in 5 months. The patient has also prescribed two doses of Glucosamine 500 mg two times a day and Calcitriol 0.25 mg once a day. The patient was advised to rest the arm, not to bore it, not to lift weight and not to make sudden movements for at least 3 months. Treatments were done with aim to help the bone and surrounding tissues to recover faster, to ease the constant pain in the shoulder and upper arm, to heal the damaged tendons and ligaments, to bring back the normal function of the shoulder and arm and prevent from further dislocations. Calcitriol was taken to mineralise the bone and Glucosamine as a supplement to help relieve the pain and stiffness in the joint, cartilage, ligaments and tendons.

Less than a year, the results came up amazing, there is no pain in the shoulder and arm, and the movement is unlimited and eased in all directions. On figure 3 and figure 4 are shown the MRI scans done after the treatment. The MRI image is with normal findings with a little piece of 3 mm still to heal on the broken part of the shoulder.

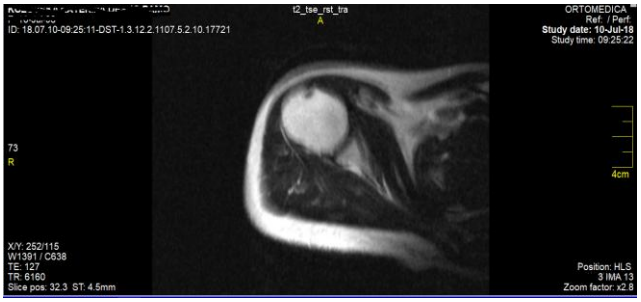

Figure 3: MRI scan done after the treatment

The results after the acupuncture treatment are showing normal tendon of m. Supraspinatus, m. Subscapularis, mm. Infraspinatus, m. Teres minor.

Normal Biceps Labral complex. The normal tendon of m. Biceps intrascapular long and labrum. Normal capsuloglagigmental complex, normal rotator cuff part and normal acromioclavicular joint. Type II morphology of the acromion is present. No present fluid is detected in the subacromial-subdeltoid bursa. Normal visualization of coracohumeral and coracoacromial ligaments. Normal quadrilateral space and normal axillary space. Normal m. Deltoideus and m. Trapezius. Present 3 mm cavity (may be seen as a cyst on the scan) in the healing process in the head of the humerus by the insertion of the supraspinal tendon.

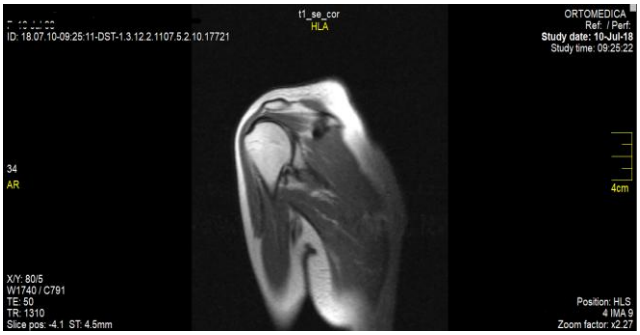

Figure 4: MRI scan done after the treatment

On figure 5 is shown the treated place and the treated points. Treatment was done on Ashi, i.e. trigger points located on the most painful places in the affected area.

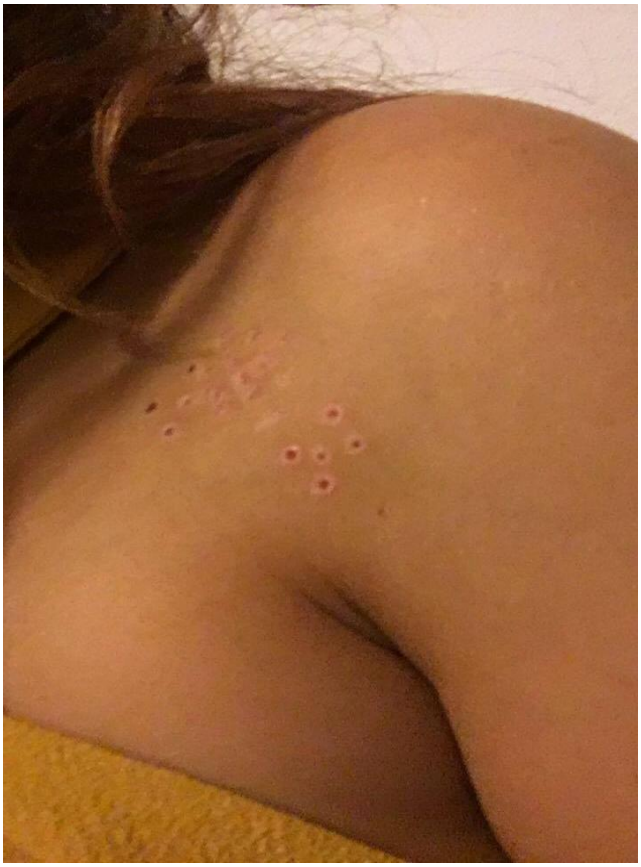

Figure 5: Treated points with fire needle acupuncture

The shoulder joint has a complex structure which allows an incredible range of motion, but on the other side the blood supply is relatively poor, and when there is injury, it heals slowly. Acupuncture treatments help with the healing process by stimulating the circulation of Qi and Blood, reducing the inflammation in the affected area, removing the pain, removing the blockages from the meridians, clearing the accompanying symptoms, strengthening the immune system and allowing free movement in the joints in everyday life without pain [8].

In conclusion, for such a serious condition as shoulder dislocation, acupuncture gave amazing and very satisfying results in this case, without the need for open surgery. The patient continues to use the arm in all daily activities, adding gradually a moderate physical activity. In the coming months, the patient's condition will be monitored and will be scheduled for routine examinations.

References

1. Welsh S. Shoulder Dislocation Surgery Treatment & Management, 2016. [www.emedicine.medscape.com]

2. Stone RK. Shoulder dislocation, 2016. [www.stoneclinic.com]

3. Warme JW. Treating Shoulder Dislocation / Subluxation (Instability) and Associated Pain with Minimally Invasive Arthroscopy, 2013. [www.orthop.washington.edu]

4. Miller J, Armfield S. Dislocated Shoulder, 2018. [www.physioworks.com.au]

5. Asheghan M et al. Investigation of the effectiveness of acupuncture in the treatment of frozen shoulder. Mater Sociomed. 2016; 28(4): 253–257. https://doi.org/10.5455/msm.2016.28.253-257 PMid:27698596 PMCid:PMC5034968

6. Vas J, Ortega C, Olmo V, Perez-Fernandez F, Hernandez L, Medina I, Seminario JM, Herrera A, Luna F, Perea-Milla E, Mendez C. Single-point acupuncture and physiotherapy for the treatment of painful shoulder: a multicentre randomised controlled trial. Rheumatology. 2008; 47(6):887-93. https://doi.org/10.1093/rheumatology/ken040 PMid:18403402

7. Vas J et al. Acupuncture and rehabilitation of the painful shoulder: study protocol of an ongoing multicentre randomised controlled clinical trial [ISRCTN28687220]; BMC Complement Altern Med. 2005; 5:19. https://doi.org/10.1186/1472-6882-5-19 PMid:16225693 PMCid:PMC1277817

8. Zhu J, Arsovska B, Velickova N. Acupuncture Treatment for Shoulder Bursitis. Imperial Journal of Interdisciplinary Research. 2016; 2(12).

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Case Report

"Two Stones on One Bird": A Case Report on Severe Biphasic Anaphylaxis Masquerading as Life-Threatening Acute Asthma

Alvin Oliver Payus[1*], Azliza Ibrahim[2], Norlaila Mustafa[2]

[1]Faculty of Medicine and Health Science, Universiti Malaysia Sabah (UMS), Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia; [2]Department of Internal Medicine, Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Jalan Yaacob Latif, 56000 Cheras, Kuala Lumpur, Malaysia

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Keywords: Anaphylaxis; Acute asthma; Biphasic reaction; Shortness of breath; Intramuscular adrenaline 0.5 mg

***Correspondence:** Alvin Oliver Payus. Faculty of Medicine and Health Science, Universiti Malaysia Sabah (UMS), Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia. E-mail: dralvinpayus@ums.edu.my

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Abstract

BACKGROUND: Anaphylaxis often misdiagnosed and treated as acute asthma, especially when it has a predominant respiratory symptom, and there are no obvious precipitants or previous allergic history. This morbid outcome is preventable if the level of suspicion for anaphylaxis is high among healthcare provider when treating a patient who is not responding to the standard management of acute asthma. A proportion of anaphylactic patient shows a biphasic reaction which potentially fatal when it is under-anticipated and prematurely discharge without adequate observation period after the recovery of the initial episode.

CASE REPORT: Here, we present a case of a young man who has childhood asthma with the last attack more than 10 years ago presented with symptoms suggestive of acute exacerbation of bronchial asthma. As the symptoms failed to improve after standard asthma management, anaphylaxis was suspected, and he was given intramuscular adrenaline 0.5 mg which leads to symptom improvement. However, he developed another attack shortly after improvement while under observation.

CONCLUSION: The objective of this case report is to emphasise the importance of keeping anaphylaxis in mind whenever a patient has treatment-refractory asthma, and also the anticipation of biphasic reaction that warrants adequate observation period especially those who are likely to have developed it.

Introduction

Acute asthma and anaphylactic reaction have a clinical presentation that similar to each other. Therefore, it is not uncommon for a patient with anaphylaxis to be treated as acute asthma and not given the appropriate treatment [1]. This potentially fatal misdiagnosis can be prevented if health care provider has a higher suspicion level for anaphylaxis when treating an acute asthma patient that refractory to standard treatment. Biphasic anaphylaxis is one of the morbid sequelae of an anaphylactic reaction, which is still poorly understood and said to occur in 1 in every 5 cases of anaphylaxis [2]. The fatality of this phenomenon can be avoided if the patient is subjected to adequate observation after the recovery of the initial symptoms, especially those who have a history of bronchial asthma or another risk factor.

Case Report

A 21-year old army officer presented with progressive shortness of breath and left-sided pleuritic chest pain for the past three days. He had a fever with coryzal symptoms five days before the onset of breathlessness which he seeks treatment from a private clinic. He has given oral amoxicillin 500 mg

three times daily, oral chlorpheniramine 4 mg three times daily, oral paracetamol 1gm four times daily and oral salbutamol 2 mg three times daily. He was also nebulised twice during the clinic visit. There was no spirometry test done during the visit. Upon further history noted that he had childhood asthma before and was only on a single short-acting reliever treatment with salbutamol inhaler, and never had a proper follow-up. The last asthma attack was more than 10 years ago. On arrival to the emergency department, he was tachypnoeic and had multiple bouts of a cough, but otherwise alert and conscious. There was a marked periorbital swelling noted as well. According to the patient, he noticed that his face was increasing swollen in the past few days, and there were some itchiness and redness too, but he did not seek any treatment for it. He was allergic to cashew nuts and diclofenac which he did not take before the complaint. His vital signs were stable with a blood pressure of 130/90 mmHg, heart rate of 140 beats per minute and oxygen saturation rate of 100% on room air via a pulse oximeter. On examination of the chest noted there was a very poor air entry over the whole both lungs with occasional rhonchi heard. Preliminary blood investigation was taken (as shown in Table 1). His chest radiograph image did not show pneumothorax or consolidation (as shown in Figure 1).

Table 1: Preliminary blood investigation taken on arrival to the emergency department showed mild leucocytosis and eosinophilia. Otherwise the haemoglobin and platelet count was normal. There is no deranged electrolyte level, and no liver or renal impairment

TEST	VALUE	NORMAL RANGE
Haemoglobin	14.1 g/d	12 – 18 g/dL
Haematocrit	43.5 %	35 – 48 %
White cell count	11.8 x 10^9/L	4.0 – 11.0 x 10^9/L
Eosinophil	0.6 x 10^9/L	0.2 – 0.5 x 10^9/L
Platelet	294 x 10^9/L	150 – 400 x 10^9/L
Urea	5.4 mmol/L	1.7 – 8.0 mmol/L
Creatinine	104 umol/L	60 – 120 umol/L
Sodium	138 mmol/L	135 – 150 mmol/L
Potassium	3.9 mmol/L	3.5 – 5.0 mmol/L
Calcium	2.3 mmol/L	2.10 – 2.55 mmol/L
Magnesium	0.90 mmol/L	0.75 – 1.10 mmol/L
ALT	42 u/L	5 – 35 u/L
ALP	70 u/L	30 – 100 u/L
pH	7.38	7.35 – 7.45
Arterial PaO_2	82 mmHg	80 - 100 mmHg
$PaCO_2$	40 mmHg	35 – 45 mmHg
HCO_3^-	26 mEq/L	22 – 28 mEq/L

ALT: Alanine Aminotransferase; ALP: Alkaline Phosphatase; PaO_2: Partial Pressure of Oxygen; $PaCO_2$: Partial Pressure of Carbon Dioxide; HCO_3^-: Bicarbonate

Given the symptoms and the background history of childhood asthma, the initial impression was severe to life-threatening acute exacerbation of bronchial asthma. He was immediately given multiple nebulizations of combined salbutamol and ipratropium bromide along with intravenous (IV) hydrocortisone 200 mg bolus, IV chlorpheniramine 10 mg bolus, and IV magnesium sulphate 2.47 mg slow infusion. Despite all the medications, the patient still has severe bronchospasm and worsening tachypnoea.

At this point, the possibility of severe anaphylaxis was entertained given the facial swelling and itchiness, and also the symptoms were refractory to the standard bronchodilator management.

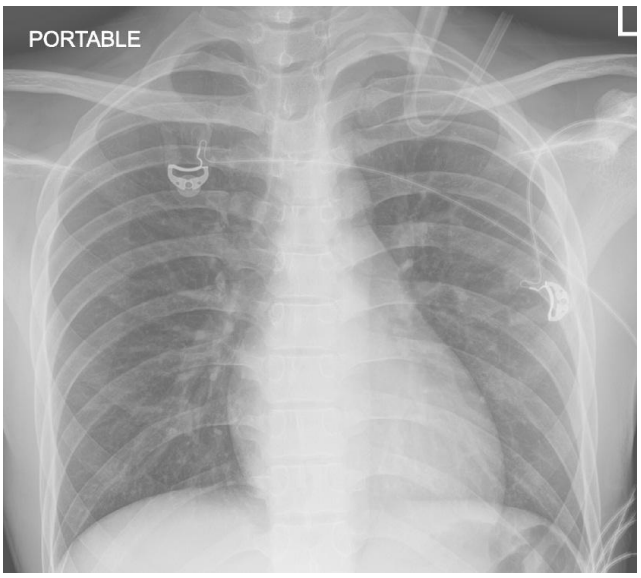

Figure 1: Chest radiograph image taken on arrival to the emergency department was normal

It could be secondary to the antibiotic he took a few days ago or may be triggered by recent upper respiratory tract infection. Therefore, intramuscular (IM) adrenaline 0.5 mg was given, and it provides rapid improvement. However, it only provides a temporary relief whereby the bronchospasm and tachypnoea resumed and necessitates a repeated IM adrenaline to be given twice. The facial swelling reduced but the bronchospasm persisted. Repeated arterial blood gas at that time showed type 1 respiratory failure with the slow rising of carbon dioxide retention. The nebulization was escalated to continuous nebulization. As the patient was increasingly fatigue and there were impending type 2 respiratory failures, the patient was subjected to invasive assisted ventilation. While intubated and mechanically ventilated, the bronchodilator nebuliser was continued. After being on assisted ventilation for 12 hours, he was then extubated and kept on closed observation in the intensive care unit. He managed to keep good oxygenation via spontaneous respiratory effort, and there was no further bronchospasm attack. He was discharged after a few days of admission with an emergency epinephrine pen and will be investigated for the potential triggers of his anaphylaxis episode in the outpatient clinic appointment. Spirometry test done before discharge was normal.

Discussion

Anaphylaxis is an acute, systemic, potentially fatal allergic reaction which driven by type I hypersensitivity reaction that requires both immediate recognition and rapid treatment. It is clinically

manifested as hives, flushing, itchiness, angioedema, wheezing, stridor, breathlessness, vomiting, diarrhoea or shock. Given the nature of its presentation, anaphylaxis with respiratory symptom predominant can be easily misdiagnosed as severe acute asthma. According to an original article by J. Rainbow and G.J. Browne (2002), patients who have the symptom of severe acute asthma may be suffering from anaphylaxis. Therefore, they mentioned a few recommendations, which are to suspect anaphylaxis in a patient with rapid onset of wheeze or not responding to the standard asthma treatment, to use adrenaline early when anaphylaxis is suspected, to confirmed anaphylaxis with radioimmunoassay and mast cell tryptase level, and to screen for precipitating allergens [1].

Some patient with anaphylaxis may show a biphasic reaction. Biphasic anaphylaxis refers to the second episode of anaphylaxis that follows the brief resolution of symptoms period after the initial anaphylaxis reaction without further exposure to the triggering factors. It is a poorly understood allergic phenomenon that has a fatal outcome and has been reported to develop in up to 20% of all cases of anaphylactic reactions [2]. There are a few possible risk factors has been suggested that increases the likelihood of biphasic anaphylaxis, namely severe initial symptoms, delayed administration of adrenaline during the initial treatment, a longer time for the initial reaction to resolve, delayed onset of the initial symptoms after exposure to the antigen, and exposure to allergens in the form of oral ingestion [3]. However, all these factors are still yet to be proven further by more study in the future. Confino-Cohen R and Goldberg A (2010) in their research of biphasic reactions following allergen immunotherapy suggested that the only difference between biphasic and uniphasic reactions was that a higher incidence of asthma and low peak expiratory flow rate could be identified in those with biphasic reactions [4]. The clinical severity of the second phase of biphasic anaphylaxis does not necessarily resemble the first. Some reported that it could be more severe or even fatal [5]. The period between the recurrence of symptoms and the initial resolution of initial symptom varies substantially and can go up to 72 hours.

In this article, our patient presented with symptoms of severe to life-threatening acute exacerbation of bronchial asthma. After treated promptly with bronchodilator nebuliser and other systemic medications, the bronchospasm failed to improve. Therefore, given the presence of a history of allergy and also taking antibiotic before the presentation, there was a possibility of severe anaphylaxis instead of acute asthma which may be induced by the antibiotic. Therefore, intramuscular (IM) adrenaline 0.5 mg was given, and it leads to rapid improvement. However, the patient showed a biphasic anaphylactic reaction whereby there was only a brief period where the bronchospasm and facial swelling improved after the first dose of IM adrenaline before the bronchospasm recur again while he was under observation, which severe enough that he needs to be intubated and mechanically ventilated.

In conclusion, this case report served to emphasise the importance of having a high level of suspicion for severe anaphylaxis whenever treating a non-responsive life-threatening bronchial asthma. Therefore, it is imperative to give a trial of adrenaline in a severe to life-threatening acute asthma patient who is refractory to standard treatment, with or without allergy history. Apart from that, it is also crucial to anticipate a biphasic reaction in a patient with a high likelihood to develop it, for example, who has underlying bronchial asthma. Therefore, a period of observation after the resolution of attack is warranted and potentially save a life.

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References

1. Rainbow J, Browne GJ. Fatal asthma or anaphylaxis? Emergency Medicine Journal. 2002; 19(5):415-7. https://doi.org/10.1136/emj.19.5.415 PMid:12204988 PMCid:PMC1725974

2. Muraro A, Roberts G, Worm M, et al. Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. Allergy. 2014; 69:1026-45. https://doi.org/10.1111/all.12437 PMid:24909803

3. Lieberman PL, Feldweg A, Simons F. Biphasic and protracted anaphylaxis, 2016.

4. Confino-Cohen R, Goldberg A. Allergen immunotherapy-induced biphasic systemic reactions: incidence, characteristics, and outcome: a prospective study. Ann Allergy Asthma Immunol. 2010; 104:73. https://doi.org/10.1016/j.anai.2009.11.001 PMid:20143649

5. Cortellini G, Corvetta A, Campi P, et al. A case of fatal biphasic anaphylaxis secondary to multiple stings: adrenalin and/or a longer observation time could have saved the patient? Eur Ann Allergy Clin Immunol. 2005; 37:343. PMid:16453966

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Case Report

Incidental Finding Of Hyperreactio Luteinalis during Caesarean Section in Twin Pregnancy

Senka Sabolovic-Rudman, Ante Omazic*, Ivka Djakovic, Hrvojka Soljacic-Vranes, Vesna Kosec

Clinical Department of Gynecology and Obstetrics, Sestre Milosrdnice University Hospital Center, Zagreb, Croatia

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Keywords: Hyperreactio Luteinalis; Twin Pregnancy; Caesarean Section

***Correspondence:** Ante Omazic. Clinical Department of Gynecology and Obstetrics, Sestre Milosrdnice University Hospital Center, Zagreb, Croatia. E-mail: dr.ante.omazic@gmail.com

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Abstract

BACKGROUND: Some benign changes of the ovaries like hyper reaction luteinalis sometimes cannot be differentiated from malignant ones without histological examination. In these cases, surgical intervention sometimes cannot be avoided. Hyperreactio luteinalis is a condition that can occur only in pregnancy. It is characterised by bilateral benign multicystic ovarian enlargement.

CASE REPORT: We present a case of misleading intraoperative findings during Cesarean section that ended with ovariectomy.

CONCLUSION: During the Caesarean section, some benign masses of the ovaries, like hyper reaction luteinalis, are difficult to differentiate from malignant disease without histological examination, requiring surgical intervention.

Introduction

Hyperreactio luteinalis (HL) is a condition that can occur only in pregnancy. It is characterised by bilateral benign multicystic ovarian enlargement. The exact aetiology is unclear but high B-hCG or B-hCG – hypersensitivity is possible causes. It is very rare [1] [2]. Treatment is non-surgical, but sometimes it requires emergency surgery in case of ovarian torsion or haemorrhage [3].

We present a case of misleading intraoperative findings during Cesarean section that ended with ovariectomy.

Case Report

A 27-year-old patient in her first twin pregnancy was admitted to our department in the 31st week of pregnancy for treatment of premature contractions. The pregnancy had been without complications to date and was followed up regularly. Standard laboratory tests were normal, and intravenous tocolysis was administered. Fetal ultrasound was normal, and a detailed ultrasound of the adnexa was not performed. The patient remained at our department up to her 33rd week of pregnancy, when her amniotic membrane ruptured spontaneously. The regular contraction started, and due to breech presentation of the first twin, delivery was performed by Caesarean section. The first twin was female, weighing 1780 g and 43 cm long, Apgar score 9/10. The second twin was male, weighing 1790 g and 44 cm long, Apgar score 9/10. During the Cesarean section surgery, multicystic and enlarged ovaries were identified, measuring 9 cm on the right and 8 cm on the left side (Figure 1 and 2). A frozen section biopsy of the right ovary was performed to rule out malignancy. This caused profuse haemorrhage that could not be controlled without ovariectomy. In the postoperative period, the patient had no complications. Histological examination of the right ovary revealed hyper reaction luteinalis. At ultrasound

examination, one-month post-surgery, the patient's left ovary was polyfollicular and measured 8 cm in diameter.

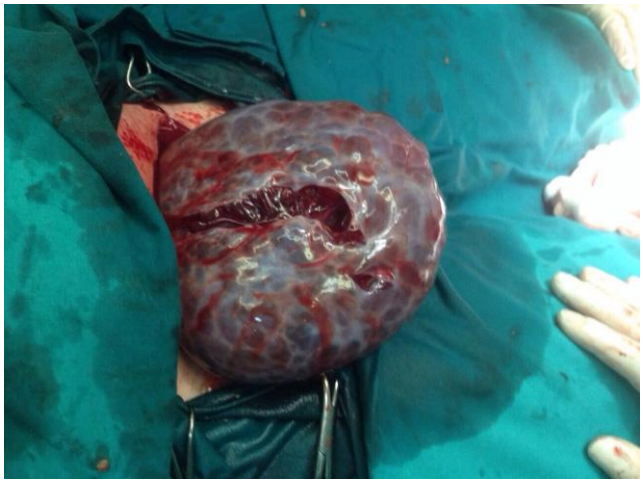

Figure 1: Intraoperative finding of right ovary

Discussion

Risk factors are multiple gestations, Rh sensitisation, twin to twin transfusion syndrome, gestational trophoblastic disease, hydropsfetalis, molar pregnancy, choriocarcinoma, polycystic ovaries, gestational diabetes, ovulation induction, clomiphene therapy, decreased the clearance of B-hCG due to renal dysfunction. The condition is most frequent in primiparas [4]. Hypothyroidism, PCOS, FSH secreting adenoma, or mutation in the FSH receptor can also lead to HL [5]. The genetic component in the development of the disease is excluded [1].

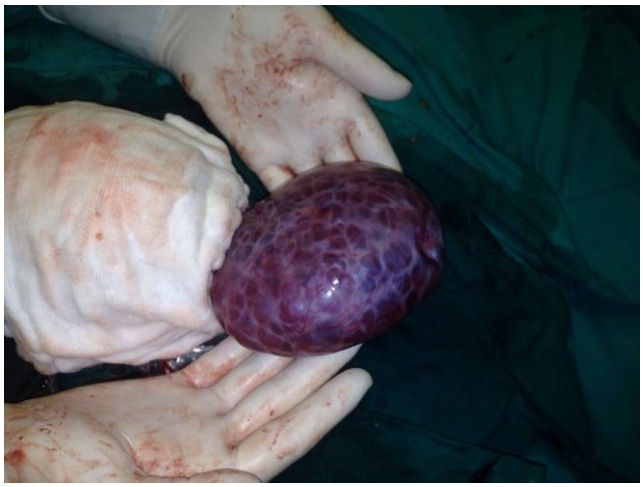

Figure 2: Intraoperative finding of left ovarii

Over 25% of cases of hyper reaction luteinalis are asymptomatic [6]. Symptoms are a low abdominal pain, nausea and vomiting, ascites, signs of virilisation, weight gain and shortness of breath[6]. Nausea and vomiting of pregnancy correlate positively with high levels of B-hCG [7]. Signs of virilisation are rare, occurring in only 30%of patients, mostly in their third trimester, and rarely in the second trimester [1], [2], [3], [4], [5], [6], [7], [8]. In our case, there were no clinical signs of maternal virilisation in pregnancy. Complications of this condition are preeclampsia, HELLP syndrome; fetal grow restriction, preterm delivery, torsion, dystocia and late onset of lactation in the post-delivery period. Remarkably high B-hCG levels might be predictive for preeclampsia as it may occur before 20 weeks' gestation [9]. Usually, it takes 2 months after childbirth for ovarian tissue to return to its normal size and ultrasonographic appearance [1]. Cases of virilized female fetuses are extremely rare [10], [11].

Diagnosis is mostly made by ultrasound, at a mean gestational age of 21.6 weeks with ovaries showing a characteristic "spoke-wheel" appearance. Finding of bilateral cysts, normal Doppler flow, and a lack of solid components differentiate HL from ovarian malignancies such as a borderline mucinous tumour of intestinal type [12], [13]. Laboratory findings may exhibit high levels of hCG, hyperandrogenism, and hyperthyroidism. The condition may be diagnosed incidentally during Cesarean sections and does not usually require any specific treatment. Adnexal masses can be identified in 0.3% of all cesarean deliveries, and most of them are incidental discoveries. In 96.7% of cases, the ovarian masses were characterized as benign, with only in 2.0% confirmed ovarian malignancies [14]. The rationale for surgical treatment is suspected malignancy, requiring histological evaluation through ovariectomy [15].

During the Caesarean section, some benign masses of the ovaries, like hyper reaction luteinalis, are difficult to differentiate from malignant disease without histological examination, requiring surgical intervention.

References

1. Malinowski AK, Sen J, Sermer M.Hyperreactio Luteinalis: Maternal and Fetal Effects. J Obstet Gynaecol Can. 2015; 37(8):715-23. https://doi.org/10.1016/S1701-2163(15)30176-6

2. Bishop LA, Patel S, Fries MH. A case of recurrent hyperreactio luteinalis in three spontaneous pregnancies. Journal of Clinical Ultrasound. 2016; 44(8):502-5. https://doi.org/10.1002/jcu.22343 PMid:26892678

3. Cho Ah-Ra, et al. Vaginal delivery in a spontaneously conceived singleton pregnancy complicated with hyperreactio luteinalis: A case report. Journal of Womens Medicine. 2011; 4(2):53–56. https://doi.org/10.5468/jwm.2011.4.2.53

4. Skandhan AK, Ravi V. Hyperreactio luteinalis: an often mistaken diagnosis. Indian J Radiol Imaging. 2014; 24:84–6. https://doi.org/10.4103/0971-3026.130711 PMid:24851012 PMCid:PMC4028923

5. Angioni S, Portoghese E, Milano F, Melis GB, Fulghesu AM.

Hirsutism and hyperandrogenism associated with hyperreactio luteinalis in a singleton pregnancy: a case report. Gynecol Endocrinol. 2007; 23:248–51. https://doi.org/10.1080/09513590701214513 PMid:17558681

6. Cavoretto P, Giorgione V, Sigismondi C, Mangili G, Serafini A, Dallagiovanna C, Candiani M. Hyperreactio luteinalis: timely diagnosis minimizes the risk of oophorectomy and alerts clinicians to the associated risk of placental insufficiency. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2014; 176:10-6. https://doi.org/10.1016/j.ejogrb.2014.02.017 PMid:24630301

7. Lee NM, Saha S. Nausea and vomiting of pregnancy. Gastroenterol Clin North Am. 2011; 40:309–334. https://doi.org/10.1016/j.gtc.2011.03.009 PMid:21601782 PMCid:PMC3676933

8. Angioni Stefano, et al. Hirsutism and hyperandrogenism associated with hyperreactio luteinalis in a singleton pregnancy: a case report. Gynecological endocrinology. 2007; 23(5):248–251. https://doi.org/10.1080/09513590701214513 PMid:17558681

9. Mehmet Akif Sargin, Niyazi Tug, Ozgur Aydin Tosun, Murat Yassa, Evrim Bostanci. Theca lutein cysts and early onset severe preeclampsia. Pan Afr Med J. 2016; 24:141. PMid:27642479 PMCid:PMC5012798

10. Masuyama H, Tateishi Y, Matsuda M, Hiramatrsu Y. Hyperreactio luteinalis with both markedly elevated human chorionic gonadotropin levels and an imbalance of angiogenic factors subsequently developed severe early-onset preeclampsia. Fertil Steril. 2009; 92(393):e1–3. https://doi.org/10.1016/j.fertnstert.2009.04.002

11. Lynn KN, Steinkeler JA, Wilkins-Haug LE, Benson CB.Hyperreactio luteinalis (enlarged ovaries) during the second and third trimesters of pregnancy: common clinical associations. J Ultrasound Med. 2013; 32(7):1285-9. https://doi.org/10.7863/ultra.32.7.1285 PMid:23804351

12. Simsek Y, et al. Severe preeclampsia and fetal virilization in a spontaneous singleton pregnancy complicated by hyperreactio luteinalis. Eur Rev Med Pharmacol Sci. 2012; 16(1):118–121. PMid:22338557

13. Van Holsbeke C, Amant F, Veldman J, De Boodt A, Moerman P, Timmerman D. Hyperreactio luteinalis in a spontaneously conceived singleton pregnancy. Ultrasound Obstet Gynecol. 2009; 33:371–373. https://doi.org/10.1002/uog.6325 PMid:19248002

14. Lambers, D.S. and Rosenn, B. Hyperreactio luteinalis complicating a normal singleton pregnancy. Am J Perinatol. 1996; 13:491–494. https://doi.org/10.1055/s-2007-994434 PMid:8989481

15. Baser E, Erkilinc S, Esin S, Togrul C, Biberoglu E, Karaca MZ, Gungor T, Danisman N. Adnexal masses encountered during cesarean delivery. Int J Gynaecol Obstet. 2013; 123(2):124-6. https://doi.org/10.1016/j.ijgo.2013.06.015 PMid:24008309

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Case Report

Allergic Contact Dermatitis, Angioneurotic Edema and Conjunctivitis in a Patient with Autoimmune Thrombocytopenia – A Clinical Case

Svetlan Dermendzhiev[1], Mariya Ivanovska[2,3*], Tihomir Dermendzhiev[2]

[1]Department of Occupational Diseases and Toxicology, Second Department of Internal Medicine, Faculty of Medicine, Medical University, Plovdiv, Bulgaria; [2]Department of Microbiology and Immunology, Faculty of Pharmacy, Medical University, Plovdiv, Bulgaria; [3]Division of Immunological Assessment of Posttraumatic Stress Disorder, Technological Center for Emergency Medicine (TCEMED), Plovdiv, Bulgaria

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Keywords: Contact dermatitis; Angioneurotic oedema; Conjunctivitis; Autoimmune thrombocytopenia

***Correspondence:** Mariya Vlado Ivanovska. Department of Microbiology and Immunology, Faculty of Pharmacy, Medical University, Plovdiv, Bulgaria; Division of Immunological Assessment of Posttraumatic Stress Disorder, Technological Center for Emergency Medicine (TCEMED), Plovdiv, Bulgaria. E-mail: marijaku87@yahoo.com

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BACKGROUND: Allergic contact dermatitis (ACD) is common in clinical practice, but the aetiology of this disease is quite varied. A leading pathogenetic mechanism is a cell-mediated immunity. The combinations of ACD with other allergic and systemic autoimmune diseases are relatively rare, but these conditions are undoubtedly a professional challenge for practitioners.

CASE REPORT: We present a case of ACD combined with other immune-allergic conditions. Aetiology and pathogenesis in these cases are not well understood.

CONCLUSION: Based on the data from the general and targeted allergic history, patient's subjective complaints, clinical picture, allergenic status, paraclinical results, and the presented photo material, the final diagnosis is as follows: Contact allergic dermatitis-acute form.

Abstract

Introduction

Contact dermatitis is the most common skin allergic disease with professional aetiology [1]. The disease is Type 4 allergic reaction after Coombs and Gell (delayed type hypersensitivity) [1] [2]. According to literature, symptomatology in most of the cases is triggered and occurs in skin areas exposed to direct contact with allergens from the work environment [1] [2] [3]. The clinical picture is characterised by burning and itchy exanthema in allergen contact areas of the skin. Sometimes the rash also involves more distant non-contact areas [1]. Angioneurotic oedema is defined as a state of pathological fluid retention in the subepidermic interstitium [1] [4]. Swelling is a symptom that occurs in many different diseases. Oedema is a condition where there is pathological fluid retention in the interstitial, i.e. in the extravascular part of the extracellular space. Pathogenetic mechanisms that determine the onset of swelling are increased hydrostatic pressure in the capillaries, decreased oncotic plasma pressure found in hypoalbuminemia, increased capillary permeability and worsened lymphatic drainage [4]. Drug oedema is most commonly caused by calcium antagonists, ACE inhibitors, angiotensin-2 receptor antagonists – sartans, diuretics (aldosterone), NSAIDs, corticosteroids, antidepressants (AD). Angioedema is an acute swelling of the deeply located connective

tissue most commonly located on the eyelids, lips, tongue, pharynx, thigh, and larynx. It rarely occurs on limbs. Several pathogenetic forms of angioedema are known: allergic IgE mediated angioedema (Oedema Quincke), non-allergic drug-induced angioedema (aspirin, ACE inhibitors, sartans), angioedema in parasitic diseases (echinococcosis, larva migrans), physical angioedema (cold, pressure, vibrations, etc.). A particular form of angioneurotic oedema is hereditary angioedema, a rare disease associated with congenital or acquired deficiency of the C1 esterase inhibitor [1]. Diagnosis of angioedema requires a lot of effort, clinical experience and in-depth knowledge. It includes a detailed history, physical examination and modern clinical and immunological tests. Differential diagnosis (DD) of angioedema includes various conditions. Localisation and the nature of the swelling focus attention on the diseases that will result in a differential diagnosis. These include immunopathological diseases such as autoimmune thrombocytopenia, immune-allergic vasculitis, malignant hematopoiesis and others. Some cases in children and adults have been reported in the literature [5] [6] [7] [8] [9].

Material and Methods

A source of information is the data from the clinical and paraclinical examinations carried out in pre-hospital and hospital care, reflected in the patient's medical records, as well as photos voluntarily provided by the patient herself.

Clinical Case

The patient is a young female aged 29, hospitalised urgently for diagnosis and treatment in the Department of Occupational Diseases and Clinical Allergology of the University Hospital "St. George" in Plovdiv at 2017. Our participant signed voluntary informed consent after a detailed explanation of all procedures and the ethics of this study. We have followed the Declaration of Helsinki and European Medicines Agency Guidelines for Good Clinical Practice.

The woman is admitted to the clinic for a rash and a heavy itching on her neck and behind her ears. Gradually, the exanthem spreads over the whole body. The patient associates the appearance of allergic symptoms with body lotion (a frequent contact allergen).

The patient reports a similar incident from a year ago, also after using a cosmetic product (face cream). The complaints are an urticarial rash on the face, neck and behind ears, concomitant episodes of pruritus in the eyes with profuse, non-exogenous conjunctival secretion, and angioneurotic swelling of the soft tissues of the face and neck several weeks previously and resolving spontaneously.

After an outpatient clinic consultation with an ophthalmologist, allergic conjunctivitis was diagnosed (Table 1).

Table 1: Haematological and biochemical tests

Haematology	Differential blood count	Biochemistry
HGB – 136 g/l	Neut. – 77.4 %	gluc – 5.1 mmol/l
RBC – 4.66 T/l	Lymph. – 16.5 %	t.prot – 70.0 g/l
HCT – 0.397 pg/l	Eos. – 0.1 %	alb – 45.0 g/l
MCH – 29.1 pg	Mono – 4.4 %	urea – 3.3 mmol/l
MCV – 85 fl	Baso – 0.3 %	crea – 78 mmol/l
WBC – 8.74 G/l		AST – 16 u/l
		ALT – 22 u/l
PLT – 153 G/l		
ESR – 22 mm/h		

There are currently no data on food, medication and insect allergy. The patient has no addictions and is not in domestic or professional contact with animals and birds.

Comorbidity of autoimmune thrombocytopenia was objectively diagnosed with immunological tests before the occurrence of the above-described mucocutaneous symptoms (Table 2).

Table 2: ANA profile3 (14Ag, PCNA)

Antigen	Method	Result
RNP/Sm	immunoblot	+++
SS-A native (60kDa)	immunoblot	+
Ro-52 recombinant	immunoblot	++
Nucleosomes	immunoblot	+
Histones	immunoblot	0
Sm	immunoblot	0

The patient is observed by a haematologist. Serum immunoglobulins and complementary fractions were added to the medical documentation during an asymptomatic period. They are in reference values (Table 3).

Table 3: Scale for interpretation of Immunoblot

Intensity	Class	explanation
0-5	0	negative
6-10	(+)	borderline
11-25	(+)	positive
26-50	(++)	strong positive
51-256	(+++)	strong positive

Family history: a mother with a drug allergy to penicillin.

The patient works as a doctor of clinical immunology at University Hospital "St. George "and Plovdiv Medical University. This does not link the triggering of allergic manifestations to work environment factors.

General condition – good. Clear consciousness. Adequate. Afebrile. White skin. Pale-pink, visible mucous membranes. Language and speech – normal.

Local status: maculopapular rash on the neck

(Figure 1), behind the ears and on the abdomen, against the background of pronounced erythema (Figure 2), accompanied by intense itching.

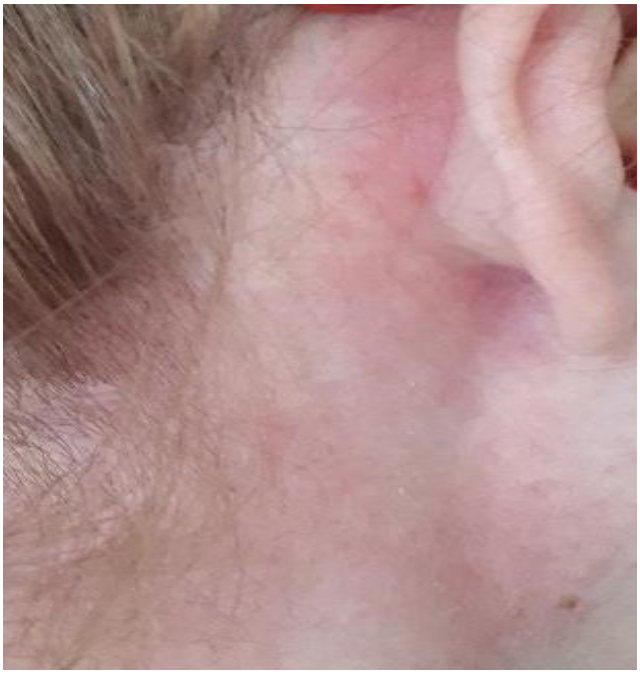

Figure 1: Maculopapular rash behind ears and neck

Peripheral lymph nodes are not palpable in accessible areas. Respiratory system: chest with proper form. Clear percussion tone. On auscultation – pure vesicular breathing without wheezing.

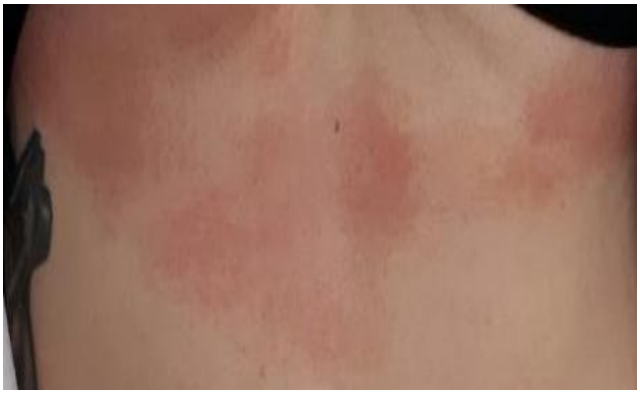

Figure 2: Rash on the upper body

Cardiovascular system: Rhythmic, nor frequent cardiac activity. Heart rate 80 beats/minute. Clear heart tones without pathological noises. Arterial pressure 120/70 mmHg. Succusio renalis bilateral (-) rep. Abdomen – at the level of the chest, soft and painless in palpation. Liver and spleen not enlarged upon palpation. Bone-muscle system – properly developed for the age.

The haematological and biochemical tests of the patient were within the reference range, except for the differential count, which showed an increase in neutrophil granulocytes with lymphopenia at normal leukocytes (Table 1). Tables 2, 3 and 4 present the results of immunological tests which confirm autoimmune thrombocytopenia and have relevance to the interpretation and differentiation of the angioedema type.

Table 4: Immunological parameters

Test	Method	Result	Reference value
Tot. IgE	ELISA	12 IU/ml	0 – 100 IU/ml
C 3	nephelometry	1.44 g/l	0.9 – 1.8 g/l
C 4	nephelometry	0.18 g/l	0.1 – 0.4 g/l
C1 esterase inhibitor (Ag)	RID	31.2 mg/dl	sera 21.0 – 39 mg/dl plasma 18.0–32 mg/dl
C1esterase inhibitor (func)		113 %	70 – 130 %

Systemic corticosteroids, antihistamines and H2 blockers at doses adequate for the clinical picture for 3 days. On the third day after admission, the patient was discharged in good general condition with a complete reversal of the exanthema. The case history points out given recommendations.

5: Skin-allergic samples with a panel of plant and animal allergens

Grass	20 min	Micro ticks	20 min
688 (5 kinds of grass)	(-)	314 (D. farinae)	(-)
687 (4 wheat)	(-)	315 (D. pteronissimus)	(-)
Trees		**Allergens of animal origin**	
696 (Beech)	(-)	507 (Cat)	(-)
702 (Birch)		509 (Dog)	(-)
701 (Willow)	(-)	506 (Feathers)	(-)
Weeds		**Controls**	
604 (Ambrosia)	(-)	Positive control (histamine)	8/5
605 (Plain wormwwod)	(-)	Negative control	(-)
665 (Plantain)	(-)		
714 (Atripliceae)	(-)		

To clarify the aetiology of the skin-mucous toxo-allergic syndrome, further studies are recommended to be performed on a "pure background" and in the absence of contraindications. The volume and duration of treatment with H1- and H2-blockers has been specified. Use of cosmetic products should be discontinued.

Table 6: Epicutaneous test with European standard series for epicutaneous testing (European Environmental and Contact Dermatitis Research Group)

No	*Allergen*	*48h* +/++/+++/++++	*72h* +/++/+++/++++
1	Potassium dichromate	-	-
2	4-phenylenediamine base (PPD)	-	-
3	Thiuram mix	-	-
4	Neomycin sulfate	-	-
5	Cobalt (||) chloride hexahydrate	-	-
6	Benzocaine	-	-
7	Nickel sulfate hexahydrate	-	-
8	Clioquinol	-	-
9	Colophony	+ +	+
10	Paraben mix	+ + +	+ +
11	N-Isopropyl-N-phenyl-3-phenylenediamine (IPPD)	-	-
12	Lanolin Alcohol	+ +	+
13	Mercapto mix	-	-
14	Epoxy resin	-	-
15	Balsam Peru	-	-
16	4-tert-Butylphenolformaldehyde resin	-	-
17	2-Mercaptobenzothiazole (MBT)	-	-
18	Formaldehyde	-	-
19	Fragrance mix I	+ + +	+ +
20	Sesquiterpene lactone mix	-	-
21	Qua ternium 15	-	-
22	2-methoxy-6-n-pentyl-4-benzoquinone (Primin)	-	-
23	5-chloro-2-methyl-4-isothiazolin-3-one (Kathon CG)	-	-
24	Budesonide	-	-
25	Tixocortol-21-pivalate	-	-
26	Methyldibromoglutaronitrile	-	-
27	Fragrance mix II	-	-
28	Lyral (alfa-hexyl cinnamal)	+ + +	+ +

Based on the data from the general and targeted allergic history, patient's subjective complaints, clinical picture, allergenic status, paraclinical results, and the presented photo material, the final diagnosis is as follows: Contact allergic

dermatitis-acute form. Status after angioneurotic oedema. Accompanying diseases: autoimmune thrombocytopenia and allergic conjunctivitis.

The results of the additional diagnostic procedures performed to determine the patient's immuno-allergic diseases have been reported in Table 5 and Table 6.

Discussion

The combination of allergy-related reactions, though less common, is not an isolated phenomenon in practice. We have described similar cases [10] [11] [12] [13] [14] [15] [16]. Some of them have an interesting aetiology, especially those related to risk factors in the work environment [10] [11] [14] [16]. In others, rare associations of allergic with non-allergic mechanisms are described, which explains the combined pathology in the same person [17] [18]. Similar clinical cases are also described in hereditary angioedema (HAE) type 1 and type 2 patients [19] [20]. There have also been reports of autoimmune hematopoiesis associated with allergic syndromes [21].

Discussion questions are whether diseases, demonstrated in the case described, are an expression of allergy, what is the relationship between them and how autoimmune hemopathy is connect with angioedema and ACD.

The allergic aetiology of angioedema in the case described is questioned because of normal blood eosinophil levels and total serum IgE (biomarkers for allergy and atopic predisposition) [1]. The same applies to negative skin-allergic specimens with a set of indoor and outdoor allergens that are a proven method in the diagnosis of allergic diseases [1].

The normal levels of the C1-esterase inhibitor (both quantitative and qualitative) and C4 (the "golden" standard in the diagnosis of HAE), as well as the negative family history, practically exclude HAE type 1 as a cause of oedema in the case described. It is probably an acquired autoimmune thrombocytopenic form of angioedema that can explain the increased consumption of C3 in immune complexes. This explanation does not contradict the normal values of C3 because the study is done in a period of clinical and immunological remission of the disease. Regarding the causes of conjunctivitis certain facts should be taken into account. Negative results from specific allergy tests do not support allergic mechanisms. Given the clinical symptoms typical of allergic conjunctivitis, the possibility of the person being sensitised to an allergen that is missing in the panel with which it was tested and the fact that no specific IgE antibodies have been tested, the allergic cause of conjunctivitis is highly probable. This is also supported by the conclusion of the ophthalmologist.

Benchmarks for the diagnosis of ACD are the results from the history, the clinical manifestations and the positive results of epicutaneous testing with the European Environmental and Contact Dermatitis Research Group.[17] The result of the patch-test is positive for Colophony, Lanolin Alcohol and highly positive for Paraben mix, Fragrance mix I and Lyral (alpha-hexyl cinnamal). Epicutaneous testing objectively targets allergic reactions in the 4th (cell-mediated) type. The patch test is considered a "golden" standard in the diagnosis of ACD [1], a disease that illustrates the fourth type of allergic reactions by Coombs and Gell.

As to the analysis of the compounds (allergens) from the epithelial samples carried out, some of them are components of the cosmetics used by the patient [22]. Benzocaine and rosin are used in the manufacture of nail polish, makeup, spirals, eye pencils, shades, lipsticks, blush and creams. This also applies to Paraben mix 4 chemicals. Individually or in combination, they are used as preservatives for the production of various cosmetic preparations (lotions, creams, makeup, lipsticks, shampoos, soaps, gels). The same applies to other allergens in the series to which the patient is objectively sensitized – Lanolin, Fragrance mix I and Lyral. Fragrance Mix I is a multi-component allergen that contains cinnamic alcohol, cinnamic aldehyde, hydroxycitronellal, amyl cinnamaldehyde, geraniol, eugenol, Isoeugenol. These compounds are a component of the fragrance of a range of cosmetic products (perfumes, deodorants, soaps, shampoos, shower gels). Wool alcohols is an essential ingredient in a range of cosmetic products (creams, lotions, lipsticks, lipsticks, shampoos).

The sensitising effect of the cosmetic products used by the patient is made by contact route. It should be taken in mind that, depending on the mode of use, cosmetic products can affect the body through different mechanisms. For example, aerosols and vapours are irritants, and some of them have a direct toxic effect on the skin and the lining of the eyes and the upper respiratory tract.

Regardless of the technology of production, the sensitising effect of these substances is preserved. Moreover, they are added to other preparations with contact-irritant and toxic-chemical action-fillers, stabilisers, preservatives, flavours, colourants.Deviations in neutrophil and lymphocyte counts in normal white blood cell counts are not a specific indicator for a specific disease state, including immune-allergic pathogenesis. The professional aetiology of immune-allergic diseases in the case described is not commented because there is no causal link between the occurrence and the clinical manifestation, with exposure to a certain risk factor in

the working environment. There are no disease-specific laboratory parameters to verify the performance of professional allergens. The occupation of the patient coincides with the diagnosed immuno-allergic pathology, which does not meet the requirements and criteria for acceptance of professional aetiology [23] [24] [25], which in turn do not correspond to the normative regulations for the administration of occupational diseases in the Republic of Bulgaria.

We present this specific clinical case because it is:

1. A rare combination of clinically proven diseases with the skin-mucosal syndrome.

2. A combination of various etiological factors and pathogenetic mechanisms.

3. The profession of the patient-an immunologist.

Although it may rarely occur in a patient, there may be symptoms of various diseases manifested by skin-mucosal syndrome, with diverse aetiology and pathogenesis. In these cases, both immune and non-immune factors and mechanisms are involved. Good knowledge of etiological factors is crucial for the timely and accurate diagnosis of immune-allergic diseases and is a key to their effective treatment.

References

1. Dimitrov V. Allergic diseases – principles, diagnosis and treatment. ARSO. 2000; 23:80-87; 32:172-175.

2. Bourke J, Coulson I. & English J. Guidelines for the management of contact dermatitis: an update. BJD British Journal of Dermatology 2009.

3. Rea JN, Newhouse ML, Halil T. (1976). Skin diseases in Lambeth. A community study of prevalence and use of medical care. Br J Prev Soc Med. 1976;30:107 – 14. https://doi.org/10.1136/jech.30.2.107

4. Mileva Zh. Edemas – a serious diagnostic problem. Medinfo, XIth year, 2011:29 – 32. [in Bulgarian].

5. Chiang M, Wei C, Muo C, Fu L, Li T, et al. Association of primary immune thrombocytopenia and common allergic diseases among children. Pediatric Research. 2015; 77(4):597 – 601. https://doi.org/10.1038/pr.2015.6 PMid:25580738

6. Stefanini M. Idiopathic thrombocytopenic purpura (ITP): an analysis of 1,122 cases. "Allergic" versus "autoimmune" forms. Nouv Rev Fr Hematol. 1990; 32(2):129 – 35. PMid:2377445

7. Wei CC, Lin CL, Shen TC, Tsai JD. Atopic dermatitis and association of risk for primary immune thrombocytopenia and autoimmune diseases among children: a nationwide population-based cohort study. Medicine. 2016; 95(29). https://doi.org/10.1097/MD.0000000000004226

8. Aster RH, Bougie DW. Drug-Induced Immune Thrombocytopenia. New England Journal of Medicine. 2007; 357:580 – 7. https://doi.org/10.1056/NEJMra066469 PMid:17687133

9. Yuki MF, Andersen Egeberg A, Gislason GH, Lone S, Thyssen JP. Autoimmune diseases in adults with atopic dermatitis. Journal of the American Academy of Dermatology. 2017; 76(2):274 – 280. https://doi.org/10.1016/j.jaad.2016.08.047 PMid:27742171

10. Dermendzhiev S, Penchev B, Deleva P, Dimitrov V. Urticaria and Angioedema in Exposed to Latex Professionals – clinical case. Allergies Hypersensitivity Asthma. 2013; 10:53 – 60 [in Bulgarian].

11. Dermendzhiev S. Severe allergic pathology in professional exposure to military industry materials. Allergies Hypersensitivity Asthma. 2014; 11:76 – 82 [in Bulgarian].

12. Dermendzhiev S, Deleva P. Allergic reactions to substances of unknown physico-chemical composition. Bulgarian Medicine. 2013; 4:46 – 50 [in Bulgarian].

13. Dermendzhiev S, Simeonova R. Rarely occurring allergic manifestations in cosmetic manipulations. Poster session presentation at the meeting of the Third National Conference on Rare Diseases, Sofia, 2013:125 – 126. [in Bulgarian].

14. Dermendzhiev S. Skin-mucous allergic manifestations in farm workers. Bulgarian Medicine. 2014; 3:32–36 [in Bulgarian].

15. Dermendzhiev S. Allergy and homeopathic medicinal products – paradox, possible risk or something else: clinical case. Allergies Hypersensitivity Asthma. 2011; 8:40–54 [in Bulgarian].

16. Dermendzhiev S. Paper dust – a risk factor for sensitization in professionally exposed individuals. Poster session presentation at the meeting of the Third National Conference on Rare Diseases, Sofia, 2013:118–120 [in Bulgarian].

17. Dermendzhiev S, Deleva P. Is the time of combined allergic syndromes coming? Case report. Acta Medica Bulgarica. 2013; 40(1):84-89 [in Bulgarian].

18. Dermendzhiev S, Arolski I, Velev V, Todorova A, Asenova K, Deleva P. Rare case of chronic urticaria and angioedema with multiple aetiology and pathogenesis. Conference paper at the meeting of the Fifth National Conference on Rare Diseases, Sofia, 2015:89 [in Bulgarian].

19. Dermendzhiev S, Sokolova R, Penchev B, Murdzheva M, Spasova M, Dermendzhiev T, Todorova A, Velev V, Arolski I. Hereditary angioedema type II with drug-induced edemas. Conference paper at the meeting of the 7th National Conference for Rare Diseases and Orphan Drugs, Sofia, 2016:140 [in Bulgarian]. PMid:27582707 PMCid:PMC4987332

20. Dermendzhiev S, Todorova A, Sokolova R, Dermendzhiev T, Penchev B, Velev V. Rare case of hereditary angioedema type I. Conference paper at the meeting of the 7th National Conference for Rare Diseases and Orphan Drugs, Sofia, 2016:141 [in Bulgarian].

21. Dermendzhiev S, Deleva P, Stoyneva-Paskaleva Z, Arolski I, Velev V, Todorova A, Penchev B. A rare case of angioedema and urticaria in a patient with essential thrombocythemia. Poster session presentation at the meeting of the Anniversary Scientific Conference 'Science for Health', Plovdiv, Bulgaria. In: Folia Med (Plovdiv). 2015; 57(Suppl 1):108.

22. Johansen JD, et al. Chemotechnique diagnostics. Patch test products & reference manual 2015, European Baseline, Series S-1000. 2015; 28–31.

23. Prakova G. Current problems in the medical expertise of occupational diseases. Health Policy and Management. 2015; 14(3):23– 30 [in Bulgarian].

24. European commission. Report on the current situation in relation to occupational diseases' systems in EU Member States and EFTA/EEA countries, in particular relative to Commission Recommendation 2003/670/EC concerning the European Schedule of Occupational Diseases and gathering of data on relevant related aspects, March 2013:150. http://ec.europa.eu/social/BlobServlet?docId=9982&langId=en

25. Ordinance No. 3 (2008, January). The conditions and procedure for the performance of the occupational medicine services issued by the Minister of Health and the Minister of Labor and Social Policy, promulgated in SG, no. 14 of 12.02.2008.

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Case Report

High-Risk BCC Of the Lower Eyelid in Patient with Presternal Located Cutaneous Melanoma and BCC Of the Shoulder: Melolabial Advancement Flap Combined with Undermining Surgical Approach As Promising Complex One Step Treatment Option!

Georgi Tchernev[1,2*], Ilia Lozev[3], Ivan Pidakev[3], Irina Yungareva[1], Tanya Naskova-Popova[4], Ivanka Temelkova[1]

[1]Medical Institute of Ministry of Interior (MVR), Department of Dermatology, Venereology and Dermatologic Surgery, General Skobelev Nr 79, Sofia, Bulgaria; [2]Onkoderma - Policlinic for Dermatology and Dermatologic Surgery, General Skobelev 26, Sofia, Bulgaria; [3]Medical Institute of the Ministry of Interior, Surgery, Sofia, Bulgaria; [4]Department of Clinical Hematology University Multiprofile Hospital for Active Treatment "Sveti Ivan Rilski", 15, Acad. Ivan Geshov Blvd., Sofia 1431, Bulgaria

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***Correspondence:** Georgi Tchernev. Medical Institute of Ministry of Interior (MVR), Department of Dermatology, Venereology and Dermatologic Surgery, General Skobelev Nr 79, Sofia, Bulgaria; Onkoderma-Policlinic for Dermatology and Dermatologic Surgery, General Skobelev 26, Sofia, Bulgaria. E-mail: georgi_tchernev@yahoo.de

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Abstract

BACKGROUND: It is assumed that the occurrence of keratinocyte and melanocytic tumours is multifactorial driven. Certain risk factors such as solar radiation, p53 protein and Melanocortin-1 receptor (MC1R) prove to be common to their development, which at the same time shows that their simultaneous manifestation in the same patients, for example, is quite possible. Such a manifestation could be observed as collision tumours within the same solitary lesion or as a simultaneous occurrence within two completely different lesions that are clearly distinguished from one another.

CASE REPORT: An 85-year-old patient is presented with three primary cutaneous tumours located in region presternal, infraorbital sinistra and scapularis extra. The lesions were removed during a single surgical session. For the high-risk basal cell carcinoma (BCC) in the lower eyelid, the so-called melolabial advancement flap was applied, and for the tumours located in the other two areas, the undermining surgical approach was applied. The subsequent histological analysis found that the case referred to two keratinocyte tumours (BCC) and one melanocyte tumour (cutaneous melanoma).

CONCLUSIONS: The patient presented is interesting with regard to 1) the simultaneous presentation of three primaries with different localization (so far not described in the world literature, namely 2 basal cell carcinomas and one melanoma in the same patient concurrently), 2) one of the basal cell tumours belongs to the group of high-risk (according to the localization) and meanwhile advanced BCC (according to the infiltration degree of the underlying tissue-infiltration of the musculature) and 3) their simultaneous successful surgical treatment in a single surgical session under local anaesthesia.

Introduction

Solar radiation could be considered a major etiologic/risk factor for the occurrence of basal cell carcinoma and malignant melanoma [1], [2]. The combination of mutations in the p53 gene and UV radiation increases the risk of development of melanoma and non-melanoma skin tumours [3], [4]. There are some regulatory proteins that may prove to be key but also common for the development of both melanomas and basal cell carcinomas [5], [6] [7], [8].

For example, the p53 protein and Melanocortin-1 receptor (MC1R) are considered as risk factors for both malignant melanoma (MM) and basal cell carcinoma (BCC), as well as for spinocellular carcinoma (SCC) development [5] [6] [9]. These data allow us to conclude that the simultaneous manifestation of melanocytic and keratinocyte cutaneous tumours should be entirely possible [10] [11].

Case report

An 85-year-old patient is presented with some concomitant diseases: arterial hypertension, chronic congestive heart failure, high grade aortic, mitral and tricuspid insufficiency, atrial fibrillation, pulmonary hypertension, cholelithiasis, hiatal hernia, iron deficiency anaemia and idiopathic thrombocytopenia. Treatment with Eltrombopag (25 mg x 1/day) is given with good results for idiopathic thrombocytopenia. The patient was hospitalised for scheduled surgical co-removal of the tumour formations located in the lower eyelid, back and sternum. During the dermatological examination, three lesions of different nature and localisation were identified. In the region pre sternalis a pigmentary lesion with irregular edges, clinically and dermatoscopically suspected for melanoma, was identified (Figure 1d and 1e). In the area, scapularis extra, an exophytic oval tumorous formation with an ulcerative and at the same time heavily bleeding surface, with a diameter of approximately 6-7,8 cm, was additionally noted (Figure 1a). In regio infraorbitalis sinistra, immediately next to the lower eyelid, an exophytic tumorous formation with a centrally located erosive surface covered with hemorrhagic crusts and a slightly raised peripheral edge were observed (Figure 1b and 3a). Surgical removal of the three formations was planned under local anaesthesia within one surgical session. The lesion located in regio presternal, suspected for malignant melanoma, was removed by elliptical excision under local anaesthesia, with a surgical safety margin of 0.5 cm in all directions (Figure 1f). The resulting surgical defect was closed by single interrupted stitches (Figure 1g).

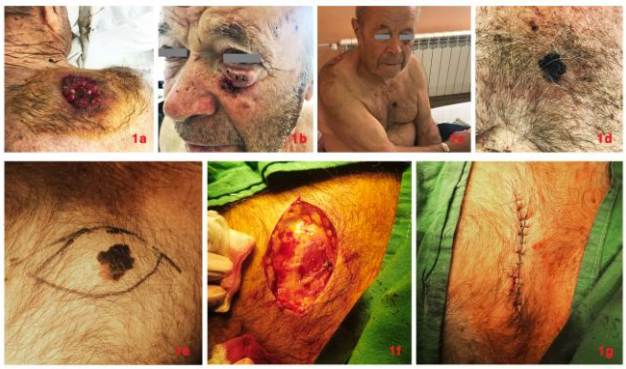

Figure 1: a) Clinical view of the lesion in regio scapularis extra-exophytic oval tumorous formation with ulcerative and at the same time heavily bleeding surface, with a diameter of approximately 7/8 cm; b) Exophytic tumorous formation with a centrally located erosive surface covered with hemorrhagic crusts and a slightly raised peripheral edge in regio infraorbitalis sinistra; c) Simultaneous clinical view of the three lesions during the first dermatological examination; d) Regio pre sternalis-pigmentary lesion with irregular edges; e) Preoperative outlining of the pigmentary lesion surgical margins; f) Intraoperative finding-elliptical excision of the melanocytic lesion; g) Postoperative view following the removal of the melanocytic lesion-closure of the defect with single interrupted stitches

The histological analysis showed that it was malignant melanoma, superficial progression type, III Clark's level, 2 mm Breslow's thickness, no ulceration, high mitotic activity, abundant lymphocytic infiltration in the stroma, no spontaneous regression, clear resection lines, IB (T2aNxM0) stage.

The lesion localised in regio scapularis extra, suspected for spinocellular carcinoma, was removed by extensive elliptic excision under local anaesthesia (Figures 2b, 2c and 2d). This was followed by careful dissection of the subcutaneous tissue to the muscles in all directions to a better adaptation of the wound edges (Figure 2e). The resulting surgical defect was recovered by stretch plastics (Figure 2f). The histological analysis found that it was a basal cell carcinoma with clear resection lines, I stage. The tumour formation in the area of regio infraorbitalis sinistra, which was the cause of hospitalisation and suspected for basal cell carcinoma, was surgically removed in stages by melolabial advancement plastics.

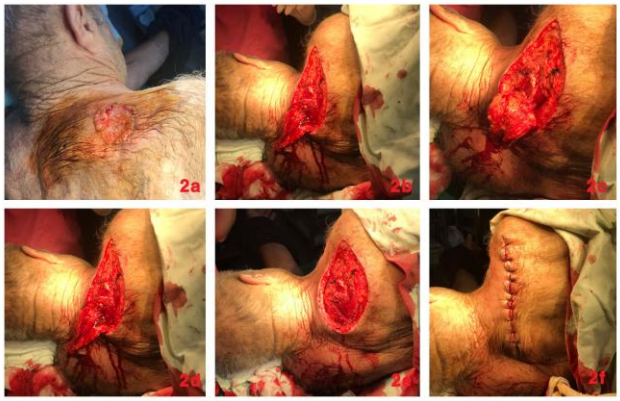

Figure 2: a) Preoperative view of the lesion in the shoulder area-disinfection; b), c), d) and e) Intraoperative view-elliptical excision of the exophytic lesion in regio scapularis extra; f) Postoperative closure of the surgical defect by stretch plastics and single interrupted stitches

The lesion was initially contoured with a surgical safety margin of 0.2-0.3 cm medially to the nose, laterally to the ear and caudally to the upper lip, and cranially due to the proximity to the lower eyelid, with a distance of approximately 0.1 cm (Figure 3a). In the second stage, the lesion was excised in the form of a quadrangle with oval edges, and a small part of the underlying muscle was also removed (Figure 3b and 3c). After stopping the bleeding, the skin integrity in the area of the resulting skin defect was restored by a melolabial advancement flap. Expansion of the defect was initiated by conducting an initial single oblong incision parallel to the melolabial fold, followed by skin erosion laterally to the incision and finally transposition of the skin upward using slight rotation (Figure 3d). The transposition flap was carefully adapted to the edge of the lower eyelid by single subcutaneous stitches, and then by skin stitches (Figure 3e and 3f). Histological verification showed

that it was an advanced high-risk basal cell carcinoma with clear resection lines, II stage.

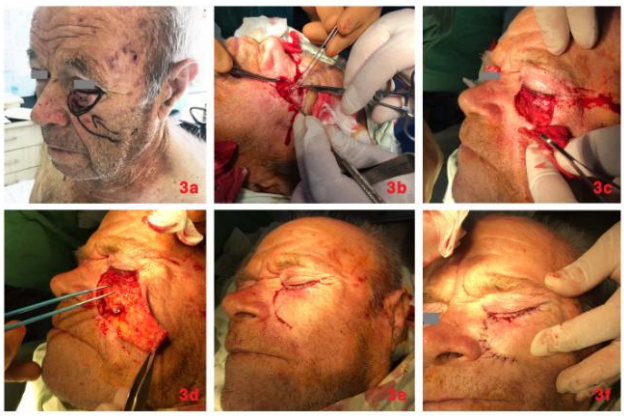

Figure 3: a) Preoperative outlining of the safety surgical margins; b), c) Oval excision of the lesion located in regio infraorbitalis sinistra; d) Intraoperative finding-stopping the bleeding by electrocautery; e) Postoperative view after the melolabial advancement flap; d) Clinical postoperative status-single interrupted stitches

At the time of the surgical intervention, there was no apparatus or laboratory evidence of progression of both melanoma and the keratinocyte tumours.

About the histologically established melanoma, it was recommended to perform a re-excision with safety surgical margin of 1.5 cm within 14 days, and detect the draining lymph node within the same surgical session.

Discussion

About the BCC and MM occurrence, UV radiation is referred to as an exogenous etiological factor of paramount importance [1], [2]. In turn, a large number of regulatory proteins are considered to be the key endogenous factors in the pathogenesis of melanoma and non-melanoma skin tumours [5], [6], [7], [8]. P53 protein and Melanocortin-1 receptor (MC1R) are identified as risk factors for the development of malignant melanoma (MM), basal cell carcinomas (BCC) and spinocellular carcinomas (SCC) [5], [6], [9]. P53 gene mutations lead to overproduction of long-life mutant forms of p53 protein, which in combination with the additional influence of sunlight significantly increases the risk of developing BCC and malignant melanoma [3], [4]. Similar dependence is seen with combined UV radiation and various gene variants of the Melanocortin-1 receptor (MC1R) [5], [6]. These common risk factors and mechanisms in the genesis of melanocytic and keratinocyte tumors suggest their possible simultaneous presentation in the same patient [1], [2], [3], [4], [5], [6], [7], [8], [9], [10], [11], [12].

The simultaneous manifestation of melanocyte and keratinocyte skin tumours may be seen in the form of the so-called 1) collision tumours – when they are established within the same lesion (clinically, dermatoscopically and/or histologically) and 2) simultaneous occurrence of two or more histologically distinct primary malignancies [12], [13]. The presence of two different malignant tumours at the same time, located within the same histological sample, is referred to as the so-called collision tumours [14], [15]. However, depending on the boundaries between cells, the simultaneous manifestation of two histologically distinct tumours is subdivided into two types: 1) collision type, in which each cellular type is distinct, and 2) intermingled type, in which the two cell types are “intimately related” [16]. The predominant number of documented cases in the world literature represents the BCC and MM combination [12], [14], [15], [16]. In this respect, their correlation is interesting, defined as parasitism, i.e. BCC colonization by MM [14], [17], [18], [19]. A two-phase and three-phase manifestation in the form of a squamomelanocytic tumour, basomelanocytic tumour or basosquamous melanocytic malignant tumour [20], [21], [22] is also possible, though relatively rare. Even in the form of a collision tumour, the ability of the malignant melanoma to provide metastasis is retained [15], [23]. In some cases, metastasis may manifest as blue nevi, thus simulating the clinical picture of a benign lesion [15]. All possible options of coexistence between melanocytic and keratinocyte tumours are most safely demonstrated histopathologically and immunohistochemically [15], [18], [20], [21].

Unlike collisional tumours, the simultaneous occurrence of two different primary histological carcinoma types is extremely rare [10], [11]. To our knowledge, we present for the first time in the world literature a unique case of a patient with simultaneous occurrence of three primary cutaneous tumours with different localisation – two keratinocyte tumours (2 basal cell carcinomas) in combination with presternal localised superficial melanoma.

Two cases of patients with BCC, SCC and MM have been documented in the world literature [10], [11]. In one case, the data simultaneously manifests the three types of tumours [10], and the other described case refers to a patient with metastatic melanoma in combination with 2 keratinocyte tumours (BCC and SCC with different localisation) [11].

Patients with BCC, MM, SCC are at increased risk of development of subsequent cutaneous tumours of the same or another type [24], [25], [26]. Cutaneous melanoma diagnosis is considered a risk of developing multiple cutaneous (pre-) malignancies [25]. Patients with BCC may subsequently develop other forms of cancer, such as testicular cancer,

breast cancer, and non-Hodgkin lymphoma [27]. This requires their regular analysis (clinical and apparatus diagnostic procedures in the framework of selected screening programs).

Basal cell carcinomas located in the so-called H-zone of the face (nasolabial fold, nasal alar, orbital area and auricular area, are considered to be high risk regarding the occurrence of possible recurrence [28].

High-risk cases substantially include long-term tumors (not defined), localized midface/ear (basalioma adjacent to the lower eyelid/this criterion is met in our patient), diameter over 2 cm (basalioma in proximity to the lower eyelid/this criterion is met in our patient also), aggressive histological subtypes, perivascular/perineural infiltration, prior radiation therapy or other types of treatment failure [29]. Advanced BCCs are defined as III stage (with musculature infiltration, as described in our patient) or IV stage tumours, and when their size is more than 5 cm, they are classified as giant BCC [29]. There is often a criteria overlap between the high-risk and advanced BCCs, as well as failure of the lesion to meet all the requirements specified in the definitions.

Both types often require the application of more sophisticated surgical techniques [28], [30].

In conclusion, concomitant surgical treatment of risk basal cell carcinoma with facial muscular infiltration combined with cutaneous melanoma of presternal localisation and additional resection of basal cell carcinoma on the shoulder is a serious challenge for most of the dermatosurgeons. We at this moment inform for the first time in the world literature about the simultaneous diagnosis of 2 keratinocyte and one melanocytic tumour in the form of primaries with different localisation, as well as their successful surgical treatment within one surgical session.

References

1. Rosso S, Zanetti R, Pippione M, Sancho-Garnier H. Parallel risk assessment of melanoma and basal cell carcinoma: skin characteristics and sun exposure. Melanoma Res. 1998; 8(6):573-83. https://doi.org/10.1097/00008390-199812000-00013 PMid:9918420

2. Armstrong B, Kricker A. The epidemiology of UV induced skin cancer. J Photochem Photobiol B. 2001; 63(1-3):8-18. https://doi.org/10.1016/S1011-1344(01)00198-1

3. Giglia-Mari G, Sarasin A. TP53 mutations in human skin cancers. Hum Mutat. 2003; 21(3):217-28. https://doi.org/10.1002/humu.10179 PMid:12619107

4. Shea C, McNutt N, Volkenandt M, Lugo J, Prioleau P, Albino A. Overexpression of p53 protein in basal cell carcinomas of human skin. Am J Pathol. 1992; 141(1):25–29. PMid:1632467 PMCid:PMC1886589

5. Box N, Duffy D, Irving R, Russell A, Chen W, Griffyths L, Parsons P, Green A, Sturm R. Melanocortin-1 receptor genotype is a risk factor for basal and squamous cell carcinoma. J Invest Dermatol. 2001; 116(2):224-9. https://doi.org/10.1046/j.1523-1747.2001.01224.x PMid:11179997

6. Kennedy C, ter Huurne J, Berkhout M, Gruis N, Bastiaens M, Bergman W, Willemze R, Bavinck J. Melanocortin 1 receptor (MC1R) gene variants are associated with an increased risk for cutaneous melanoma which is largely independent of skin type and hair color. J Invest Dermatol. 2001; 117(2):294-300. https://doi.org/10.1046/j.0022-202x.2001.01421.x PMid:11511307

7. Tchernev G, Orfanos CE. Downregulation of cell cycle modulators p21, p27, p53, Rb and proapoptotic Bcl-2-related proteins Bax and Bak in cutaneous melanoma is associated with worse patient prognosis: preliminary findings. J Cutan Pathol. 2007; 34(3):247-56. https://doi.org/10.1111/j.1600-0560.2006.00700.x PMid:17302609

8. Fecker LF, Geilen CC, Tchernev G, Trefzer U, Assaf C, Kurbanov BM, Schwarz C, Daniel PT, Eberle J. Loss of proapoptotic Bcl-2-related multidomain proteins in primary melanomas is associated with poor prognosis. J Invest Dermatol. 2006; 126(6):1366-71. https://doi.org/10.1038/sj.jid.5700192 PMid:16528364

9. Koseoglu RD, Sezer E, Eyibilen A, Aladag I, Etikan I. Expressions of p53, cyclinD1 and histopathological features in basal cell carcinomas. J Cutan Pathol. 2009; 36(9):958-65. https://doi.org/10.1111/j.1600-0560.2009.01204.x PMid:19187116

10. Hagedorn M, RuBwurm R, Sommer B, Thomas C. Simultaneous Occurrence Of A Basal Cell Carcinoma, Squamous Cell Carcinoma And Malignant Melanoma In A Patient. The American Journal of Dermatopathology. 1994; 16(1):101. https://doi.org/10.1097/00000372-199402000-00043

11. Grampurohit V, Dinesh U, Rao R. Multiple cutaneous malignancies in a patient of xeroderma pigmentosum. J Cancer Res Ther. 2011; 7(2):205-7. https://doi.org/10.4103/0973-1482.82932 PMid:21768716

12. Medeiros P, Alves N, Silva C, Faria P, Barcaui C, Pi-eiro-Maceira J. Collision of malignant neoplasms of the skin: basosquamous cell carcinoma associated with melanoma. An Bras Dermatol. 2015; 90(3 Suppl 1):39–42. https://doi.org/10.1590/abd1806-4841.20153845 PMid:26312670 PMCid:PMC4540504

13. Nakahara H, Kitamura R, Shirasuna K. Simultaneous malignant melanoma and squamous cell carcinoma of the oral cavity: a case report. J Oral Maxillofac Surg. 1995; 53(12):1455-7. https://doi.org/10.1016/0278-2391(95)90676-2

14. Mancebo S, Marchetti M, Hollmann T, Marghoob A Busam K, Halpern A. Melanoma in situ colonizing basal cell carcinoma: a case report and review of the literature. Dermatol Pract Concept. 2015; 5(1):25–30. https://doi.org/10.5826/dpc.0501a04 PMid:25692077 PMCid:PMC4325687

15. King R, Lyons J, Meyers A, Googe P, Page R, Gupta V. Primary invasive melanoma and basal cell carcinoma (collision tumor) with blue nevus-like cutaneous metastases. J Cutan Pathol. 2007; 34(8):629-33. https://doi.org/10.1111/j.1600-0560.2006.00677.x PMid:17640233

16. Braun-Falco M. Combined malignant melanoma and basal cell carcinoma tumor of the intermingled type. J Cutan Pathol. 2007; 34(9):731-5. https://doi.org/10.1111/j.1600-0560.2006.00703.x PMid:17696923

17. Burkhalter A, White W. Malignant melanoma in situ colonizing basal cell carcinoma. A simulator of invasive melanoma. Am J Dermatopathol. 1997; 19(3):303–7. https://doi.org/10.1097/00000372-199706000-00019 PMid:9185921

18. Wang H, Benda P, Piepkorn M. Parasitism of basal cell carcinoma by lentigo maligna melanoma. J Am Acad Dermatol. 2003; 48(5 Suppl):S92–4. https://doi.org/10.1067/mjd.2003.237 PMid:12734489

19. Goeser M, Dimaio D. A colonization of basal cell carcinoma by malignant melanoma in situ resembling a malignant basomelanocytic tumor. Am J Dermatopathol. 2014; 36(11):e179–82. https://doi.org/10.1097/DAD.0000000000000047 PMid:24752214

20. Miteva M, Herschthal D, Ricotti C. A rare case of a cutaneous squamomelanocytic tumor: revisiting the histogenesis of combined neoplasms. Am J Dermatopathol. 2009; 31(6):599–603. https://doi.org/10.1097/DAD.0b013e3181a88116 PMid:19590411

21. Erickson L, Myers J, Mihm M. Malignant basomelanocytic tumor manifesting as metastatic melanoma. Am J Surg Pathol. 2004; 28(10):1393–96. https://doi.org/10.1097/01.pas.0000135526.19189.31 PMid:15371958

22. Cornejo K, Deng A. Malignant melanoma within squamous cell carcinoma and basal cell carcinoma: is it a combined or collision tumor?--a case report and review of the literature. Am J Dermatopathol. 2013; 35(2):226-34. https://doi.org/10.1097/DAD.0b013e3182545e27 PMid:22588546

23. Sharma S, Agrawal U, Gupta P, Bhatnagar A, Jairajpuri Z. Malignant melanoma and basal cell carcinoma of the face: a rare coexistence. Ann Saudi Med. 2013; 33(3):304-6. https://doi.org/10.5144/0256-4947.2013.304 PMid:23793437 PMCid:PMC6078527

24. van der Leest R, Hollestein L, Liu L, Nijsten T, de Vries E. Risks of different skin tumour combinations after a first melanoma, squamous cell carcinoma and basal cell carcinoma in Dutch population-based cohorts: 1989-2009. J Eur Acad Dermatol Venereol. 2018; 32(3):382-389. https://doi.org/10.1111/jdv.14587 PMid:28898461

25. van der Leest R, Flohil S, Arends L, de Vries E, Nijsten T. Risk of subsequent cutaneous malignancy in patients with prior melanoma: a systematic review and meta-analysis. J Eur Acad Dermatol Venereol. 2015; 29(6):1053-62. https://doi.org/10.1111/jdv.12887 PMid:25491923

26. Flohil S, van der Leest R, Arends L, de Vries E, Nijsten T. Risk of subsequent cutaneous malignancy in patients with prior keratinocyte carcinoma: a systematic review and meta-analysis. Eur J Cancer. 2013; 49(10):2365-75. https://doi.org/10.1016/j.ejca.2013.03.010 PMid:23608733

27. Frisch M, Hjalgrim H, Olsen J, Melbye M. Risk for subsequent cancer after diagnosis of basal-cell carcinoma. A population-based, epidemiologic study. Ann Intern Med. 1996; 125(10):815-21. https://doi.org/10.7326/0003-4819-125-10-199611150-00005 PMid:8928988

28. Yalcin O, Sezer E, Kabukcuoglu F, Kilic A, Sari A, Cerman A, Altunay I. Presence of ulceration, but not high risk zone location, correlates with unfavorable histopathological subtype in facial basal cell carcinoma. Int J Clin Exp Pathol. 2015; 8(11):15448–15453. PMid:26823913 PMCid:PMC4713699

29. Wollina U, Steinbach F, Verma S, Tchernev G. Penile tumours: a review. Journal of the European Academy of Dermatology and Venereology. 2014; 28(10):1267-76. https://doi.org/10.1111/jdv.12491 PMid:24684236

30. Choi J, Kim Y, Kim H, Nam S, Woong Y. Distribution of Basal Cell Carcinoma and Squamous Cell Carcinoma by Facial Esthetic Unit. Arch Plast Surg. 2013; 40(4):387–391. https://doi.org/10.5999/aps.2013.40.4.387 PMid:23898436 PMCid:PMC3724000

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Case Report

Eruptive Xanthomas – Two Case Reports With Distinct Features

Uwe Wollina[1], André Koch[1], Gesina Hansel[1], Jacqueline Schönlebe[2]

[1]Department of Dermatology and Allergology, Städtisches Klinikum Dresden, Dresden, Germany; [2]Institute of Pathology "Georg Schmorl", Städtisches Klinikum Dresden, Dresden, Germany

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Keywords: Eruptive Xanthomas; Metabolic Disorders; Chylomicronemia; Diabetes mellitus; Hyperlipidemia

***Correspondence:** Uwe Wollina. Department of Dermatology and Allergology, Städtisches Klinikum Dresden, Dresden, Germany. E-mail: uwollina@gmail.com

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Abstract

BACKGROUND: Eruptive xanthomas are rare and often asymptomatic. On the other hand, these cutaneous lesions are a red flag for serious underlying metabolic disorders that demand an early diagnosis to prevent morbidity and mortality.

CASE REPORT: We report on two male patients, aged 23 and 27 years, who presented with eruptive xanthomas. Clinical, histological and laboratory investigations disclosed a metabolic syndrome in the younger patient and an alcohol-induced chylomicronemia in the elder one. Two types of macromorphology of cutaneous lesions were observed. Treatment was tailored according to underlying pathologies and resulted in significant improvement of the metabolic parameters and improvement of skin lesions.

CONCLUSION: Dermatologists should be aware of the diagnostic importance of eruptive xanthomas for serious metabolic disorders.

Introduction

Xanthomas are cutaneous lesions developing as a result of local storage of lipids. Xanthomas can be classified according to the clinical presentation of the individual lesion or by the mode of appearance. Eruptive xanthomas are uncommon and represent an important clinical sign for serious metabolic disorders. Low-density lipoprotein particles are preferably stored in foam cells and giant cells [1]. A particular subtype is neutrophilic eruptive xanthomas, mostly but not exclusively seen in cases of immunosuppression or immunodeficiency [2].

Clinically, the lesions appear as rapidly evolving papules with a red-to-yellowish hue and are about 1-5 mm in diameter. They generally form across the extensor surfaces of the arms and legs, as well as across the buttocks, and may involve palmoplantar skin along the creases. The lesions can be tender, often they remain asymptomatic, but early lesions may be pruritic. Eruptive xanthomas may be associated with diabetes mellitus, hypercholesterolemia, hypertriglyceridemia, lipemia retinalis, or hepatosteatosis [3], [4]. Another but unusual cause is tattooing that causes a Koebner phenomenon [5], [6]. The recognition of underlying metabolic disorders is necessary to prevent conceivable fatal medical conditions such as coronary artery disease or pancreatitis [7].

Case reports

Case 1: A 23-year-old male presented with multiple non-pruriginous papules, that developed during five years. He was a heavy cigarette smoker without alcohol abuse. His medical history was remarkable for acute pancreatitis one year before of unknown cause. His father died from myocardial infarction.

On examination, we found an obese male patient with a body-mass-index of 35.9. He had

disseminated reddish to yellowish papules of variable diameter (from several millimetres to 1 cm) arranged in stripes on his trunk and the extremities (Figure 1). Predominant sites were the neck, lower back, buttocks, palmar area, and the extension sites of the arms.

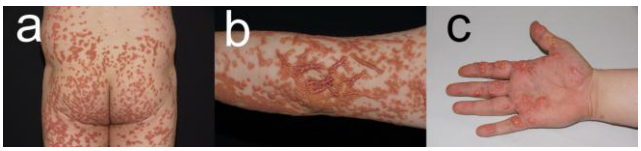

Figure 1: Eruptive xanthomas with unrecognised metabolic syndrome; a) Multiple papules on the buttocks and lower back; b) extension sites of the arms and c) palms

We took a biopsy for histopathological examination. The investigations revealed foam cells and some Touton giant cells in the upper dermis. The foam cells were CD68 positive, while stains for S100 and CD1a remained negative (Figure 2). The diagnosis of xanthomas was made.

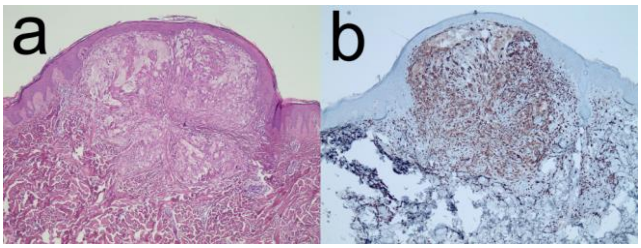

Figure 2: Histology of eruptive xanthomas; a) Hematoxylin-eosin stain with foam cells of the upper dermis; b) Positive staining for CD68 (Original magnification x 4)

Laboratory investigations: Blood glucose 16.7 mmol/l; HbA1c 11.6 % (normal range 4-6); cholesterol 16.5 mmol/l (normal range < 5.2); triglycerides 4.66 mmol/l (< 2.3), very low-density lipoprotein (VLDL) was extremely increased. Blood cell counts, liver enzymes, lipoprotein (a), and clotting parameters were in the normal range. Blood serum was lipemic (Figure 3).

Figure 3: Lipemic serum (right) versus normal serum (left)

Imaging: X-ray of the chest and ultrasound of the abdomen were unremarkable. Repeated blood pressure measurements revealed hypertension. The patient was treated by an interdisciplinary approach for diabetes mellitus type II and hyperlipidemia. We started with subcutaneous insulin and certoparin injections to stimulate the lipoprotein lipase. After significant improvement of the glucose metabolism, the treatment was switched to combined oral medication of metformin and sitagliptin. The hypertension was treated with oral ramipril. We also recommended a low-lipid diet. At the end of his hospital stay, blood glucose was 5.7 mmol/l and cholesterol 3.67 mmol/l. The cutaneous lesion started to diminish.

Case 2: A 27-year-old male presented with multiple, moderate pruritic cutaneous plane yellowish plaques which developed throughout 2 weeks. His medical history was positive for pollinosis and nodular struma. The family history disclosed no risk factors for cardiovascular or cutaneous disorders. He had regular alcohol consume.

On examination, we observed an otherwise healthy non-obese male patient with disseminated yellowish plane cutaneous lesions on trunk and flexion sites of extremities (Figure 4). Palms, soles and face were spared.

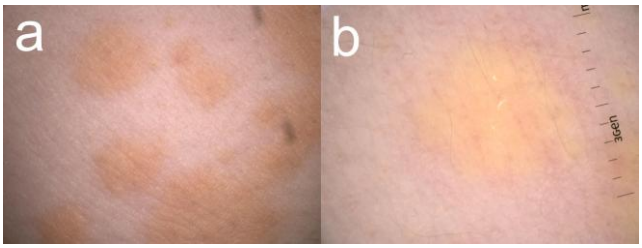

Figure 4: Eruptive xanthomas with chylomicronemia; a) Flat yellowish plaques; b) Dermoscopy with polarised light (Dermogenius) demonstrating structureless plaque without erythema or vascular proliferation

We took a skin biopsy. Histopathologic examination revealed lipid storing macrophages in the upper and mid-dermis, foam cells were absent. Infiltrates composed of lymphocytes and mast cells with some intermingled neutrophils and eosinophils were localised perivascular. The macrophages were CD 68-positive but lacked S-100 or CD1a. The diagnosis of eruptive xanthomas was confirmed.

Laboratory investigations: Triglycerides 9.84 mmol/l (normal range < 2.3); cholesterol 15.94 mmol/l (normal range < 5.2); lipoprotein (a) 0.74 g/l (normal range < 0.3); triglyceride/ cholesterol ratio 12.62 (normal range < 1.5); gamma-glutamyl transferase 6.66 µkat/l (normal range < 1). Liver transaminases and eosinophil count were slightly increased. Blood glucose levels and pancreatic lipase were in the normal range. Imaging: Chest-X-ray and abdominal

ultrasound unremarkable. The diagnosis of an alcohol-induced chylomicronemia with eruptive xanthomas was made. A reduction of alcohol consumption was recommended what significantly reduced hyperlipidemia. In the interdisciplinary consultation, medical drug therapy was not recommended since the reduction or avoidance of substance abuse leads to normalisation. There was no clue for familial chylomicronemia syndrome.

Discussion

Eruptive xanthomas may be the result of a variety of metabolic disorders, medical drugs (glucocorticoids, retinoids, estrogens), secondary insulin resistance, and alcohol abuse. The most common causes represent chylomicronemia and hypertriglyceridemia either due to lipoprotein lipase deficiency (Type I hyperlipoproteinemia) or familial hyperlipoproteinemia (Type V hyperlipoproteinemia). In diabetic patients unresponsive to insulin, an acquired lipoprotein lipase deficiency may develop [8]. Rare syndromes associated with eruptive xanthomas are the Berardinelli-Seip syndrome [9], the von Gierke syndrome (glycogen storage disease type I) [10], or the primary lipoprotein-lipase deficiency [11].

LDL is one of the major carriers of cholesterol. Circulating LDL particles in the blood stream realise the cholesterol transport to those cells that are requiring lipids. These cells express higher levels of the LDL-receptor (LDLR) that mediates uptake of LDL particles by receptor-mediated endocytosis. For plane xanthomas, development of foam cells is associated with the uptake of LDL particles that are modified due to increased residence time in plasma by over-expressed macrophage scavenger receptor (SR) [12]. Sortilin, a transmembrane receptor expressed by macrophages that binds LDL and support intracellular LDL uptake, is another driver for their transformation into foam cell [13]. In eruptive xanthomas, foam cells may develop due to inflammatory stress. Thereby, LDL receptor negative feedback regulation induced by intracellular cholesterol becomes disrupted [14].

We presented two young male patients with eruptive xanthomas with a slightly different clinical appearance of the cutaneous lesions. In case # 1 the lesions were reddish to yellowish papules with a preference of the extension sites of the arms. The underlying pathology was an unrecognised metabolic syndrome. In case # 2, the lesions were flat, moderate pruritic, yellowish without an erythematous note and preferred the flexion sites of the arms. The underlying pathology was alcohol-induced chylomicronemia. The treatment was tailored according to the individual needs. Diagnosis of underlying metabolic disorders and their correction by treatment is an appropriate method to improve eruptive xanthomas.

References

1. Zak A, Zeman M, Slaby A, Vecka M. Xanthomas: clinical and pathophysiological relations. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2014; 158(2):181-8. https://doi.org/10.5507/bp.2014.016 PMid:24781043

2. Smith KJ, Yeager J, Skelton HG. Histologically distinctive papular neutrophilic xanthomas in HIV-1+ patients. Am J Surg Pathol. 1997; 21(5):545-9. https://doi.org/10.1097/00000478-199705000-00006 PMid:9158678

3. Santiago L, Pinho A, Cardoso JC. Eruptive xanthomas: a cardinal manifestation of the serious metabolic disease. Acta Med Port. 2018; 31(4): 219-22. https://doi.org/10.20344/amp.9126 PMid:29855416

4. Zabeen B, Khaled Z, Nahar J, Baki A, Amin F, Akhter S, Begum T, Azad K, Nahar N. Hypertriglyceridemia associated with eruptive xanthomas and lipemia retinalis in newly diagnosed diabetes mellitus. Mymensingh Med J. 2013; 22(3):591-5. PMid:23982556

5. Brazzelli V, Rivetti N, Carugno A, Barruscotti S, Croci GA, Perani G, Borroni G. Eruptive xanthomas after extensive tattooing: a case report and literature review. G Ital Dermatol Venereol. 2015; 150(6):770-1. PMid:25014584

6. Gao H, Chen J. Eruptive xanthomas presenting in tattoos. CMAJ. 2015; 187(5):356. https://doi.org/10.1503/cmaj.140383 PMid:25623644 PMCid:PMC4361110

7. Saraceno R, Dattola A, Pietroleonardo L, Pitocco R, Fida M, Chimenti S. Eruptive xanthomas and pancreatitis: clinical, dermatoscopy, confocal and pathological correlation. G Ital Dermatol Venereol. 2017; 152(4):394-6. PMid:28621119

8. Seremet S, Gurel MS. Miscellaneous skin disease and the metabolic syndrome. Clin Dermatol. 2018; 36(1):94-100. https://doi.org/10.1016/j.clindermatol.2017.09.016 PMid:29241760

9. Machado PV, Daxbacher EL, Obadia DL, Cunha EF, Alves Mde F, Mann D. Do you know this syndrome? Berardinelli-Seip syndrome. An Bras Dermatol. 2013; 88(6):1011-3. https://doi.org/10.1590/abd1806-4841.20132178 PMid:24474121 PMCid:PMC3900363

10. Zakon SJ, Oyamada A, Rosenthal IH. Eruptive xanthoma and hyperlipemia in glycogen storage disease (von Gierke's disease). AMA Arch Derm Syphilol. 1953; 67(2):146-51. https://doi.org/10.1001/archderm.1953.01540020024005 PMid:13029898

11. Gagné C, Brun LD, Julien P, Moorjani S, Lupien PJ. Primary lipoprotein-lipase-activity deficiency: clinical investigation of a French Canadian population. CMAJ. 1989; 140(4):405-11. PMid:2914262 PMCid:PMC1268664

12. Giry C, Giroux LM, Roy M, Davignon J, Minnich A. Characterization of inherited scavenger receptor overexpression and abnormal macrophage phenotype in a normolipidemic subject with planar xanthomas. J Lipid Res. 1996; 37(7):1422-35. PMid:8827515

13. Patel KM, Strong A, Tohyama J, Jin X, Morales CR, Billheimer J, Millar J, Kruth H, Rader DJ. Macrophage sortilin promotes LDL uptake, foam cell formation, and atherosclerosis. Circ Res. 2015; 116(5):789-96. https://doi.org/10.1161/CIRCRESAHA.116.305811 PMid:25593281 PMCid:PMC4602371

14. Ye Q, Lei H, Fan Z, Zheng W, Zheng S. Difference in LDL receptor feedback regulation in macrophages and vascular smooth muscle cells: foam cell transformation under inflammatory stress. Inflammation. 2014; 37(2):555-65. https://doi.org/10.1007/s10753-013-9769-x PMid:24297394

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Case Report

Multiple Primary Cutaneous Melanomas in a Bulgarian Patient: The Possible Role of One Step Melanoma Surgery (OSMS) As the Most Adequate Treatment Approach!

Georgi Tchernev[1,2*], Ivanka Temelkova[1]

[1]Medical Institute of Ministry of Interior (MVR), Department of Dermatology, Venereology and Dermatologic Surgery, General Skobelev Nr 79, Sofia, Bulgaria; [2]Onkoderma - Policlinic for Dermatology and Dermatologic Surgery, General Skobelev 26, Sofia, Bulgaria

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Keywords: Melanoma; OSMS; Treatment outcome; Survival benefit; Surgery

***Correspondence:** Georgi Tchernev. Medical Institute of Ministry of Interior (MVR), Department of Dermatology, Venereology and Dermatologic Surgery, General Skobelev Nr 79, Sofia, Bulgaria; Onkoderma-Policlinic for Dermatology and Dermatologic Surgery, General Skobelev 26, Sofia, Bulgaria. E-mail: georgi_tchernev@yahoo.de

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Abstract

BACKGROUND: Simultaneous occurrence of multiple primary melanomas is a rare, however possible phenomenon, and it is believed that older, male, white, Atypical Mole Syndrome carriers (sporadic and familial) are part of the possible risk factors for its occurrence. In these patients, it is possible to observe involutional changes or (partial/complete) regression of melanocytic lesions, which are likely to be caused by the generation of a spontaneous immune reaction against specific tumour antigens.

CASE REPORT: A 58-year-old male patient is presented with two melanocytic lesions located in the right clavicle (subclavicular area) and left the scapular area that meets clinically and dermatoscopically the requirements for malignant melanoma. The lesions were removed by a radical excision with 0.5 cm surgical safety margin in all directions. During the subsequent histological verification it was established it was melanoma with a tumour thickness of 1 mm that in one case, and the other 2 mm. A week later, according to the recommendations of the current guidelines, a re-excision was performed with a surgical safety margin of 1.5 cm in all directions. Considering the complications that are possible during the parallel removal of a draining lymph node recommended for these tumour thicknesses, the patient definitively refused its detection and removal.

CONCLUSION: A rare case of a Bulgarian patient with multiple primary, however partially involutional melanomas existing for over 20 years, is described. The individual recommendations of dermato-oncologists for the patient (according to AJCC) were not in favour of radicality but of a more sparing, individualised approach, appropriate for that patient. Unfortunately, the approach we used, in this case, was inconsistent with the AJCC or OSMS guidelines. The reason for individualising the approach in the case we have described is due to the variability of the recommendations for surgical treatment of melanomas laid down in the AJCC.

Introduction

The incidence of the so-called multiple primary melanomas (MPM) is between 1.3 and 8.0% [1]. The presence of primary melanoma increases the risk of subsequent development of the same melanocytic lesion, which most often occurs several years after the diagnosis of the primary formation and usually affects parts of the body that is different from its initial location [2]. The family history of malignant melanoma, as well as the presence of dysplastic nevus (in the relevant patient groups), are thought to be risk factors for MPM occurrence [1], [2], [3], [4]. These result in two interesting groups of patients with multiple primary melanomas: 1) within the so-called sporadic form of the syndrome of dysplastic nevi-AMS (atypical mole syndrome), 2) Familial atypical multiple melanoma (FAMMM) syndromes, 3) Xeroderma pigmentosum should also be considered a disease, in which there may be multiple primary melanomas, 4) Complete involution of malignant melanomas (MMs) is an extremely rare phenomenon; on the other hand, partial regression occurs in 10% to 35% of the cases, regardless of Breslow tumour thickness, 5) In this respect, clinical and histopathological criteria for MMs regression have been established [5]. Each patient with malignant melanoma or multiple primary melanomas should be considered a combination of

individual specificities that should determine the choice of strategy for subsequent treatment [6].

Case report

A 58-year-old male patient is presented in good general status. The patient was hospitalised for the first time in Oncoderma Dermatology and Dermatologic Surgery Clinic, Sofia, for surgical removal of two pigment lesions located in the right clavicle (subclavicular area) and left scapular area. The lesions occurred 15 years ago, but within the last year, the patient has observed an increase in the intensity of the black colour and size of one of the lesions, as well as involution in the scapular area. During the dermatological examination, two melanocytic lesions with uneven boundaries and irregular distribution of the pigment were found in the right subclavicular and left scapular areas (Figures 1a-1f).

Clinically and dermatoscopically, these findings met the requirements for a malignant melanocytic lesion. Further tests were carried out: 1) paraclinical – without substantial changes, 2) lymph node ultrasound – no enlarged lymph nodes were visualized in the neck, two axil and inguinal area; 3) lung and heart radiology – preserved transparency of pulmonary parenchyma without active disease alterations – no focal changes were observed; clear costophrenic sinuses; heart shadow – normal, 4) S-100-0.036 (< 0.1); LDH – 314.00 IU (240.00-480.00).

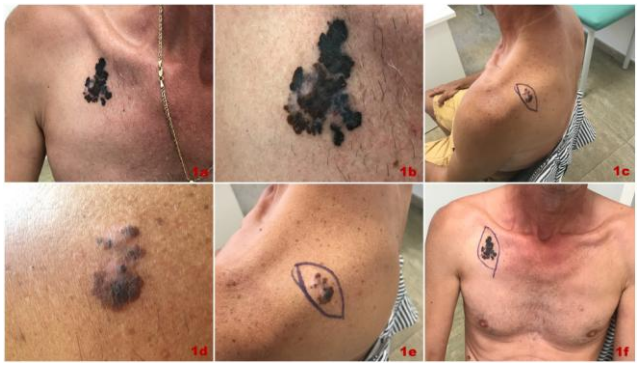

Figure 1: a), b) Clinical picture of primary cutaneous melanoma located on the right- subclavicular. Lesion with uneven pigmentation and uneven boundaries; c), d) Clinical view of melanoma showing uneven pigmentation, uneven edges and data about involution located in the left scapular area; e), f) Outlining the 0,5 cm operational security boundaries in all directions, preoperative finding

The lesion localised to the right, in the subclavicular area, was removed by radical excision. A surgical margin of 0.5 cm was used in all directions (Figure 2a). The resulting surgical defect was recovered by a single interrupted suture (Figure 2b). Histological verification showed that it was mixed, superficially advancing and nodular malignant melanoma, Clark's level III, Breslow's thickness 2 mm, no ulceration, high mitotic activity, moderate stromal lymphocytic reaction, no spontaneous regression, T2aN0M0 stage.

In the left scapular area, a primary excision of the melanocytic lesion was performed with a surgical safety margin of 0.5 cm in all directions (Figure 2c). The defect was corrected using stretch plastics (Figure 2d). The histological examination of the lesion removed from the left scapular area showed superficially advancing malignant melanoma, Clark's level II, Breslow's thickness 1 mm, no ulceration, T1bN0M0 stage.

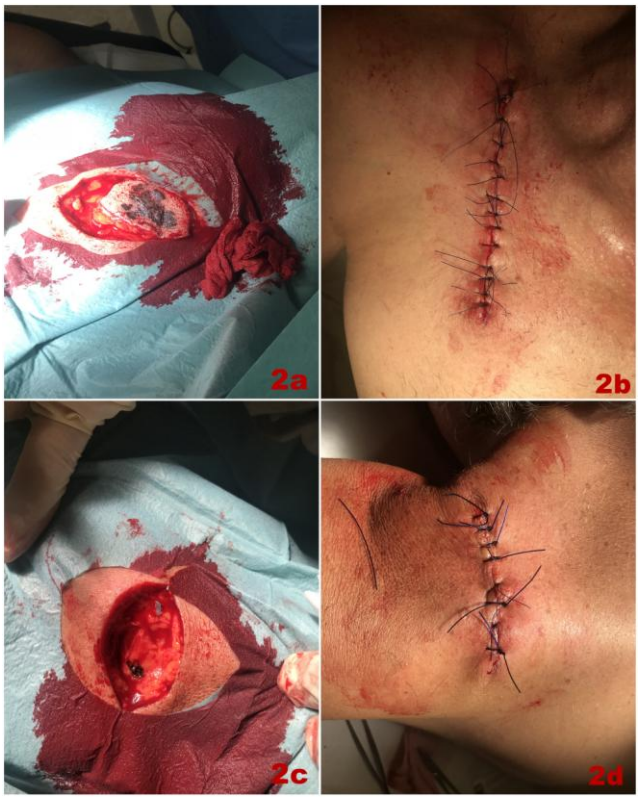

Figure 2: a), c) Intraoperative finding of the two lesions removed by elliptical excision; b), d) Postoperative clinical picture of surgical defects closed by single interrupted sutures

A week later, following the recommendations of the applicable guidelines, a re-excision was performed in the primary excision zones with 1.5 cm surgical safety margin in all directions (Figures 3a-3f). Immediately before the re-excision, the patient was consulted in the Oncology Ward, where the need for parallel removal of draining lymph node during the re-excisions was rejected, based on 1) the lack of explicit evidence of locoregional dissemination of the process, 2) complains that occurred more than 20 years ago, as well as 3) the involutional nature of one of the lesions. Postoperative period – calm, without complications. The patient was referred to the oncology unit for regular ultrasound controls and the introduction of systemic Interferon therapy.

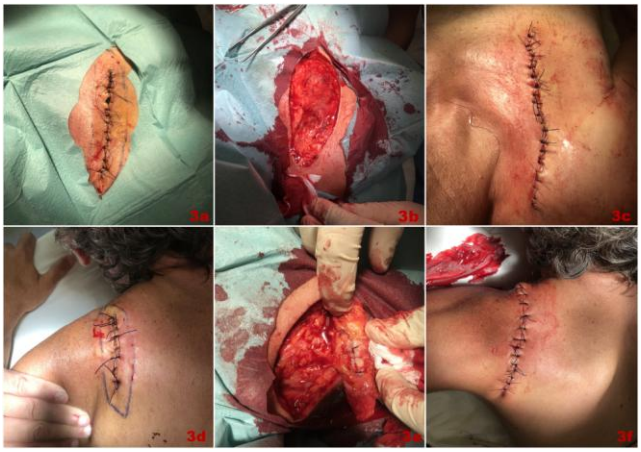

Figure 3: a), d) Preoperative outline of 1.5 cm operational security boundaries in all directions for primary excision sites directly before re-excision; b), e) Intraoperative view of elliptical re-excision; c), f) Postoperative clinical picture of surgical defects closed by single interrupted sutures after re-excision

Discussion

Simultaneous occurrence of multiple primary melanomas (MPM) in the same patient is a rare, however well-recognisable phenomenon with variable incidence, according to the available literature [7], [8] [9], [10]. It is believed that older, male, white, history of melanoma, thin primaries and history of another cancer are risk factors for development MPMs [6], [10]. Also, the identification of Atypical Mole Syndrome carriers (sporadic and familial) is essential, as it is considered the most important phenotypic risk factor for cutaneous melanoma and there are documented cases of MPM within the familial and sporadic syndrome of dysplastic nevi [3].

MPM patients are also at risk of developing subsequent primary melanomas, which most often occurs within the first year [1], [10]. Apart from additional melanoma, the probability of developing nonmelanoma tumours in melanoma patients should be noted as well [11].

It is interesting whether there is a difference in the prognosis between the patients with single and multiple primary melanomas [12]. So far, melanoma thickness, as well as some additional features, such as the presence of ulceration, mitoses and scalp location, has been used as a prognostic factor [12].

Based on the available literature from research, some conclusions have been laid down, without a unanimous opinion:

1. According to some authors, the mean Breslow's thickness of the first melanoma is significantly higher than the mean Breslow's thickness of the second primary melanoma [13].
2. No difference is observed in the presence or absence of mitoses, a marker of tumour proliferation, in SPM (Single primary melanomas) and MPM [14].
3. CDKN2A mutation status and family history of melanoma significantly affect outcomes of MPM patients, and worse outcomes have been noted in patients with multiple primary melanomas (MPMs) than in patients with single primary melanomas [15].
4. Multiple invasive lesions seem more at risk of death than melanoma [16], but according to others:
5. Thicker SPM, however, had higher fatality than thicker MPM [12], and
6. The presence of multiple primary melanomas does not appear to be a negative prognostic factor [17].

However, it is generally accepted that melanoma patients require close follow-up to detect not only metastases but also subsequent primaries in their earliest phases [17].

Spontaneous regression of malignant tumours is extremely rare, but it is still a possible phenomenon [18], [19], [20]. It may be partial or complete [21]. Regression is defined as a much more common phenomenon in cases of malignant melanoma than in other types of tumours [21]. It may typically be observed in patients with no evidence of metastases development, but also the event of metastatic melanomas [18], [20], [22]. While primary non-metastatic melanomas are thought to have a possible spontaneous regression in up to 50% of cases, the percentage of metastases is quite different, and regression is only observed in 0.23% of the cases of metastatic forms [20], [21]. Clinically, the presence of hyper- to hypo-pigmented macules, patches, papules and plaques, measuring from 0.4 to 3.0 cm in diameter, showing enlargement, friability, and bleeding, speaks in favour of emerging regression [5]. In turn, histopathological signs defining the regression as an event include atrophic epidermis, dermal aggregation of many melanophages, lymphocytic infiltrate, reactive vascular proliferation, and surrounding fibrosis [5].

Regarding the factors considered as leading to spontaneous regression, surgical trauma, infection, vaccination (BCG and rabies vaccines), immunological factors, blood transfusion and various endocrine factors are being mentioned [21]. Now there is a widespread perception that specific cell-mediated immunity against melanoma cells are involved in regression mechanisms and that mainly the lymphocytes are responsible for this tumour regression [19]. This has been investigated histologically and by immunofluorescence (direct and indirect) in the cases of Halo nevus, where it is clear

that in addition to T cell-mediated immunity (CD8 + cells outnumbered CD4 + cells), IgM antibodies against nevus cells as well as melanoma cells and cultured melanocytes may be involved in regression [23].

It is not yet clear how spontaneous immune response is induced and why, for example, in specific events of melanoma and congenital melanocytic nevus combination, regression only affects melanoma, while nevus remains undisturbed by the immunological response [24].

According to some authors, patients who develop multiple asynchronous melanomas, namely the first melanoma, produce an immunisation effect with increased immunity against certain antigens expressed by tumour-associated melanocytes [25]. According to other authors, the presence of regression may be considered a favourable prognostic factor in patients with AJCC stage I-II melanoma. However this statement is still controversial [26], [27].

The approach adopted for treatment of malignant melanoma includes surgical resection with adequate excision margins, with or without lymph node biopsy [28]. At this stage, a large proportion of dermatologists follow the recommendations of the American Joint Committee on Cancer, according to which primary melanoma surgery is based on Breslow tumour thickness and includes resection of 0.5 cm for MIS, 1.0 cm for melanomas ≤ 1.0 mm thick, 1-2 cm for melanoma thickness of 1.01-2 mm, 2 cm margins for melanoma thickness of 2.01-4 mm, and 2cm margins for melanomas > 4 mm thick [29]. Based on the established guidelines, treatment begins with resection of the melanocytic lesion with a surgical margin of 0.4-0.5 cm in all directions, followed by re-excision (as in our patient) with or without parallel drainage lymph node (depending on the established postoperative tumor thickness), which however is not individualized and often leads to ambiguity and hesitation, and hence to difficulty in choosing a therapeutic approach, as in the patient we described. [30]. The role of SUNN (sentinel lymph node biopsy) continues to be studied, and its use is currently recommended for Stage IB and Stage II melanomas [29]. In some cases, preoperative high-frequency ultrasound diagnosis helps determine the limits of surgical margins, indications for lymph node biopsy, and the need for re-excision [31].

In the event of hesitation, One step melanoma surgery (OSMS) is found to be a very useful approach, since it clearly defines the surgical safety margins in a) melanoma *in situ* resection with 1 cm surgical safety margin, b) tumours 1 to 2 mm thick resection with 1 cm surgical safety margin, and c) thickness of 2 to 4 mm – 2 cm surgical safety margin, where, in the event of b) and c), it is obligatory or highly recommended to perform, simultaneously or in parallel, detection and removal of the draining lymph node (regardless of whether the node is echographically enlarged); d) tumour thickness above 4 mm – resection with 2 cm in all directions and if there are no enlarged lymph nodes, 2 cm resection is sufficient, together with control/instrumental examinations every two months, but in the presence of enlarged lymph nodes, it is recommended that they will be removed and examined for the BRAF V600mutation, followed by computed tomography scan (CT) with contrast or PET scan. Interestingly, the abovementioned guidelines for One step melanoma surgery do not change and completely overlap with AJCC recommendations (relative to the result or efficiency score), however OSMS is a less traumatic method requiring additional preoperative preparation and judgment [32], [33], [34], [35], [36].

It is believed that in the case of melanomas with a thickness of over 4mm ELND is not of paramount importance as the draining lymph nodes may not be affected [32]. The reasons for this may be different: 1) accessory parallel lymphatic pathways available, on the one hand, and the other; 2) tumour cells may have passed the draining lymph node without stopping there, or there has already been primary haematogenous dissemination without involving the lymph nodes and pathways [32]. However, it should be taken into account that OSMS applies to all melanoma patient groups, which makes it more appropriate therapeutic endpoint [32].

One stage melanoma surgery results in a reduction of the number of steps of the so-called melanoma treatment programs by reducing the number of stages in which an error (or deviation from the guidelines) could occur [35]. Meanwhile, OSMS saves the patients repeated surgical interventions and relieved them emotionally and financially [32], [36], [37].

In conclusion, one step melanoma surgery is an approach with clearly defined surgical safety (in all melanoma patient groups) and behaviour against draining lymph nodes based on the preoperative ultrasound tumour thickness or clinical and dermatoscopic judgement (when the latter are explicitly in favour of thin melanoma diagnosed in certain patients). It is a better therapeutic solution for all cutaneous melanoma patient groups that allows for the best control of the disease within one surgical session. The case is indicative of the errors or omissions resulting from surgical treatment approaches recommended or not fully defined by AJCC. Or, if we need to be even more precise, this case is indicative of the risks that arise during a possible two-step or two-stage surgical approach.

In the One step melanoma surgery, these possibilities are virtually non-existent, they are limited to the absolute minimum by the creation of certain algorithms. Optimising surgical approaches is a personal choice for the clinician. When guidelines are not so strict and precise, they provide an unjustified field of freedom for the clinician’s actions, and this

may be confusing for the patients themselves. Thus, there is an increased possibility of inadequate and unexplained approaches or solutions (as the case we have described). This is also the reason why some dermatologists in the relevant units are increasingly using the recommendations for OSMS that limit the clinician's freedom of action but also refine his/her actions. Or reduce the possibilities for errors.

References

1. Ferrone C, Porat L, Panageas K, Berwick M, Halpern A, Patel A, Coit D. Clinicopathological features of and risk factors for multiple primary melanomas. JAMA. 2005; 294(13):1647-54. https://doi.org/10.1001/jama.294.13.1647 PMid:16204664

2. Johnson T, Hamilton T, Lowe L. Multiple primary melanomas. J Am Acad Dermatol. 1998; 39(3):422-7. https://doi.org/10.1016/S0190-9622(98)70318-4

3. Tchernev G, Ananiev J, Cardoso J, Chokoeva A, Philipov S, Penev P, Lotti T, Wollina U. Multiple primary cutaneous melanomas in patients with FAMMM syndrome and sporadic atypical mole syndrome (AMS): what's worse? Wien Med Wochenschr. 2014; 164(15-16):302-7. https://doi.org/10.1007/s10354-014-0295-8 PMid:25096163

4. Fölster-Holst R, Schubert C, Christophers E. [Multiple melanoma in xeroderma pigmentosum]. Hautarzt. 1994; 45(8):554-61. https://doi.org/10.1007/s001050050126 PMid:7960757

5. Yamada S, Nawata A, Yoshioka M, Hiraki T, Higashi M, Hatanaka K, Tanimoto A. Complete regression of primary cutaneous malignant melanoma associated with distant lymph node metastasis: a teaching case mimicking blue nevus. BMC Res Notes. 2016; 9:366. https://doi.org/10.1186/s13104-016-2174-4 PMid:27456492 PMCid:PMC4960676

6. Slingluff C, Vollmer R, Seigler H. Multiple primary melanoma: incidence and risk factors in 283 patients. Surgery. 1993; 113(3):330-9. PMid:8441968

7. Cheng S, Nambi R. Simultaneous occurrence of five primary melanomas and six melanomas in situ in a patient. Clin Exp Dermatol. 2012; 37(2):210-1. https://doi.org/10.1111/j.1365-2230.2011.04171.x PMid:22340700

8. Remy W, Bockendahl H. [Simultaneous occurrence of two primary melanomas in a patient]. Hautarzt. 1975; 26(6):327-9. PMid:1165196

9. Savoia P, Quaglino P, Verrone A, Bernengo M. Multiple primary melanomas: analysis of 49 cases. Melanoma Res. 1998; 8(4):361-6. https://doi.org/10.1097/00008390-199808000-00010 PMid:9764812

10. Moore M, Geller A, Warton E, Schwalbe J, Asgari M. Multiple primary melanomas among 16,570 patients with melanoma diagnosed at Kaiser Permanente Northern California, 1996 to 2011. J Am Acad Dermatol. 2015; 73(4):630-6. https://doi.org/10.1016/j.jaad.2015.06.059 PMid:26298295

11. Bower M, Scoggins C, Martin R, Mays M, Edwards M, Reintgen D, Ross M, Urist M, Noyes R, Sussman J, Hagendoorn L, Stromberg A, McMasters K. Second primary melanomas: incidence and outcome. Am Surg. 2010; 76(7):675-81. PMid:20698369

12. Kricker A, Armstrong B, Goumas C, Thomas N, From L, Busam K, Kanetsky P, Gallagher R, Marrett L, Groben P, Gruber S, Anton-Culver H, Rosso S, Dwyer T, Berwick M; GEM Study Group. Survival for patients with single and multiple primary melanomas: the genes, environment, and melanoma study. JAMA Dermatol. 2013; 149(8):921-7. https://doi.org/10.1001/jamadermatol.2013.4581 PMid:23784017 PMCid:PMC3815536

13. Buljan M, Situm M, Bolanca Z, Zivković MV, Mihić L. Multiple primary melanoma: epidemiological and prognostic implications; analysis of 36 cases. Coll Antropol. 2010; 34(Suppl 2):131-4. PMid:21302712

14. Hwa C, Price L, Belitskaya-Levy I, Ma M, Shapiro R, Berman R, Kamino H, Darvishian F, Osman I, Stein J. Single versus multiple primary melanomas: old questions and new answers. Cancer. 2012; 118(17):4184-92. https://doi.org/10.1002/cncr.27407 PMid:22246969

15. Helgadottir H, Tuominen R, Olsson H, Hansson J, Höiom V. Cancer risks and survival in patients with multiple primary melanomas: Association with family history of melanoma and germline CDKN2A mutation status. J Am Acad Dermatol. 2017; 77(5):893-901. https://doi.org/10.1016/j.jaad.2017.05.050 PMid:28818438

16. Rowe C, Law M, Palmer J, MacGregor S, Hayward N, Khosrotehrani K. Survival outcomes in patients with multiple primary melanomas. J Eur Acad Dermatol Venereol. 2015; 29(11):2120-7. https://doi.org/10.1111/jdv.13144 PMid:25864459

17. Savoia P, Quaglino P, Verrone A, Bernengo M. Multiple primary melanomas: analysis of 49 cases. Melanoma Res. 1998; 8(4):361-6. https://doi.org/10.1097/00008390-199808000-00010 PMid:9764812

18. Martín J, Pinazo I, Monteagudo C, Markovic J, Allende A, Jordá E. Spontaneous regression of multiple melanocytic nevi after melanoma: report of 3 cases. Am J Dermatopathol. 2014; 36(11):e183-8. https://doi.org/10.1097/DAD.0000000000000033 PMid:25343215

19. Micksche M, Cerni C, Gebhart W, Kokoschka E. [Halo Nevus (Morbus Sutton): model of an immunological tumor regression]. Osterr Z Onkol. 1975; 2(2-3):73-81. PMid:1243163

20. Schlabe J, Shah KA, Sheerin F, Payne MJ, Fasanmade AA. Complete spontaneous regression of a metastatic melanoma of the mandible: a case report and follow-up recommendations. International journal of oral and maxillofacial surgery. 2018; 47(12):1519-22. https://doi.org/10.1016/j.ijom.2018.06.007 PMid:29970290

21. Cervinkova M, Kucerova P, Cizkova J. Spontaneous regression of malignant melanoma - is it based on the interplay between host immune system and melanoma antigens?. Anticancer Drugs. 2017; 28(8):819-830. https://doi.org/10.1097/CAD.0000000000000526 PMid:28609309

22. Kappauf H, Esser G. Metachronous Spontaneous Remission of Melanoma Lung Metastasis and Mediastinal Lymph Node Metastases. Oncol Res Treat. 2018; 41(3):135-138. https://doi.org/10.1159/000485626 PMid:29485417

23. Tokura Y, Yamanaka K, Wakita H, Kurokawa S, Horiguchi D, Usui A, Sayama S, Takigawa M. Halo congenital nevus undergoing spontaneous regression. Involvement of T-cell immunity in involution and presence of circulating anti-nevus cell IgM antibodies. Arch Dermatol. 1994; 130(8):1036-41. https://doi.org/10.1001/archderm.1994.01690080102015 PMid:8053701

24. Mărgăritescu I, Chiriţă A, Vasilescu F. Completely regressed primary cutaneous melanoma - difficulties in diagnosis and classification. Rom J Morphol Embryol. 2014; 55(2 Suppl):635-42. PMid:25178337

25. Martín J, Pinazo I, Mateo J, Escandell I, Jordá E, Monteagudo C. Assessment of regression in successive primary melanomas. Actas Dermosifiliogr. 2014; 105(8):768-73. https://doi.org/10.1016/j.ad.2014.01.006 PMid:24880710

26. Ribero S, Osella-Abate S, Sanlorenzo M, Savoia P, Astrua C, Cavaliere G, Tomasini C, Senetta R, Macripò G, Bernengo M, Quaglino P. Favourable prognostic role of regression of primary melanoma in AJCC stage I-II patients. Br J Dermatol. 2013; 169(6):1240-5. https://doi.org/10.1111/bjd.12586 PMid:23952011

27. Fontaine D, Parkhill W, Greer W, Walsh N. Partial regression of primary cutaneous melanoma: is there an association with sub-clinical sentinel lymph node metastasis?. Am J Dermatopathol. 2003; 25(5):371-6. https://doi.org/10.1097/00000372-200310000-

00002 PMid:14501285

28. Dabek R, Baletic N, McUmber H, Nahed B, Haynes A, Eberlin K, Bojovica B. Development of a primary melanoma in situ within a full-thickness skin graft overlying a free muscle flap: a case report. Case Reports Plast Surg Hand Surg. 2018; 5(1):23–26. https://doi.org/10.1080/23320885.2018.1452615 PMid:29707609 PMCid:PMC5917313

29. Leilabadi S, Chen A, Tsai S, Soundararajan V, Silberman H, Wong A. Update and Review on the Surgical Management of Primary Cutaneous Melanoma. Healthcare (Basel). 2014; 2(2):234-49. https://doi.org/10.3390/healthcare2020234 PMid:27429273 PMCid:PMC4934469

30. Tchernev G, Chernin S, Lozev I, Lotti T, Stavrov K, Temelkova I, Pidakev I. Innovative One Step Melanoma Surgical Approach (OSMS): Not a Myth-It's a Reality! Case Related Analysis of a Patient with a Perfect Clinical Outcome Reported from the Bulgarian Society for Dermatologic Surgery (BULSDS)! Open Access Maced J Med Sci. 2018; 6(4):673-674. https://doi.org/10.3889/oamjms.2018.194 PMid:29731939 PMCid:PMC5927502

31. Fernández I, de Troya M, Fúnez R, Rivas F, Blanco G, Blázquez N. Preoperative 15-MHz ultrasound assessment of tumor thickness in malignant melanoma. Actas Dermosifiliogr. 2013; 104(3):227-31. https://doi.org/10.1016/j.adengl.2012.06.025

32. Tchernev G, Temelkova I, Stavrov K. One Step Melanoma Surgery (OSMS) Without Using Ultrasonography for Preoperative Tumour Thickness Measurement? - "A Question that Sometimes Drives Me Hazy: Am I or Are the Others Crazy!" Open Access Maced J Med Sci. 2018; 6(6):1085-1090. https://doi.org/10.3889/oamjms.2018.236 PMid:29983807 PMCid:PMC6026427

33. Balch M, Urist M, Karakousis P, Smith J, Temple J, Drzewiecki K, Jewell R, Bartolucci A, Mihm Jr, Barnhill R. Efficacy of 2-cm surgical margins for intermediate-thickness melanomas (1 to 4 mm). Results of a multi-institutional randomized surgical trial. Ann Surg. 1993; 218(3):262-7. https://doi.org/10.1097/00000658-199309000-00005 PMid:8373269 PMCid:PMC1242959

34. Balch M, Soong J, Bartolucci A, Urist M, Karakousis P, Smith J, Temple J, Ross I, Jewell R, Mihm C, Barnhill L, Wanebo J. Efficacy of an elective regional lymph node dissection of 1 to 4 mm thick melanomas for patients 60 years of age and younger. Ann Surg. 1996; 224(3):255-63. https://doi.org/10.1097/00000658-199609000-00002 PMid:8813254 PMCid:PMC1235362

35. Tchernev G. One Step Melanoma Surgery for Patient with Thick Primary Melanomas: "To Break the Rules, You Must First Master Them!" Open Access Maced J Med Sci. 2018; 6(2):367–371. https://doi.org/10.3889/oamjms.2018.084 PMid:29531606 PMCid:PMC5839450

36. Tchernev G. One Step Surgery for Cutaneous Melanoma: "We Cannot Solve Our Problems with the Same Thinking We Used When We Created Them?" Open Access Maced J Med Sci. 2017; 5(6):774–776. https://doi.org/10.3889/oamjms.2017.168

37. Tchernev G, Chokoeva A. New Safety Margins for Melanoma Surgery: Nice Possibility for Drinking of "Just That Cup of Coffee"? Open Access Maced J Med Sci. 2017; 5(3):352-358. https://doi.org/10.3889/oamjms.2017.068

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Case Report

A Successful Tracheal Resection and Anastomosis in Papillary Thyroid Carcinoma with Tracheal Invasion

I Gede Budhi Setiawan, Putu Anda Tusta Adiputra*

Surgical Oncology Division, Department of Surgery, Faculty of Medicine Udayana University, Sanglah General Hospital, Denpasar, Bali, Indonesia

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Keywords: Thyroid cancer; Well-differentiated thyroid carcinoma (DTC); Tracheal invasion

***Correspondence:** Putu Anda Tusta Adiputra. Surgical Oncology Division, Department of Surgery, Faculty of Medicine Udayana University, Sanglah General Hospital, Denpasar, Bali, Indonesia. E-mail: andatusta@unud.ac.id

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Abstract

BACKGROUND: Well-differentiated thyroid carcinoma (DTC) can be locally aggressive, invading aerodigestive tract. The rationale for aggressive surgical resection in this clinical setting is supported by a long-term local control with a positive impact on survival.

CASE REPORT: A 60-year-old male patient was consulted by a digestive surgeon of unaware thyroid enlargement. Physical and imaging examination showed a suspect of thyroid malignancy. During surgery, we found that a tumour had invaded the anterior side of the trachea. Resection of three tracheal rings was performed, with end-to-end anastomosis. Surgical outcome regarding nervous preservation and parathyroid glands was good as well as cosmetic aspect. During one-year follow-up, no indication of tumour recurrence was found. The management of locally invasive DTC has been controversial yielding the palliative surgery modalities. Advances in surgical technique have given a new perspective of resection in a difficult case. This case report was managed by sleeve resection with end-to-end anastomosis which showed a satisfactory outcome functionally and cosmetically.

CONCLUSION: Sleeve resection with primary reconstruction of the trachea is a simple one-stage procedure which can adequately address the problem of tracheal invasion by thyroid cancer.

Introduction

A well-differentiated thyroid carcinoma can be locally aggressive, invading the aerodigestive tract, such as larynx, trachea, and or oesophagus [1]. In the case where thyroid cancer invaded the trachea, wide resection is needed to achieve minimal residual tumour [2]. In tracheal resection and reconstruction, removal of the invaded airway segment should always be attempted whenever feasible [3].

This case report presented a favourable outcome of a management case of thyroid cancer invading the tracheal with radical resection and end-to-end anastomosis.

Case Report

A 60-year-old male patient was consulted by a digestive surgeon of unware thyroid enlargement. He denied any symptoms of hemoptysis, cough, neck discomfort, nervousness, weight loss, palpitation, or fatigue. He was previously admitted to the digestive department due to cholelithiasis. On neck examination, we palpated a well-defined multinodular painless 5 x 7 cm solid mass. The mass was found to be mobile on swallowing. There was no evidence of cervical lymph nodes enlargement. There was no other remarkable clinical finding.

Cervical x-ray showed inferolateral tracheal deviation to the left side at the level of cervical VII (Figure 1). Ultrasonography showed a large solid mass on the right lobe up to isthmus that caused the tracheal deviation, suspicious of a benign lesion. No

metastases lesion was found on chest x-ray examination. Laboratory examination revealed a normal level of thyroid hormone. FNA biopsy showed a follicular neoplasm of the thyroid.

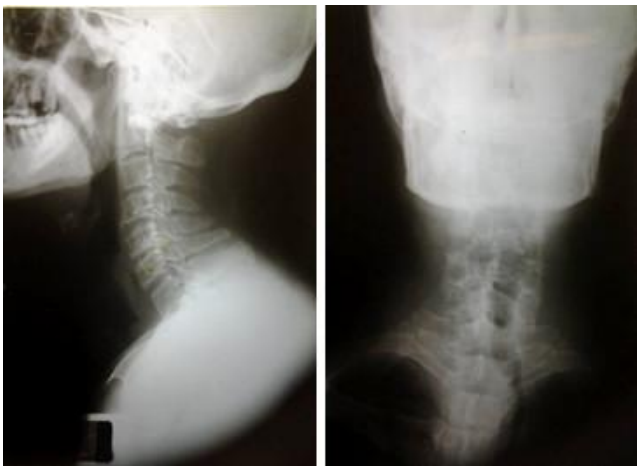

Figure 1: Cervical x-ray showed inferolateral tracheal deviation

Laparoscopic cholecystectomy was performed, followed by total thyroidectomy. During intubation, the orotracheal tube was inserted easily by the anesthesiologist. During surgery, we found that a tumour had invaded the anterior side of the trachea (Figure 2). Tracheostomy below the tumour level has been decided during the surgery, and a sterile tube was inserted into tracheostomy to replace the orotracheal tube.

Intraoperative endoscopy showed invasion of a tumour up to anterior part of the trachea, but the vocal cord was normal. Resection of three tracheal rings (Figure 3) was performed, with end-to-end anastomosis (Figure 4). Bilaterally the recurrent laryngeal nerves were monitored and preserved. Before completing anastomosis, a new orotracheal tube was reinserted to replace the tracheal tube. During neck exploration, we did not find enlarged lymph nodes.

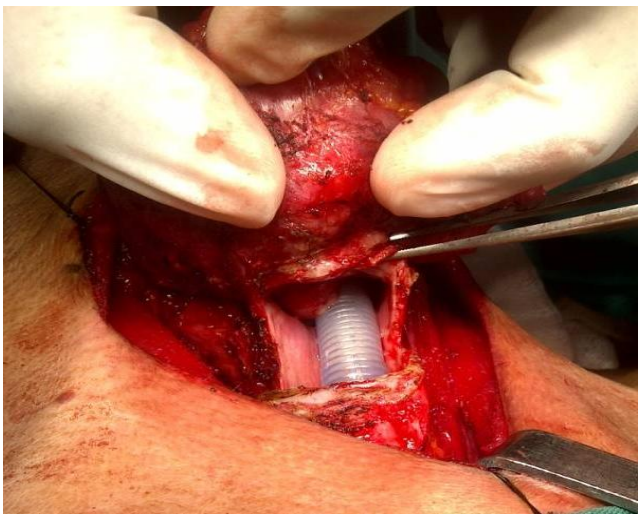

Figure 2: Tumor had invaded the anterior side of the trachea

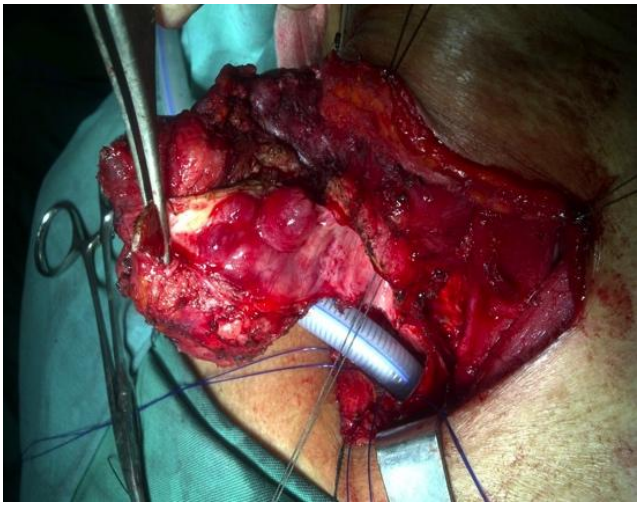

Figure 3: Resection of three tracheal rings

Pathology report revealed papillary thyroid carcinoma with tracheal invasion, with a clear margin of the edge of tracheal resection. After resection, the patient was positioned with a fully flexed head (chin to chest position) for about 3 weeks, the orotracheal tube placed for 4 days as the stent, and a nasogastric tube placed for 7 days for feeding. The postoperative condition was uneventful, and the patient recovered satisfactorily.

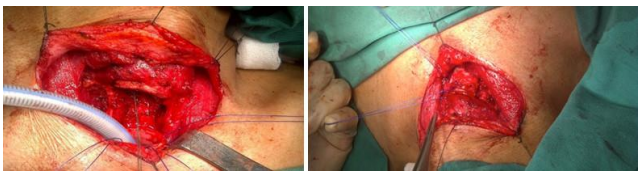

Figure 4: Sleeve resection with end-to-end anastomosis

Surgical outcome regarding preservation of nervous and parathyroid glands as well as the cosmetic aspect was good (Figure 5). During 3-year follow-up, evidence of tumour recurrence and complication was not found (Figure 6).

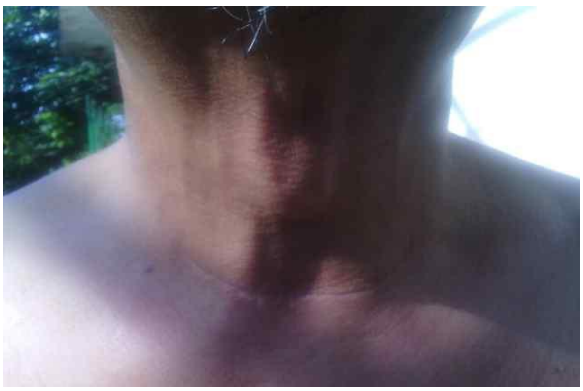

Figure 5: Surgical outcome cosmetically was good

Discussion

Papillary thyroid carcinoma represented the most common type of thyroid cancer (83%) [4]. Mutations occurred in papillary thyroid carcinoma were BRAF/RAS, RET/PTC, PIK3CA, TP53, TSHR, PTEN, GNAS, and CTNNB1 [5]. The most aggressive behaviours were gene mutation of BRAF V600E, a part of MAP kinase cascade, which promotes proto-oncogenes [6]. Besides that, it also caused dysfunction of iodine absorption of follicular cells, blunted the radioiodine therapy [7].

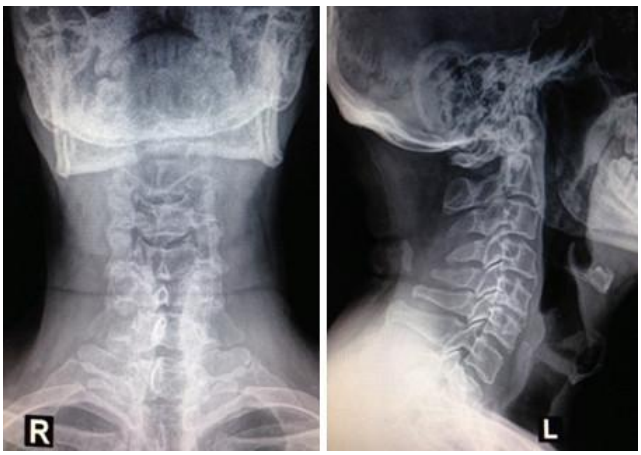

Figure 6: Cervical X-Ray in 3-year follow up

Around 1-8% patients will overcome the aerodigestive tract invasion [8]. From McCaffrey et al., [9] study, it was shown that the most commonly involved aerodigestive were the strap neck muscles, recurrent laryngeal nerve, rings of trachea, oesophagus, lateral neck structures, and larynx. The frequency of thyroid cancer involving tracheal was not well documented in Sanglah General Hospital was not well recorded. Not surprising, patients with thyroid cancer extending to trachea often had bad results due to airway obstruction [10]. This condition dropped 45% of ten years survival in thyroid cancer [1].

Thyroid malignancy was shown to invade directly to the thyroid cartilage. In this case report, the invasion was found to the anterior side. This can occur through invasion from isthmus or paratracheal lymph nodes, until extracapsular extension [11]. The invasion of trachea occurred gradually from outer layer to inner layer [12].

This case report was categorized in stage IV according to Shin et al., [13] classification for thyroid cancer involving aerodigestive tract and recommended a complete resection. Pappalardo et al., [14] reported that the survival for stage IV thyroid cancer with aerodigestive tract involvement ranged from 18 to 108 months. However, by MACIS score calculation, this case has = (0.08 x age) + (0.3 x tumor size in cm) + 1 (if incomplete excision) + 1 (if locally invasive) + 3 (if distant metastases) = (0.08 x 60) + (0.3 x 7) = 4.8 + 2.1 = 6.9, thus the survival was 89% [15].

Generally, the resection surgery for thyroid cancer aims to reach the complete excision and control the invasion [16]. The best goal is to preserve all the thyroid health structures [17]. However, the survival rate was only 15-39% with 1.2% mortality, worse that incomplete resection [14], [15]. The most common surgical complications were airway obstruction, infection, bleeding, and anastomotic dehiscence [18]. This made the surgery for locally-invasive DTC has been controversial. Thus, palliative surgery had been the most common surgical procedure or treatment for many years. Nowadays, advances in surgical techniques have improved the radicality of surgery, which excellent preservation of vocal cord function and survival satisfactory [19].

Various methods have been carried out by surgeons for reaching the best outcome for the patient. The there most common procedure was two stages surgery for thyroid carcinoma invading the trachea [20]. First was resection of a tumour and second was reconstruction closure of the tracheal defect using cartilage graft and musculocutaneous or latissimus dorsi flap [21]. However, reconstruction of the tracheal wall is challenging due to unstable tracheal lumen [22]. Another method for simpler cases was segmental resection of the tracheal and end to end anastomosis. If the case were too advanced, total laryngectomy and permanent tracheotomy would likely to be done [23].

Pappalardo et al., [14] reported a successful radical resection of extrathyroidal thyroid cancer which invaded the tracheal lumens until the blood vessels with 9 years of satisfactory survival and functionality. Shigemitsu et al., [22] reported a successful case also with a partial tracheal resection with latissimus dorsi musculocutaneous flap reconstruction. Endo et al., [23] reported that a single staged thyroidectomy with auricular cartilage and sternohyoid reconstruction successfully cured the patient [24]. In Tsai et al., [24] study, thyroid and en bloc tracheal resection with end to end anastomosis is the treatment of choice of a thyroid tumour invading the trachea. This method showed the highest survival rate, reported to be 78%.

Patients with inoperable thyroid cancers are usually treated by RAI (radioactive iodine) therapy and external beam radiation therapy [25]. The surgery method needs to be tailored to each in an individual with three main principles, such as to accomplish the possible complete resection, prevent airway obstruction, and prolonged the survival. An incomplete tumour resection has a bad outcome and increases the risk of recurrent. Tangential tumour resection (shaving) is indicated if no invasion in transmural of the trachea.

Due to the anterior invasion of the tumor, we decided to carry out resection of three tracheal rings. End to end anastomosis was performed to ensure the

intact vascularity around. Bilaterally the recurrent laryngeal nerves were carefully monitored and preserved. Surgical outcome regarding preservation of nervous and parathyroid glands as well as cosmetic aspect was good. This case report will add information of another resection with end-to-end anastomosis with a favourable outcome.

The successful management of well-differentiated thyroid cancer requires meticulous preparation, because of the vitality of the surrounding structures. The goal of resection was to achieve the larger extension as possible with restoration of functionality for the patient.

In conclusion, sleeve resection with primary reconstruction of the trachea is a simple one-stage procedure which could adequately address the problem of tracheal invasion by thyroid cancer.

References

1. Price DL, Wong RJ, Randolph GW. Invasive thyroid cancer: management of the trachea and esophagus. Otolaryngol Clin North Am. 2008; 1155-8. https://doi.org/10.1016/j.otc.2008.08.002 PMid:19040976 PMCid:PMC2750808

2. Grillo HC, Zannini P. Resectional management of airway invasion by thyroid carcinoma. Ann Thorac Surg. 1986; 42:287-98. https://doi.org/10.1016/S0003-4975(10)62737-3

3. Yang CC, Lee CH, Wang LS. Resectional treatment for thyroid cancer with tracheal invasion: A long-term follow-up study. JAMA Surgery. 2000; 135:704-7.

4. Tuttle RM, Ball DW, Byrd D. Thyroid carcinoma. J Natl Compr Canc Netw. 2010; 8:1228-74. https://doi.org/10.6004/jnccn.2010.0093 PMid:21081783

5. Nikiforova MN, Wald AI, Roy S, Durso MB, Nikiforov YE. Targeted next-generation sequencing panel (ThyroSeq) for detection of mutations in thyroid cancer. J Clin Endocrinol Metab. 2013; 98:1852-60. https://doi.org/10.1210/jc.2013-2292 PMid:23979959 PMCid:PMC3816258

6. Xing M, Westra WH, Tufano RP. BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer. J Clin Endocrinol Metabol. 2005; 90:6373-9. https://doi.org/10.1210/jc.2005-0987 PMid:16174717

7. Ito Y, Yoshida H, Kihara M, Kobayashi K, Miya A, Miyauchi A. BRAF mutation analysis in papillary thyroid carcinoma: is it useful for all patients? World J Surg. 2013; 38:679-87. https://doi.org/10.1007/s00268-013-2223-2 PMid:24052184

8. Brauckhoff M, Dralle H. Extrathyroidal thyroid cancer: results of tracheal shaving and tracheal resection. Chirurg. 2011; 82:134-40. https://doi.org/10.1007/s00104-010-1975-6 PMid:21153528

9. McCaffrey TV, Bergstralh EJ, Hay ID. Locally invasive papillary thyroid carcinoma: 1940-1990. Head Neck. 1994; 16:165-72. https://doi.org/10.1002/hed.2880160211 PMid:8021137

10. Pearson FG, Cooper JD, Nelems JM, Van Nostrand AWP. Primary tracheal anastomosis after resection of the cricoid cartilage with preservation of recurrent laryngeal nerves. J Thorac Cardiovasc Surg. 1975; 70:806-16. PMid:1186272

11. Urken ML. Prognosis and management of invasive well-differentiated thyroid cancer. Otolaryngol Clin N Am. 2010; 43:301-28. https://doi.org/10.1016/j.otc.2010.02.002 PMid:20510716

12. Kim H, Jung HJ, Lee SY, Kwon TK, Kim KH, Sung MW, et al. Prognostic factors of locally invasive well-differentiated thyroid carcinoma involving the trachea. Eur Arch Otorhinolaryngol. 2016; 273:1919-26. https://doi.org/10.1007/s00405-015-3724-4 PMid:26198285

13. Shin DH, Mark EJ, Suen CSH, Grillo HC. Pathologic staging of papillary carcinoma of the thyroid with airway invasion based on the anatomic manner of extension to the trachea: a clinicopathologic study based on 22 patients who underwent thyroidectomy and airway resection. Human Pathology. 1993; 24:866-70. https://doi.org/10.1016/0046-8177(93)90136-5

14. Pappalardo V, La Rosa S, Imperatori A, Rotolo N, Tanda ML, Sessa A, et al. Thyroid cancer with tracheal invasion: a pathological estimation. Gland Surg. 2016; 5:541-5. https://doi.org/10.21037/gs.2016.10.02 PMid:27867870 PMCid:PMC5106376

15. McCaffrey JC. Aerodigestive tract invasion by well-differentiated thyroid carcinoma: diagnosis, management, prognosis, and biology. Laryngoscope. 2006; 116:1-11. https://doi.org/10.1097/01.MLG.0000200428.26975.86 PMid:16481800

16. Rotoko N, Cattoni M, Imperatori A. Complications from tracheal resection for thyroid carcinoma. Gland Surg. 2017; 6:574-8. https://doi.org/10.21037/gs.2017.08.05 PMid:29142850 PMCid:PMC5676159

17. Shenoy AM, Burrah R, Rao V, Chavan P. Tracheal resection for thyroid cancer. J Laryngol Otol. 2012; 126:594-7. https://doi.org/10.1017/S002221511200059X PMid:22494608

18. Piazza C, Del Bon F, Barbieri D, Grazioli P, Paderno A, Perotti P, et al. Tracheal and Crico-Tracheal Resection and Anastomosis for Malignancies Involving the Thyroid Gland and the Airway. Ann Otol Rhinol Laryngol. 2016; 125:97104-6. https://doi.org/10.1177/0003489415599000 PMid:26296930

19. Chernichenko N, Shaha AR. Role of tracheal resection in thyroid cancer. Curr Opin Oncol. 2012; 24:29-34. https://doi.org/10.1097/CCO.0b013e32834d6dd7 PMid:22048058

20. Ebihara M, Kishimoto S, Hayashi R, Miyazaki M, Shinozaki T, Daiko H, et al. Window Resection of the Trachea and Secondary Reconstruction for Invasion by Differentiated Thyroid Carcinoma. Auris Nasus Larynx. 2011; 38:271-5. https://doi.org/10.1016/j.anl.2010.09.003 PMid:21093183

21. Nakahira M, Nakatani H, Takeuchi S, Higashiyama K, Fukushima K. Safe Reconstruction of a Large Cervico-Mediastinal Tracheal Defect with a Pectoralis Major Myocutaneous Flap and Free Costal Cartilage Grafts. Auris Nasus Larynx. 2006; 33:203-6. https://doi.org/10.1016/j.anl.2005.09.009 PMid:16289423

22. Shigemitsu K. Naomoto Y, Haisa M, Yamatsuji T, Noguchi H, Kataoka M, et al. Case of Thyroid Cancer Involving the Trachea: Treatment by Partial Tracheal Resection and Repair with a Latissimus Dorsi Musculocutaneous Flap. Jpn J Clin Oncol. 2000; 30:235-8. https://doi.org/10.1093/jjco/hyd053 PMid:10857502

23. Endo K, Ueno T, Kondo S, Wakisaka N, Murono S, Yoshizaki T. Successful Treatment of Thyroid Carcinoma Invading the Trachea as a Single-Stage Procedure: A Case Report. Case Reports in Clinical Medicine. 2015; 4:50-4. https://doi.org/10.4236/crcm.2015.42012

24. Tsai YF, Tseng YL, Wu MH. Aggressive resection of the airway invaded by thyroid carcinoma. Br J Surg. 2005; 92:13827-9. https://doi.org/10.1002/bjs.5124 PMid:16044411

25. Honings J, Stephen AE, Marres HA, Gaissert HA. The role of oral RAI in airway invasion has not been investigated separately. The management of thyroid carcinoma invading the larynx or trachea. Laryngoscope. 2010; 120:682-9. https://doi.org/10.1002/lary.20800 PMid:20213659

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Case Report

Retained Surgical Items in Inguinal Canal: A Case Report and Literature Review

Amer Hashim Al Ani[1], Mohammad Bakri Hammami[2*], Obaidah M. Mukhles Adi[2]

[1]Department of General Surgery, Sheikh Khalifa Medical City, Ajman, United Arab Emirates; [2]College of Medicine, University of Sharjah, Sharjah, United Arab Emirates

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Keywords: Retained surgical items; Inguinal canal; Foreign bodies; Case report; Retained Gauze; Surgical complications

***Correspondence:** Mohammad Bakri Hammami. College of Medicine, University of Sharjah, Sharjah, United Arab Emirates. E-mail: dr.mohammadbakri@outlook.com

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Abstract

BACKGROUND: Retained surgical items (RSI) are rare medical challenges with serious complications and medicolegal implications. Knowledge and preventive measures for these rare events are currently not sufficient to limit their increasing incidence. Gauzes and sponges constitute most of RSI. Forceps, needles and pins may be found too. Diagnosis of these events is challenging and often missed due to nonspecific clinical findings.

PRESENTATION OF CASE: We present here a 49-year-old patient who presented to the clinic with a history of chronic scrotal sinus on the same side of a repeatedly repaired inguinal hernia 4 months before admission. He underwent exploration of the inguinal canal as elective surgery. Exploration of the inguinal canal revealed missed surgical gauze left during the previous hernia repair. The gauze was removed, and the inguinal canal was repaired. The postoperative period was uncomplicated.

CONCLUSION: Retained surgical items are completely preventable near-events. Although they are rare entities, clinicians must have a high index of suspicion for any postoperative, in patients presenting with pain, sinus or palpable masses.

Introduction

Retained surgical items (RSI) are a medical challenge, not only because of the severe complications and morbidity they can cause but also because of their serious medicolegal implications. RSI is grossly underreported by surgeons, although they are reportable events. This is because of a surgeon's fear of legal issues or unwillingness to denounce an error [1]. The average costs to repair and remove RSI can range anywhere from 60000$ per hospital stay to millions of dollars after settle malpractice claims [2] [3]. It's estimated that the incidence of retained foreign bodies is 0.3 to 1 per 1000 abdominal operation, and 1 in 8000 to 18000 of all inpatient operations, that's one or more cases per year in a big hospital [4]. The commonest RSI are surgical sponges and gauzes (termed "Gossypiboma"), but also needles, scissors, forceps and other objects were reported in the literature [1], [5]. Retained instruments that are kept under aseptic conditions with minimal reaction can be retained for years before they produce significant symptoms and reactions that lead to their discovery [6], [7]. There're principally 2 main types of reaction that cause complications in those patients; an aseptic fibrins response that results in adhesions, pseudotumor effect, intestinal obstruction and granulomas. Another type is an exudative response giving rise to an abscess formation that will result in peritonitis, fistula formation abdominal mass and gut perforation [1], [6] [8]. It's evident that to decrease the incidence of RSI, the focus should be directed towards 3 major issues, locating missing items after the incorrect count, improving team compliance and attentiveness, and reducing the risk of false-correct surgical counts [4].

In this prospective, single Centre, case report study, we present an interesting case of a retained surgical item in the inguinal canal. The case was managed in Al Bashir teaching hospital in Amman, Jordan in 2010. The patient was followed up in the same hospital.

Case Presentation

A 49-year old male patient presented to the ER with signs and symptoms of intestinal obstruction. The patient has a previous history of an open-heart surgery 4 years before admission, chronic renal failure on dialysis 2 years ago, and a right inguinal hernia that was repaired 13 years ago, recurred one year before the presentation and was repaired again. Six months after the last inguinal repair, an inguinal sinus discharge was noted on the right inguinal region.

Examination showed a stable patient with normal vital signs. Two sinuses in the right scrotum were noted, and a mesh-related sinus infection at the surgical site was suspected. Lab workout showed a normal WBC count (6.3 x 10^3/mm^3), elevated serum creatinine (718 µmol/L), elevated BUN (14.6 mmol/L) and mild anaemia (11.2 g/DL). Other labs were within normal. CT scan with contrast wasn't performed due to elevated BUN and creatinine in this patient (Figure 1).

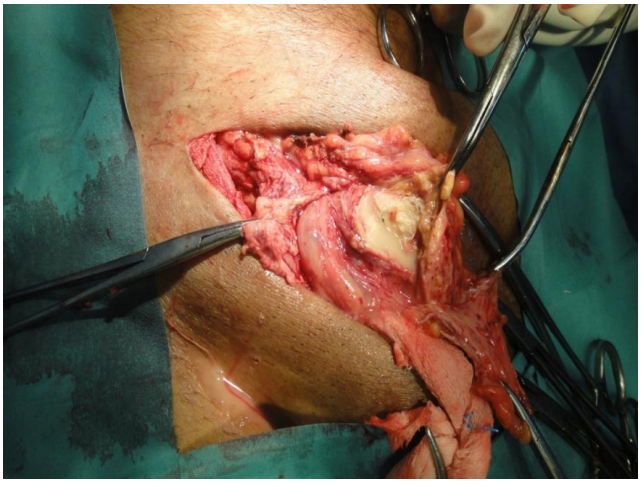

Figure 1: Open herniotomy reveals retained gauze with signs of inflammation and infection

Consent was obtained, and emergent exploration of the inguinal canal was done. Surgery confirmed the presence of a surgical gauze from a previous repair that was removed successfully (Figure 2). Postoperative period was smooth. The patient did well during follow up for one year.

Discussion

Retained surgical items -although highly underreported- is a dangerous medical error that carries significant morbidity and mortality, and therefore must be tackled seriously. Surgical gauze (Gossypiboma) constitutes most of the retained items due to its small size and amorphous structure, as in our first case. When soaked with blood, gauzes lose their original shape and colour and become unrecognisable in the surgical field and hard to find. [10]. Most Gossybipoma incidents happened in abdominal and pelvic surgeries [2], as in our case, where deepness of the region and folds of viscus can hide blood-soaked gauzes and make them harder to find. Other cavities include the vagina and the thorax among other places [11]. Other retained items include artery forceps, irrigation sets, rubber tubes and pieces of broken instruments [12].

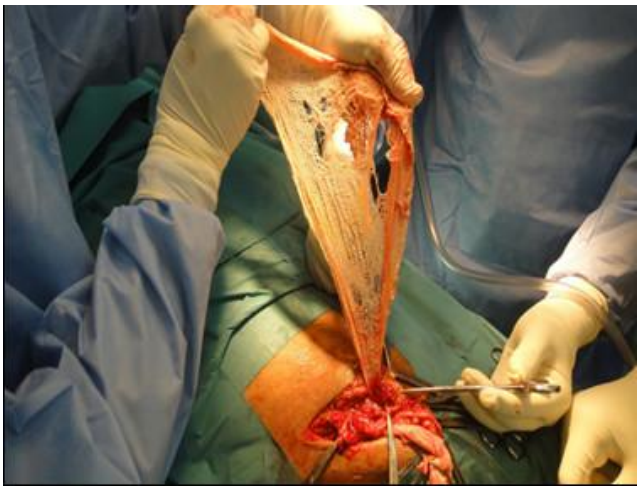

Figure 2: Removal of gauze during open herniotomy

Age and sex of the patient were not significantly related to the risk of retaining surgical items. Factors that appear to increase the risk of RSI significantly includes longer surgery durations, emergency surgeries, intraoperative complications and unexpected events [4], [11]. A recent meta-analysis study was done by Susan et al., (2014) [13] showed that additional surgical events increase the risk of RSI including incorrect\not performed the surgical count, intraoperative blood loss > 500 and more than one surgical team involved in the procedure. These events were found to increase the risk of miscommunication among different teams and error during safety checks of the patient. There were mixed opinions regarding the role of BMI of the patient [2], [11], [14], [15], [16]. However, the most recent meta-analysis studies concluded a significant direct relationship between the risk of RSI and BMI of patients. [3] In our case, we couldn't retrieve the history of the previous operations which were done in other hospitals. Moreover, our patient had normal BMI (below 30).

Presentation of retained surgical items can range from day of surgery to 28 years later, with a median date of detection at 21st day after surgery [11]. In our case, the patient presented after 1 year from the original operation. A study was done by Stawicki (2009) [6] revealed that the most common presenting complaints include abdominal pain (25.8%), abscess (21.2%), Nausea and vomiting (15.2%), wound

complication (15.2%) and masses (12.1%). In our case, the patient presented with persistent abdominal pain, intestinal obstruction, nausea and vomiting.

Diagnosis of RTI is difficult because of low clinical suspicion and since most surgical gauzes are radiolucent on imaging [10]. Previous literature emphasised the importance of using a CT scan with IV contrast for diagnosis of retained surgical items [7] [17]. This modality should be done routinely for patients with intestinal obstruction and surgeons must put RSI on their differential list in a patient with a history of previous operations. In our case, the patient didn't undergo CT scan with IV contrast due to his elevated BUN and creatinine. G Nasir (2008) [18] suggested the use of gauzes and packs that's marked by radiopaque lines to detect missing instruments. A similar study was done by Fabian (2004) [19] experimented the use of electronic tagging of surgical sponges to prevent their retention. The results showed 100% accuracy with no false positives at all. This implies the efficacy of alternative options to solve the problem of invisibility of surgical gauzes on radio imaging.

In conclusion, retained surgical items continue to be a significant challenge for surgeons due to the serious complications if they are discovered late. Those preventable mistakes place a big burden on the health system financially and logistically. However, with good teamwork and an accurate modern counting system, these can easily be prevented. Surgeons must have a high index of suspicion and retained surgical items should be in the differential diagnosis of any postoperative patient who presents with pain, infection, or palpable masses.

References

1. Ugochukwu AI, Edeh AJ. Retained intra-abdominal artery forceps–An unusual cause of intestinal strangulation. North American journal of medical sciences. 2011; 3(7):339. https://doi.org/10.4297/najms.2011.3339 PMid:22540110 PMCid:PMC3336885

2. Elsharydah A, Warmack KO, Minhajuddin A, Moffatt-Bruce SD. Retained surgical items after abdominal and pelvic surgery: Incidence, trend and predictors-observational study. Annals of medicine and surgery. 2016; 12:60-4. https://doi.org/10.1016/j.amsu.2016.11.006 PMid:27895909 PMCid:PMC5121141

3. Hempel S, Maggard-Gibbons M, Nguyen DK, Dawes AJ, Miake-Lye I, Beroes JM, Booth MJ, Miles JN, Shanman R, Shekelle PG. Wrong-site surgery, retained surgical items, and surgical fires: a systematic review of surgical never events. JAMA surgery. 2015; 150(8):796-805. https://doi.org/10.1001/jamasurg.2015.0301 PMid:26061125

4. Stawicki SP, Moffatt-Bruce SD, Ahmed HM, Anderson III HL, Balija TM, Bernescu I, Chan L, Chowayou L, Cipolla J, Coyle SM, Gracias VH. Retained surgical items: a problem yet to be solved. Journal of the American College of Surgeons. 2013; 216(1):15-22. https://doi.org/10.1016/j.jamcollsurg.2012.08.026 PMid:23041050

5. Oluwole O, Akinnagbe AM, Nwana EJ, Ogolekwu I, Yilkudi M. Gossypiboma: a cause of iatrogenic fecal entero-cutaneous fistula. Journal of Medicine in the Tropics. 2015; 17(1):34. https://doi.org/10.4103/2276-7096.148694

6. Stawicki SP, Evans DC, Cipolla J, Seamon MJ, Lukaszczyk JJ, Prosciak MP, Torigian DA, Doraiswamy VA, Yazzie NP, Gunter Jr OL, Steinberg SM. Retained surgical foreign bodies: a comprehensive review of risks and preventive strategies. Scandinavian Journal of Surgery. 2009; 98(1):8-17. https://doi.org/10.1177/145749690909800103 PMid:19447736

7. Suwatanapongched T, Boonkasem S, Sathianpitayakul E, Leelachaikul P. Intrathoracic gossypiboma: radiographic and CT findings. The British journal of radiology. 2005; 78(933):851-3. https://doi.org/10.1259/bjr/61657645 PMid:16110111

8. Asuquo ME, Ogbu N, Udosen J, Ekpo R, Agbor C, Ozinko M, Emelike K. Acute abdomen from gossypiboma: A case series and review of literature. Nigerian Journal of Surgical Research. 2006; 8(3-4). https://doi.org/10.4314/njsr.v8i3-4.54901

9. Agha RA, Fowler AJ, Rammohan S, Barai I, Orgill DP and the PROCESS Group. The PROCESS Statement: Preferred Reporting of Case Series in Surgery. International Journal of Surgery 2016; 36(Pt A):319-323.

10. Chana-Rodríguez F, Ma-anes RP, Rojo-Manaute J, Moran-Blanco LM, Vaquero-Martín J. Suppl 1: M7: Retained Sponge: A Rare Complication in Acetabular Osteosinthesis. The open orthopaedics journal. 2015; 9:321. https://doi.org/10.2174/1874325001509010321 PMid:26312116 PMCid:PMC4541466

11. Gawande AA, Studdert DM, Orav EJ, Brennan TA, Zinner MJ. Risk factors for retained instruments and sponges after surgery. New England Journal of Medicine. 2003; 348(3):229-35. https://doi.org/10.1056/NEJMsa021721 PMid:12529464

12. Yorke J, Agbeko E, Amoah G, Abantanga FA. Case Report: Intestinal Obstruction Secondary to an Intra-Abdominal Foreign Body. Journal of Medical and Biomedical Sciences. 2013; 2(4):1-5. https://doi.org/10.4314/jmbs.v2i4.1

13. Moffatt-Bruce SD, Cook CH, Steinberg SM, Stawicki SP. Risk factors for retained surgical items: a meta-analysis and proposed risk stratification system. journal of surgical research. 2014; 190(2):429-36. https://doi.org/10.1016/j.jss.2014.05.044 PMid:24953990

14. Stawicki SP, Cook CH, Anderson III HL, Chowayou L, Cipolla J, Ahmed HM, Coyle SM, Gracias VH, Evans DC, Marchigiani R, Adams RC. Natural history of retained surgical items supports the need for team training, early recognition, and prompt retrieval. The American Journal of Surgery. 2014; 208(1):65-72. https://doi.org/10.1016/j.amjsurg.2013.09.029 PMid:24524864

15. Freitas PS, Silveira RC, Clark AM, Galvão CM. Surgical count process for prevention of retained surgical items: an integrative review. Journal of clinical nursing. 2016; 25(13-14):1835-47. https://doi.org/10.1111/jocn.13216 PMid:27104785

16. Firstenberg MS, Stawicki SP. Vignettes in Patient Safety-Volume 2, 2018.

17. O'Connor AR, Coakley FV, Meng MV, Eberhardt S. Imaging of retained surgical sponges in the abdomen and pelvis. American journal of roentgenology. 2003; 180(2):481-9. https://doi.org/10.2214/ajr.180.2.1800481 PMid:12540456

18. Nasir G. Missed instrument and surgical sponge (gauze and pack). Int J Surg. 2008; 20(1):14-20.

19. Fabian CE. Electronic tagging of surgical sponges to prevent their accidental retention. Surgery. 2005; 137(3):298-301. https://doi.org/10.1016/j.surg.2004.10.003 PMid:15746781

ID Design Press, Skopje, Republic of Macedonia
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Dental Science

Effect of Denture Base Reinforcement Using Light Cured E-Glass Fibers on the Level of Salivary Immunoglobulin A

Shady M. El Naggar[1*], Mohamed I. Seif El Nasr[2], Hassan M. Sakr[2], Sherihan M. Eissa[3], Asmaa N. Elboraey[3], Amani R. Moussa[3]

[1]Removable Prosthodontics, Faculty of Oral and Dental Medicine, South Valley University, Qena Governate, Egypt; [2]Removable Prosthodontics, Faculty of Dental Medicine Al-Azhar-Boys University, Cairo, Egypt; [3]Fixed & Removable Prosthodontics Department, National Research Centre (NRC), Giza, Egypt

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Keywords: denture base reinforcement; light cured E-glass fibres; salivary immunoglobulin A (S-IgA); complete dentures

***Correspondence:** Shady M El Naggar. Removable Prosthodontics, Faculty of Oral and Dental Medicine, South Valley University, Qena Governate, Egypt. E-mail: dr_shady_1985@dent.svu.edu.eg

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Abstract

BACKGROUND: A gap still exists between in vitro and clinical studies concerning the biocompatibility of the material in the oral environment and their potential to cause immunological undesirable side effects. The uses of glass fibres to improve the mechanical properties of acrylic resin denture base polymers are well documented in vitro.

AIM: The present study aimed to evaluate the effect of denture base reinforcement using light-cured E- glass fibres mesh on the level of salivary immunoglobulin A (S-IgA) in patients wearing complete dentures.

MATERIAL AND METHODS: Fourteen completely edentulous patients, in need of complete dentures, participated in the study. The patients were divided into two groups (n = 7) according to the treatment protocol. In the first group, patients received conventional heat-cured acrylic resin dentures. In the second group, the mandibular dentures were reinforced using light cured resin impregnated E glass fibres mesh. In both groups, salivary samples were collected using passive drool technique. The level IgA was assessed by enzyme-linked immunosorbent assay (ELISA) technique at different time intervals. Statistical analysis was carried out using one-way ANOVA followed by Tukey`s post-hoc test and independent t-test. The significant level was set at $P \leq 0.05$.

RESULTS: Acrylic resin dentures and reinforced ones demonstrated an increase in the mean values of IgA level at the end of the follow-up intervals. And this increase was statistically significant ($P \leq 0.05$). Although, the reinforced dentures revealed higher mean values, there was no statistically significant difference between the two groups ($P > 0.05$)

CONCLUSIONS: Within the limitations of the present study, the following could be concluded: (1) the insertion of complete dentures induced changes in the level of IgA; and (2) denture base reinforcement using light cured resin impregnated E-glass fibres mesh had a similar effect to that of heat cured acrylic resin on the level of IgA.

Introduction

For decades, acrylic resin complete dentures have been used to improve the quality of life for completely edentulous patients. The considerable cost, aesthetics, and ease of processing and repair of acrylic resin materials are the major reasons for its clinical success. However, fracture of dentures has been a challenging problem that occurred in 68% of the cases after 3 years [1] [2]. Maxillary denture fractures are mostly caused by fatigue and impact failure, while 80 % of mandibular dentures are fractured by impact [3].

Several attempts have been made to improve the mechanical properties of acrylic resin denture materials among them is the incorporation of reinforcing E-glass fibres. Although several studies documented the significant improvement in the flexural strength, impact strength and fatigue resistance of the acrylic resin material were attained. The processing of the fibres seems to be difficult and technique-sensitive [4] [5] [6] besides their poor

bonding to the acrylic resin which is another shortcoming [3].

Advanced E-glass fibres mesh that is specially treated and impregnated with light cured resin have been recently introduced. A part from its strengthening and esthetic advantages it provides better bonding to the acrylic resin and, simpler manipulation through the use of a light curing system.

The reinforcement process involves the incorporation of new material into the dental prostheses [7]. Biocompatibility and mechanical characteristics are the principal parameters for the applications of new materials in the dentistry. The interaction between the oral environment and new materials may result in the release of biodegradation products which may cause local or systemic undesirable side effects [8].

Saliva is a complex mixture of secretions from major and minor Salivary glands where all the fixed and removable prostheses are immersed in[9]. Salivary immunoglobulin A (S-IgA) is the main immunoglobulin secreted by the immune system in the oral cavity. It compromises the first line of specific immune defence against pathogens, toxins and antigens at mucosal surfaces. The largest amount of sIgA is secreted by the minor salivary glands [10].

The effect of glass fibre reinforcement on the mechanical properties of acrylic resins has been investigated extensively [5] [6] [7]. However, a gap still exists between in vitro and clinical investigations concerning the materials biocompatibility and their potential to cause immunogenic adverse effects [11].

Therefore, the purpose of the present study was to evaluate the effect of denture base reinforcement using light-cured E-glass fibres meshwork on the level of salivary immunoglobulin A (S-IgA) in patients wearing complete dentures.

Material and Methods

Fourteen completely edentulous patients, in need of complete dentures, for the first time, were selected from the patients out clinic, Oral and dental medicine, Al Azhar University-Boys. The patient's age ranged from 45 to 60 years. The study protocol was approved by the Medical Research Ethics Committee (MREC: 18/081) of the National Research Centre. An informed written consent according was obtained from each participant before clinical procedures. All the participants were fulfilling the following inclusion criteria:

1. No previous denture experiences.
2. All of them were free of any systemic or local diseases that induce immune-competence reactions.
3. All were non-smokers.
4. They did not use any drugs that affect the oral mucosa.
5. They all were Angle Class I maxilla-mandibular relation.

Complete dentures construction

Patients were divided into two groups. In the first group (I) the acrylic resin (Across one, Dental & Medical Co, Egypt) complete maxillary and mandibular dentures without base reinforcement were delivered to the patients. The dentures were constructed following conventional procedures. In the second group (II), only the mandibular dentures were reinforced using a light cured resin impregnated with E-glass fibres meshwork (fibre force®, SYNCA, Canada). For the mandibular dentures, the reinforcement mesh was adjusted to the master cast with a 0.6 mm wax spacer.

On the waxed cast, four tissue stops were created using light-cured acrylic resin (SYNCA, Canada) supplied by the manufacturer. Then, the mesh was adapted to the cast using a vacuum unit (EZ VAC, SYNCA, Canada) and cured using the LED light curing unit (Dental Lab LED Light Cure Box, Taiwan) at 430 to 500 nm for 2 minutes. The cured meshwork was removed from the cast, flushed with boiling water stream to remove any wax remnants and dried.

The lower dentures were processed using the conventional method as the first group except that before flask closure the meshwork was placed on the master cast then packing and curing was performed. Thus, the meshwork bonded chemically to the denture acrylic resin

Immunologic evaluation

Salivary Sample collection: The samples were collected at four different time intervals: immediately before denture insertion (as control), two hours, three days and 7 days after complete denture delivery. Before collection of salivary samples, all patients were instructed not to ingest food or drinks (except water) at least for 60 minutes. The samples were collected between 10-11 AM to prevent any differences in the concentration of the saliva due to the circadian rhythm [12].

Ten millilitres of patient's saliva was collected in sterile plastic containers using passive drool technique [12] [13]. Then they were centrifuged at

3000 rpm for 15 minutes and the samples supernatant stored in a deep freezer at -20 degree and they were kept for analysis. The level of salivary IgA in the samples was assessed by using enzyme-linked immunosorbent assay (ELISA) (kit: Peritest, Robonik India) and expressed as mg/dL.

Statistical analysis was performed using with SPSS 20® (Statistical Package for Social Science) and Microsoft Excel 2010. Data were represented as means (M) and standard deviation (SD).

Comparison of data within each group was made using one-way ANOVA followed by Tukey`s post-hoc test to evaluate the effect of time on the level of salivary IgA. Also, independent t-test was performed to detect the significance between both groups for each follow-up visit. The significant level was set at P ≤ 0.05.

Results

Table 1 and 2 showed the IgA level during the follow-up periods of group I and II**.** Comparison of the effect of denture base reinforcement using mesh and conventional dentures on the level of salivary IgA was presented in Table 3.

In group I, when comparing the mean values of IgA before denture insertion and 2 hrs. Following denture use, there was an increase in the mean value; however, this increase was statistically insignificant (P > 0.05). On the other hand, after 3 and 7 days of denture use, there were an increase in the mean values of IgA and this increase was statistically significant (P > 0.05).

Table 1: the level of salivary IgA in group I

Time	M ± SD (g/L)	P-value
Before Denture Insertion (control)	90^{a} ± 6.24	0.00**
After Denture Insertion by 2 Hours	92^{a} ± 3.02	
After 3 Days	113^{b} ±7.47	
After 7 Days	132^{c} ±8.9	

M: Mean, SD: Standard Deviation, P: Probability level; Means with the same superscript letter were insignificant different; Means with the different superscript letter were significantly different; **significant difference.

Similarly, group II demonstrated an increase in the mean values of IgA following denture insertion. And this increase was statistically significant after 3 and 7 days of denture use (P < 0.05).

Table 2: the level of salivary IgA in group II

Time	M ± SD (g/L)	P-value
Before Denture Insertion (control)	94^{a} ± 5.4	0.00**
After Denture Insertion by 2 Hours	96^{a} ± 4.2	
After 3 Days	122^{b} ±8.7	
After 7 Days	142^{c} ±9.1	

M: Mean, SD: Standard Deviation, P: Probability level; Means with the same superscript letter were insignificant different; Means with the different superscript letter were significantly different; **significant difference.

Comparison of conventional dentures and reinforced ones (Table 3) revealed that reinforced light cured dentures showed higher mean values of IgA level during the follow-up periods. However, the increase in IgA means values were statistically insignificant (P < 0.05).

Table 3: Comparison of salivary IgA levels between the two groups

	M ± SD			
	Before Denture Insertion	After Denture Insertion by 2 Hours	After 3 Days	After 7 Days
Group I (Acrylic resin dentures)	90 ± 6.24	92 ± 3.02	113 ±7.47	132 ±8.9
Group II (Reinforced dentures with resin-impregnated E-glass fibres)	94 ± 5.4	96 ± 4.2	122 ±8.7	142 ±9.1
P-value	0.2239*	0.0633*	0.06*	0.0598*

M: Mean, SD: Standard Deviation, P: Probability level.

Discussion

S-IgA has an important role in the defensive mechanism where it participates in the immune reaction and protection of the mucous membrane of the oral cavity [10]. Furthermore, S-IgA concentration is related to the physiological status of the body [14] and oral cavity. The decrease in the level of S-IgA was reported to be associated with the increased risk for oral pathology [15]. On the other hand, the increase of S-IgA level had been correlated with low caries rate [16].

This study was planned to explore whether or not dentures reinforced with light cured resin impregnated E-glass fibres would affect the level of S-IgA in vivo compared to conventional acrylic resin dentures.

Only, healthy completely edentulous male patients with age ranging from 45 to 60 years participated in the study to exclude the possible effect of sex, age and general health status on the level of S-IgA [13] [14] [15] [16] [17] [18].

To assess levels of S-IgA in saliva, unstimulated salivary samples were collected using passive drool method in the present study as it is simple, inexpensive, acceptable for the analysis and more tolerable to the patients [19]. Furthermore, the procedure of salivary stimulation increased the salivary flow rate which in turn decreases the concentration of S-IgA [20] [21].

In the present study, the measurement of the S-IgA levels was performed using ELISA technique as it is a highly accurate method to detect salivary biomarkers including S-IgA [19].

The use of light-cured resin impregnated E-glass fibres mesh was selected in the study as it eliminates the difficulties in the manipulation of E-glass fibres. Curing of the reinforcing mesh using a light curing unit is another advantage to be considered. Besides, it provides better fracture resistance as supported by previous studies [22] [7].

The findings of the present study revealed that insertion of complete dentures caused a progressive continuous increase in the S-IgA levels till the end of the follow-up periods in both groups. These findings are by the previous study conducted by Corega et al., 2014 [23], and Youness et al., 2015 [24], who observed that insertion of orthodontic appliances increased the concentration of S-IgA. The apparent increase in the S-IgA could be attributed to two main reasons. The first reason could be due to hyper-salivation and the stress associated with the insertion of the new prostheses [25]. The second could be attributed to the leaching of toxic compounds from an acrylic resin material as residual monomers and plasticizers [26] [27] which, may initiate immunogenic response. However, the present finding is in disagreement with a previous study conducted by wessam et al., who reported that insertion of complete dentures made of different denture base materials, resulted in the decrease of the level of S-Ig A.

Although, the results also demonstrated an increase in S-IgA level in patients wearing reinforced denture bases compared to conventional dentures. The differences were not statistically significant.

Therefore, it could be proposed that denture acrylic resin material rather than E-glass reinforcement mesh may be the main cause for an increased level of S-Ig A in both groups. Furthermore, the increase S-Ig A level in reinforced dentures could be attributed to the impregnation process of glass fibres acrylic resin as reported by Sipahi et al., 2006 [28]. Who mentioned that this requires an increase in the ratio of monomer.

Within the limitation of the present study, it could be concluded that:

1. Insertion of complete dentures constructed from heat cured acrylic resin affects the level of salivary IgA which initiates the immune response.

2. Reinforcement of dentures with light cured resin impregnated E-glass fibres mesh showed no significant differences compared to heat cured acrylic resin. However, further clinical studies for a prolonged follow-up periods are required to detect the possible side effects such as allergic responses and systemic toxicity.

3. Laboratory investigations are also needed to assess cell cytotoxicity.

References

1. Franklin P, Wood DJ, Bubb NL. Reinforcement of poly (methyl methacrylate) denture base with glass flake. Dental materials. 2005; 21(4):365-70. https://doi.org/10.1016/j.dental.2004.07.002 PMid:15766583

2. Bertassoni LE, Marshall GW, de Souza EM, Rached RN. Effect of pre-and postpolymerization on flexural strength and elastic modulus of impregnated, fiber-reinforced denture base acrylic resins. The Journal of prosthetic dentistry. 2008; 100(6):449-57. https://doi.org/10.1016/S0022-3913(08)60263-2

3. Hari Prasad A, Kalavathy MH. Effect of glass fiber and silane treated glass fiber reinforcement on impact strength of maxillary complete denture. Ann Essen Dent. 2011; 4:7-12. https://doi.org/10.5368/aedj.2011.3.4.1.2

4. Alla RK, Sajjan S, Alluri VR, Ginjupalli K, Upadhya N. Influence of fiber reinforcement on the properties of denture base resins. Journal of Biomaterials and Nanobiotechnology. 2013; 4(1):91. https://doi.org/10.4236/jbnb.2013.41012

5. Fonseca RB, Favarão IN, Kasuya VB, Abrão M, Da Luz N FM, Naves LZ. Influence of glass fiber wt% and silanization on mechanical flexural strength of reinforced acrylics. J Mat Sci Chem Eng. 2014; 2:11–15. https://doi.org/10.4236/msce.2014.22003

6. Golbidi F., Pozveh M A. Flexural Strength of Polymethyl Methacrylate Repaired with Fiberglass.J Dent (Tehran). 2017; 14: 231–236.

7. Gad MM, Fouda SM, Al-Harbi FA, Kangas RN, Raustia A. PMMA denture base material enhancement: a review of fiber, filler, and nanofiller addition. Int J Nanomedicine. 2017: 3801-38012. https://doi.org/10.2147/IJN.S130722 PMid:28553115 PMCid:PMC5440038

8. Bettencourt AF, Neves CB, de Almeida MS, Pinheiro LM, e Oliveira SA, Lopes LP, Castro MF. Biodegradation of acrylic based resins: A review. dental materials. 2010; 26(5):e171-80.

9. Dawes C. Salivary flow patterns and the health of hard and soft oral tissues. The Journal of the American Dental Association. 2008; 139:18S-24S. https://doi.org/10.14219/jada.archive.2008.0351 PMid:18460676

10. Brandtzaeg P. Do salivary antibodies reliably reflect both mucosal and systemic immunity? Annals of the New York Academy of Sciences. 2007; 1098:288–311. https://doi.org/10.1196/annals.1384.012 PMid:17435136

11. Celebi N, Yuzugullu B, Canay S, Yucel U. Effect of polymerization methods on the residual monomer level of acrylic resin denture base polymers. Polym Adv Technol. 2008; 19:201–6. https://doi.org/10.1002/pat.996

12. Evans EW, Hayes C, Palmer CA, Bermudez OI, Cohen SA, Must A. Dietary intake and severe early childhood caries in low-income, young children. Journal of the Academy of Nutrition and Dietetics. 2013; 113(8):1057-61. https://doi.org/10.1016/j.jand.2013.03.014 PMid:23706351 PMCid:PMC4045487

13. Gomar-Vercher S, Simón-Soro A, Montiel-Company JM, Almerich-Silla JM, Mira A. Stimulated and unstimulated saliva samples have significantly different bacterial profiles. PLoS ONE. 2018; 13(6): 1-12. https://doi.org/10.1371/journal.pone.0198021 PMid:29856779 PMCid:PMC5983451

14. Phillips AC, Carroll D, Drayson MT, Der G. Salivary immunoglobulin a secretion rate is negatively associated with cancer mortality: The West of Scotland Twenty-07 Study. PLoS One. 2015; 10(12):e0145083. https://doi.org/10.1371/journal.pone.0145083 PMid:26699127 PMCid:PMC4689578

15. Giuca MR, Pasini M, Tecco S, Giuca G, Marzo G. Levels of salivary immunoglobulins and periodontal evaluation in smoking patients. BMC Immunology. 2014; 15-5. https://doi.org/10.1186/1471-2172-15-5

16. Mithra H, Darshana D, Chitharanjan S, Aditya S.Correlation between dental caries and salivary immunoglobulin in adult Indian population: An in vivo study. J Restor Dent. 2013; 1:22-25. https://doi.org/10.4103/2321-4619.111229

17. Minicucci EM, Pires RB, Vieira RA, Miot HA, Sposto MR. Assessing the impact of menopause on salivary flow and xerostomia. Aust Dent J. 2013; 58:230-4. https://doi.org/10.1111/adj.12057 PMid:23713645

18. Khan SF, KattiG,Baba I, Khan N. Age- related changes of salivary IgA among healthy subjects. J Indian Acad Oral Med Radiol. 2015; 27:203-205. https://doi.org/10.4103/0972-1363.170138

19. De La Rica R., Stevens M. Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye. Nature Nanotech. 2012; 7:821-824. https://doi.org/10.1038/nnano.2012.186 PMid:23103935

20. Shifa S, Muthu MS, Amarlal D, Prabhu VR. Quantitative assessment of IgA levels in the unstimulated whole saliva of caries-free and caries-active children. Journal of Indian Society of Pedodontics and Preventive Dentistry. 2008; 26(4):158. https://doi.org/10.4103/0970-4388.44031 PMid:19008624

21. Beltzer EK, Fortunato CK, Guaderrama MM, Peckins MK, Garramone BM, Granger DA. Salivary flow and alpha-amylase: collection technique, duration, and oral fluid type. Physiology & Behavior. 2010; 101(2):289-96. https://doi.org/10.1016/j.physbeh.2010.05.016 PMid:20515701

22. Pfeiffer P ,Grube L. In vitro resistance of reinforced interim fixed partial dentures. J Prosth Dent. 2003; 89:170–174. https://doi.org/10.1067/mpr.2003.29 PMid:12616237

23. Corega, C, VaidaL, Feştilă D, RigoniG, Albanese, M., D'agostino, A., Chiarini, G., Barone, A., Covani, U., Nocini, P.F., & Bertossi, D. Salivary levels of IgA in healthy subjects undergoing active orthodontic treatment. Minerva stomatologica. 2013; 12:11-15.

24. Youness SR, Hussein JS, Refaat WS, El Hariri HM. Effect of Orthodontic Treatment on Salivary Immunoglobulin A Levels among a group of healthy Egyptian Children. J Dent Med Sci (IOSR-JDMS). 2015; 14:2279-0861.

25. Tango RN, Arata A, Borges AL, Costa AK, Pereira LJ, Kaminagakura E. The Role of New Removable Complete Dentures in Stimulated Salivary Flow and Taste Perception. Journal of Prosthodontics. 2018; 27(4):335-9. https://doi.org/10.1111/jopr.12507 PMid:27434551

26. Boecker AF, Morton D, Poser S, Dette KE. Release of dibenzoyl peroxide from polymethyl methacrylate denture base resin: an invitro study. Dent Mater. 2008; 24:1600.

27. Goiato MC, Freitas E, Sonego M. Acrylic Resin Cytotoxicity for Denture Base--Literature Review. Advances in clinical and experimental medicine: official organ Wroclaw Medical University. 2015; 24(4):679-86. https://doi.org/10.17219/acem/33009 PMid:26469114

28. Sipahi C, Ozen J, Ugur Ural A, Dalkiz M, Beydemir B. The effect of two fibre impregnation methods on the cytotoxicity of a glass and carbon fibre-reinforced acrylic resin denture base material on oral epithelial cells and fibroblasts. Journal of oral rehabilitation. 2006; 33(9):666-73. https://doi.org/10.1111/j.1365-2842.2006.01648.x PMid:16922740

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Dental Science

Different Materials Used as Denture Retainers and Their Colour Stability

Sherif A. Sadek[1*], Wessam M. Dehis[2], Hala Hassan[3]

[1]Department of Prosthodontics, Faculty of Oral and Dental Medicine, Cairo University, Egypt; [2]Department of Fixed and Removable Prosthodontics, National Research Center, Egypt; [3]Department of Prosthodontics, Faculty of Oral and Dental Medicine, Modern University for Technology and Information, Egypt

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Keywords: Removable Partial Denture; Clasps; Direct retainer; Physical properties; Color stability; Cobalt Chromium; Thermoplastic Acetal; Versacryl; Thermopress

***Correspondence:** Sherif A Sadek. Department of Prosthodontics, Faculty of Oral and Dental Medicine, Cairo University, Egypt. E-mail: sherifalysadek@gmail.com

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Abstract

BACKGROUND: Retainers are of great importance for the longevity of the prosthetic removable partial denture during various functions especially the esthetic one. The key of successful clasp selection is to select a direct retainer that will control tipping and torquing forces on the abutment teeth, provide retention against reasonable dislodging forces and are compatible with both tooth and tissue contour together with the aesthetic desire of the patient.

AIM: This study aims to compare different clasp material to enhance the choice of the clasp based on the aesthetic point of view.

METHODS: The colour evaluation of the tested materials had been evaluated by computer aided technique with digital camera with 3 Mega Pixels of resolution.

RESULTS: In the current research, the technique of colour evaluation was carried out to compare different clasp materials to enhance the choice of the clasp based on the aesthetic point of view. Most commonly, Removable Partial Denture (RPD) retainers are fabricated identically from the metal framework's alloy as Cobalt Chromium (CoCr) alloy although it is unaesthetic. This esthetic problem has been overcome by other methods and by utilising different materials, these included covering the retainers with tooth-coloured acrylic resin, as well as the introduction of esthetic materials as; Thermoplastic Acetal, Versacryl, and Thermopress.

CONCLUSION: It has been concluded that the non-metallic Acetal resin clasp shows superior physical properties regarding colour stability.

Introduction

The most important calibre of esthetics is colour. Usually, denture base gets contaminated with various materials intraorally. The acrylic resin denture base material is highly potential to soak up distinct contaminants. Moreover, it is subjected to sorption, the practicability of both absorption and adsorption of liquids relying on the ecological circumstances then leading to possible discolouration, so colour stability is one of the most important factors in denture base materials selection [4].

The esthetic dental restorations play a great role in recent communities not only for females but also for males, due to the emphasis of physical look. Dental implant succeeded in expanding such scope, yet it is not highly recommended for the tremendous scale of patients, especially those who are suffering from some medical, psychological and financial problems [5].

Esthetic removable partial dentures (RPDs) are considered as the best and most compatible preference remedy for these subjects in replacing their lost teeth with superior esthetics. One of the major problems of RPDs was the display of the clasp assemblies. Etching the retainer's arm and overlaying it with a tooth-coloured resin coat is one of many recent ways employed to solve this issue. Moreover, as the physical appearance of these esthetic retainers is of vital essentiality, yet their mechanical properties play a great role in their success and intraoral utilisation [6].

The difficulty in using of acrylic resins or resin composite to veneers on metals of RPD appears in the diversities between both their potentiality to inflect

and coefficients of thermal expansion. Non-noble metals possess durability and resist remarkable flection. However, extreme disfigurement takes place to resins concerning both their physical and thermal status, as the matrix becomes fragile beyond its elastic borders. The resin composite matrix also tends to be brittle beyond its elastic limit. As a sequel, the capacities of both metals and resins for plastic disfigurement are in a broad conflict. Latest concerns extend to the impact of the intraoral masticatory strength together with both the adjustability and extra magnitude of the veneered retainers formed by the assembling of the covering matter. Exaggerated declining in the retainer's length and thickness should be averted to secure the stiffness and shorten the fracture of the retainer as well as provide maximum esthetics [7].

The rotational path RPD is a simple method that abolishes the utilisation of the intraoral esthetically unpleasant retainers anteriorly. It employs an anterior immobile part of the framework and an ordinary pliable posterior retentive retainer as the retentive components. Retainers' least utilisation, the superiority of esthetics and minimal accumulation of plaque are the main merits of such design. However, both clinical and laboratory procedures in demand for the rotational path RPD are skillfully precise [8] [9] 10].

Lingual retention provides an equivalent magnitude of invisible retention as that situated on the buccal surface. Such retention is achieved by substituting the bracing arm with parallel guide standard surfaces and accurately designed rests both with the retentive element placed lingually [11]. Denture clasps constructed from both a substance matching the tooth shade and thermoplastic resin have been utilised on a wide scale to both enhance the resemblance in metallic retainers' structures and support esthetics [12] [13] [14].

Polyoxymethylene (POM) which is well known as Acetal resin, an injection-moulded resin also acts as a standby to the classical PolyMethylMethAcrylate (PMMA). Fabrication of POM takes place by the polymerisation of formaldehyde. The homopolymer polyoxymethylene is a series of alternating methyl sets united by an oxygen whit. Besides that, it behaves elastically on a wide scale which allows it to be utilised as the suitable material for retainer construction. This is due to its superior proportional limit with the minimal viscous flow [12].

POM has been consumed globally in dentistry as an offset for both PMMA and metals in tremendous of prosthetic employments since two decades ago. The most commonly effective appliances were the esthetic clasps of RPD [14] [15] [16], cast posts and cores as well as brackets [17].

An attempt to perform aesthetic removable partial denture is the usage of Valplast as a retentive clasp for esthetic reasons since it belongs to Nylon family. They are thin and light in weight, resistant to fracture and esthetically good due to their pink and translucent shade which matches that of natural dentition and gingiva. Its indications extend to the cosmetic improvement of teeth that appear elongated due to gum recession and for acrylic resin's allergic patients [18]. Moreover, thermoplastic resin injection materials (Thermopress) are characterised by being easily manipulated and providing esthetically acceptable results [19].

Colour values can be measured by visual methods of specifying colour (subjective method) or by the aid of instruments (objective method). Besides that, Color is a sensation captured by our eyes in three dimensions; three terms were needed to explain colour measurements [20]. Hue recognises each colour from another, while Lightness (Value) distinguishes light colours from dark ones and Chroma describes how different colour is from grey [21].

This study aims to compare different clasp material to enhance the choice of the clasp based on the esthetic point of view.

Material and Methods

An ideal model of the maxillary partially edentulous case (Kennedy class III) usually utilised for educational purposes was selected as a master model replicating the anatomical features of the teeth.

Duplication of the model was carried out to fabricate a stone cast with both the maxillary premolar and molar into wax and ready to be to be surveyed before casting the wax into metal. Surveying was essential to provide mesially (8 mm) and lingual guide planes (6 mm) to create a (0.25 mm) undercut area on the distobuccal surface and prepare an occlusal rest seat (2 mm) deep on the mesiocclusal surface for the molar tooth. Moreover, on the distobuccal surface, both distal (8 mm) and lingual guide planes (6 mm) were fabricated as well as, (0.25 mm) undercut area on the mesiobuccal surface and an occlusal rest seat (2 mm) deep (Figure 1).

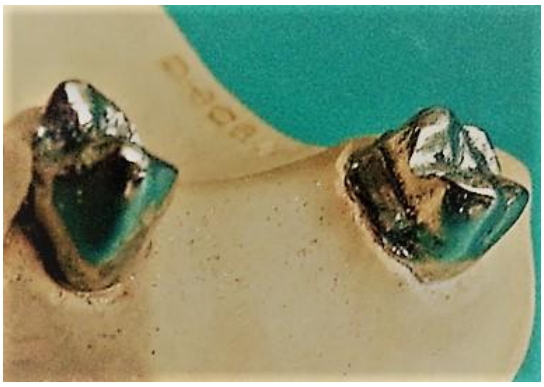

Figure 1: Surveyed metal teeth models

The specimens included five premolar clasps with (0.25 mm) undercut, and five molar clasps with (0.50 mm) undercut.

The five different clasps used for each abutment tooth were fabricated from Cobalt Chromium (CoCr) metal (Vitllium; Cobalt Chrome alloy): Cobalt 64%, Chromium 28%, Molybdenum 5.5%. Type 1-ADA, Spec. No. 14 From (Dentorium Co., U.S.A.), Versacryl (Keystone Industries GmbH, Sigen, Germany), Valplast (Valplast Denture Acrylic Resin for Flexible Dentures, IRIS, Tianjin IRIS Int. Trade Co. Ltd., Industrial district, Tianjin, China), Acetal resin (Acetal Copolymer, Flexible Acrylic Dental Clasps, Korea Engineering Plastics Co. Ltd., KEPITAL Europe GmbH, Wiesbaden, Germany) and Thermopress (Thermopress, Thermopress 400, Bredent): From (Gmbh & Co.KG, Germany). Each type of these clasps was fabricated as recommended by the manufacturer attaching to them a wax plate (4 × 7 × 3 mm) which was attached to the minor connector parallel to the path of insertion. The plate was utilised later for maintaining the clasp in the testing machine (Figure 2).

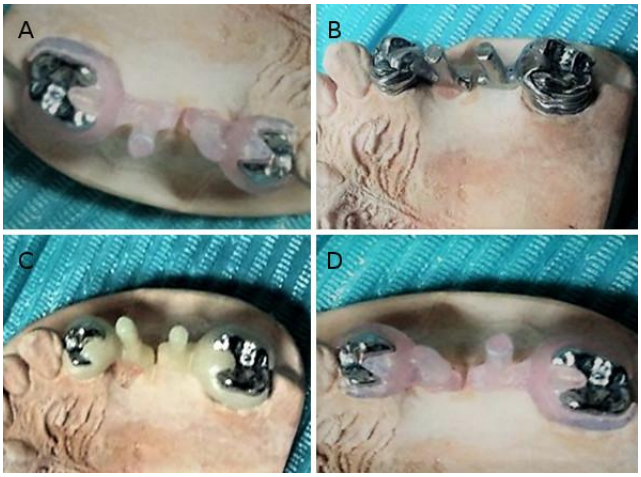

Figure 2: The Specimens; A) Acetal Clasps; B) Metal Clasps; C) Thermopress Clasps; D) Versacryl Clasps

Images Used in The Current Study Were Taken with The Following Image Acquisition System:

1) Computer-aided technique which was a combination of a digital camera (*Scope Capture Digital Microscope, Guangdong Co. Ltd., China.*) and image processing software (*Image J 1.43U, National Institute of Health, USA*) was the method used to register the color of any pixel of the specimen's image using three color sensors per pixel.

2) Digital camera with 3 Mega Pixels of the resolution was utilised to evaluate the colour. This camera was placed vertically at a distance of (2.5 cm) from the samples. The angle between the axis of the lens and the sources of illumination is approximately 45º.

3) Illumination was achieved with 8 LED lamps (*Adjustable by Control Wheel*) with a colour index (Ra) close to 95%.

4) The images were taken at maximum resolution (2272 × 1704 pixels) and connected with an IBM compatible personal computer consuming a fixed magnification of 90X. The images were recorded with a resolution of 1280 × 1024 pixels/image. Digital microscope images were cropped to 350 × 400 pixels utilising Microsoft office picture manager to specify/ standardise area of colour measurement.

5) To calibrate the digital colour system, the colour values of the 32 colour charts were measured.

6) Once the system was calibrated, it was possible to infer the Lab Color Space (Lab) values by the Red (R), Green (G) or Blue (B) (RGB) measurements from the camera without utilising the colourimeter along with image processing software (Figure 3).

Specification:

Image sensor	1.3 Mega Pixels
Still capture resolution	1600x1200 (2M), 1280x1024(1.3M), 1024x960, 1024x768, 800x600, 640x480, 352x288, 320x240, 160x120
Video capture resolution	1600x1200 (2M), 1280x1024(1.3M), 1024x960, 1024x768, 800x600, 640x480, 352x288, 320x240, 160x120
Focus Range	Manual focus from 10mm to 500mm
Frame Rate	Max 30f/s under 600 Lus Brightness
Magnification Ratio	20x to 200x
Video format	AVI
Photo format	JPEG or BMP
Light source	8 LED (adjustable by control wheel)
PC interface	USB2.0
Power source	5V DC from USB port
Operation system	Windows2000 SP4/XP/Vista/Win7, Mac OS X 10.5 or above
OSD language	English, German, Spanish, Korean, French, Russian
Bundle software	MicroCapture
Size	110mm (L) x 33mm (R)

Figure 3: Scope Capture Digital Microscope

A total number of 10 specimens were used in this research and divided into two different groups; 5 specimens in each group.

The First Group (Coffee)

Specimens of this group were 5 in number, and all were stored in Coffee solution. This solution was prepared by pouring (20 g) of coffee (*Nescafe*

Classic, Nestle, Egypt) into (1000 ml) of boiled water. Then the solution was stirred every (30 min.) for (10 sec.) until it cooled down till room temperature. Proper filtration of the Coffee mix was carried out later on through a filter paper. The specimens were immersed into (20 ml) of each immersion media and kept in the incubator (*PSA, Advanced technology, Cairo, Egypt.*). The solution was freshened once every 3 days to reduce the precipitation of particles in the staining solutions, and the solutions were stirred once a day **(22).**

The Second Group (Distilled Water)

This group included 5 specimens that were stored in Distilled Water.

Colour Evaluation

1. Colour measurement of each specimen (T0) was performed employing computational technique with a combination of a digital camera and image processing software.

2. The specimens were placed in containers as mentioned above. Subsequent colour measurements were Taken After 4 Weeks of Immersion (Tfim). All the specimens were kept in the incubator during measurements at 37 °C.

3. The mean of colour change for each material was calculated with the aid of C.I.E. *L a b* uniform colour scale. The magnitude of the total colour difference is formulated by Δ*E*.

4.

$$\Delta E_{CIELAB} = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

∆*E* the magnitude of total colour difference.

Δ *L* is the luminance or lightness component that ranges from (0 to 100).

Δ *a* is a chromatic component acting as parameter and ranges from (green to red).

Δ *b* is a chromatic component acting as parameter and ranges from (blue to yellow).

Δ *a and* Δ *b* both range from (-120 to 120).

Data were presented as the mean and standard deviation (SD) and explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Colour Parameters revealed a parametric distribution, so One-Way ANOVA was utilised to study the difference between tested materials on mean Color Parameters followed by Tokay's posthoc test for pairwise comparison when ANOAV is significant. Dependent t-test was utilized to compare between different tested solutions for each material.

The significance level was set at P ≤ 0.05. Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA.) Statistics Version 23 for Windows.

Results

Difference between the mean of the luminance or lightness component (∆L) of different tested materials in both Coffee and Distilled Water groups

Mean, and standard deviation (SD) for the luminance or lightness component (∆L) for different tested materials were presented in Table 1.

Versacryl (-2.63 ± 1.33) displayed the highest mean of the luminance or lightness component (∆L) followed by Acetal resin (-4.52 ± 0.25) and Valaplast (-5.2 ± 0.75) then followed by Thermopress (-7.09 ± 0.23) at p ≤ 0.001 for Coffee.

Versacryl (4.03 ± 0.31), Thermopress (3.66 ± 0.44) and Valaplast (4.07 ± 0.24) revealed the highest mean of the luminance or lightness component (∆L) compared to Acetal resin (1.68 ± 0.31) at p ≤ 0.001 for Distilled Water.

Table 1: Mean and standard deviation (SD) of the luminance or lightness component (∆L) for different tested materials in first and second groups

		Material								
	Groups	Acetal resin		Thermopress		Versacryl		Valaplast		p-value
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
∆L	Coffee	-4.52^{b}	.25	-7.09^{c}	.23	-2.63^{a}	1.33	-5.20^{b}	.75	≤0.001*
	Distilled Water	1.68^{b}	.31	3.66^{a}	.44	4.03^{a}	.31	4.07^{a}	.24	≤0.001*
	p-value	≤0.001*		≤0.001*		≤0.001*		≤0.001*		

Means with the same letter within each row are not significantly different at p = 0.05. * = Significant, NS=Non-significant.

Difference between the mean of the chromatic component (∆a) that ranges from (green to red) of different tested materials in both Coffee and Distilled Water groups

Mean, and standard deviation (SD) for the chromatic component (∆a) for different tested materials were presented in Table 2.

Versacryl (1.69 ± 0.51) showed the highest mean of the chromatic component (∆a) followed by Acetal (0.64 ± 0.34) followed by Thermopress (0.08 ± 0.34) and then Valaplast (-3.2 ± 0.34) at p ≤ 0.001 for Coffee.

Versacryl (0.12 ± 0.21) and Acetal resin (0.06 ± 0.26) displayed the highest mean of the chromatic component (∆a) compared to Valaplast (-2.79 ± 0.32) and Thermopress (-3.79 ± 0.39) at p ≤ 0.001 for Distilled Water.

Table 2: Mean and standard deviation (SD) of the chromatic component (∆a) for different tested materials in first and second groups

	Groups	Material								p-value
		Acetal resin		Thermopress		Versacryl		Valaplast		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
∆a	Coffee	.64[b]	.34	.08[c]	.34	1.69[a]	.51	-3.20[d]	.34	≤0.001*
	Distilled Water	.06[a]	.26	-3.79[c]	.39	.12[a]	.21	-2.79[d]	.32	≤0.001*
p-value		0.01*		≤0.001*		≤0.001*		0.086 NS		

Means with the same letter within each row are not significantly different at p = 0.05. * = Significant, NS = Non-significant

Difference between the mean of the chromatic component (∆b) that ranges from (blue to yellow) of different tested materials in both Coffee and Distilled Water groups

Mean, and standard deviation (SD) for the chromatic component (∆b) for different tested materials were presented in Table 3.

Versacryl (3.82 ± 0.54) and Valapalst (4.25 ± 0.53) displayed the highest mean for the chromatic component (∆b) followed by Acetal resin (0.252 ± 0.23) and then Thermopress (-1.812 ± 0.315) at p ≤ 0.001 for coffee.

Valaplast (1.93 ± 0.50) and Acetal resin (1.86 ± 0.19) showed the highest mean for the chromatic component (∆b) compared to Versacryl (-3.42 ± 0.60) and Thermopress (-3.23 ± 0.189) at p ≤ 0.001 for Distilled Water.

Table 3: Mean and standard deviation (SD) of the chromatic component (∆b) for different tested materials in first and second groups

	Groups	Material								p-value
		Acetal resin		Thermopress		Versacryl		Valaplast		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
∆b	Coffee	.2520[b]	.2336	-1.8120[c]	.3148	3.8200[a]	.5445	4.2520[a]	.5315	≤0.001*
	Distilled Water	1.8633[a]	.1935	-3.2320[b]	.1891	-3.4260[b]	.6070	1.9320[a]	.5073	≤0.001*
p-value		≤0.001*		≤0.001*		≤0.001*		≤0.001*		

Means with the same letter within each row are not significantly different at p = 0.05. * = Significant, NS = Non-significant.

Difference between the mean of the magnitude of the total colour difference (∆E) of different tested materials in both Coffee and Distilled Water groups

Mean, and standard deviation (SD) for the magnitude of the total colour difference (∆E) for different tested materials were presented in Table 4.

Thermopress (7.33 ± 0.209) and Valaplast (7.45 ± 0.89) revealed the highest mean for the magnitude of the total colour difference (∆E) accompanied by Acetal resin (4.588 ± 0.227) and Versacryl (5.10±0.368) at p≤0.001 for Coffee.

Thermopress (6.19 ± 0.42) displayed the highest mean for the magnitude of the total colour difference (∆E) followed by Versacryl (5.32 ± 0.34) and Valaplast (5.324 ± 0.28) followed by Acetal resin (2.52 ± 0.29) at p ≤ 0.001 for Distilled Water.

Table 4: Mean and standard deviation (SD) of the total colour difference (∆E) for different tested materials in first and second groups

	Groups	Material								p-value
		Acetal resin		Thermopress		Versacryl		Valaplast		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
∆E	Coffee	4.5880[b]	.2270	7.3360[a]	.2096	5.1080[b]	.3685	7.4540[a]	.8917	≤0.001*
	Distilled Water	2.5233[c]	.2943	6.1960[a]	.4211	5.3200[b]	.3496	5.3240[b]	.2813	≤0.001*
p-value		≤0.001*		0.001*		0.386 NS		0.001*		

Means with the same letter within each row are not significantly different at p = 0.05. * = Significant, NS=Non-significant.

Discussion

Colour evaluation techniques provide valuable information regarding the physical properties of the materials tested. However, none of the in-vitro techniques can expose the tested materials to conditions similar to that of the oral environment (in-vivo) such as pH value and temperature variations [2].

Recently, thermoplastic polymers are widely employed as denture base materials due to their translucency, flexibility, higher strength, lack of free monomers and biocompatibility [17]. Based on the literature review, studies have mainly focused on the mechanical properties of these materials, but limited published information is available on the colour stability of thermoplastic polymers after ageing [7] [23]. Colour stability is an important factor for dental materials, colour alterations due to the ageing process or any damage to the denture base material affect the esthetic results [24].

Thermoplastic Acetal is one of the thermoplastic resins that was first introduced in 1971 as an unbreakable one. During this era, quick injection methods developed the initial tooth coloured retainers which are flexible, don't need periodic adjusting to keep them tight and they were very much appreciated by the patients [25].

Achieving optimal superior esthetics usually takes place by utilising Acetal clasps since their colour matches with that of teeth, and it was documented for their high ability to eliminate expending of metal clasps which improve esthetics. Due to their low modulus of elasticity, they can be employed in larger undercuts than recommended for chromium-cobalt alloy and also exert minimal stresses on abutment teeth. This might be clinically useful due to the importance of both esthetics and periodontal health. As well as, maintaining retentive integrity, stability and protecting the health of teeth which have been a hard task to achieve are from its remarkable merits [26] [27].

Moreover, they offer the additional essential advantage of declining the potentiality for allergic reactions to metals that take place with most subjects especially the highly sensitive ones [26] [28].

The merits mentioned above allow Acetal

resin to be an ideal substance for pre-formed partial dentures' retainers, their frameworks and single pressed unilateral ones. Moreover, it fabricates the transitional bridges, occlusal splints and implant pillars. Besides that, it combats the occlusal overstrain which allows it to be suitable enough to preserve the vertical dimension through the interim restorative remedy [12] [29] [30].

Regarding the cobalt chromium alloy denture base material, it has been well known since very long time ago for its poor esthetics, especially when utilised as RPD clasps, this was due to both their undesirable metallic colour that is highly remarkable and thickness to provide the required amount of retention. Moreover, many considerations clarified that such alloy when being dipped intraorally, the metal tends to ionise. An oxide layer is fabricated on the alloy's superficies by absorption of Oxygen, which worsen much more it's very poor esthetics and declines by the time its metallic colour [31].

If one or more metals as CoCr denture base material (Vitallium) are dipped intraorally, the mineral tends to ionise. The diversity in the alloy's power tends to transfer its ions to electrolytes which then stimulate the metal to melt. The oxide layer is created on the alloy's superficies by absorption of Oxygen, which prohibits excess melting for its remaining constituents. A superficial film coating all the intraoral surfaces and fabricated by sedimentation of both salivary proteins and glycoproteins are named Biofilm [31] [32] [33] [34].

Further studies have to be carried out regarding various thicknesses and designs of both clasps and frameworks for Acetal successful dental application [32]. As the technology of injecting Acetal clasp into a mould is quite new for dentistry yet the literature is poor. Only a few data on the subject were published to date [27] [35].

Regarding the Versacryl, it has been recommended that utilising esthetic retainers in RPDs provide the patient with a metal-free smile, yet its colour stability is not that much superior as the Acetal's one [(36)].

In conclusion, (1) the most colour stable retainer and with excellent esthetics was the Acetal clasps; (2) Versacryl is of good esthetics yet its colour stability is not that much outstanding as the Acetal's one; (3) both Thermopress and Valaplast were the worst among the materials utilized in colour stability although they are considered as esthetic clasps; (4) Cobalt Chromium (CoCr) metal clasps are the poorest regarding esthetics.

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References

1. McGiveny GP, Castleberry AB. McCraken's Removable partial prosthodontics, 12th ed. St Louis. Mosby Co. 2012; 17(68):236-241.

2. Avon SL, Goulet JP, Deslauriers N. Removable acrylic resin disk as a sampling system for the study of denture biofilms in vivo. The Journal of prosthetic dentistry. 2007; 97(1):32-8. https://doi.org/10.1016/j.prosdent.2006.12.001 PMid:17280889

3. Özcan M. The use of chairside silica coating for different dental applications: a clinical report. The Journal of prosthetic dentistry. 2002; 87(5):469-72. https://doi.org/10.1067/mpr.2002.124365 PMid:12070506

4. Silva PM, Acosta EJ, Jacobina M, Pinto LD, Porto VC. Effect of repeated immersion solution cycles on the color stability of denture tooth acrylic resins. Journal of Applied Oral Science. 2011; 19(6):623-7. https://doi.org/10.1590/S1678-77572011000600013 PMid:22230997 PMCid:PMC3973464

5. Donovan TE, Derbabian K, Kaneko L, Wright R. Esthetic considerations in removable prosthodontics. Journal of Esthetic and Restorative Dentistry. 2001; 13(4):241-53. https://doi.org/10.1111/j.1708-8240.2001.tb00270.x PMid:11572508

6. de Delgado MM, Garcia LT, Rudd KD. Camouflaging partial denture clasps. Journal of Prosthetic Dentistry. 1986; 55(5):656-60. https://doi.org/10.1016/0022-3913(86)90050-8

7. Snyder HA, Duncanson Jr MC, Johnson DL, Bloom J. Effects of clasp flexure on a 4-META adhered light-polymerized composite resin. International Journal of Prosthodontics. 1991; 4(4). PMid:1811631

8. Firtell DN, Jacobson TE. Removable partial dentures with rotational paths of insertion: problem analysis. Journal of Prosthetic Dentistry. 1983; 50(1):8-15. https://doi.org/10.1016/0022-3913(83)90157-9

9. Jacobson TE. Rotational path partial denture design: A 10-year clinical follow-up—Part I. Journal of Prosthetic Dentistry. 1994; 71(3):271-7. https://doi.org/10.1016/0022-3913(94)90466-9

10. Byron JR, Frazer RQ, Herren MC. Rotational path removable partial denture: an esthetic alternative. General dentistry. 2007; 55(3):245-50. PMid:17511371

11. Pardo-Mindan S, Ruiz-Villandiego JC. A flexible lingual clasp as an esthetic alternative: a clinical report. Journal of Prosthetic Dentistry. 1993; 69(3):245-6. https://doi.org/10.1016/0022-3913(93)90099-A

12. Fitton JS, Davies EH, Howlett JA, Pearson GJ. The physical properties of a polyacetal denture resin. Clinical materials. 1994; 17(3):125-9. https://doi.org/10.1016/0267-6605(94)90135-X

13. Turner JW, Radford DR, Sherriff M. Flexural properties and surface finishing of acetal resin denture clasps. Journal of prosthodontics. 1999; 8(3):188-95. https://doi.org/10.1111/j.1532-849X.1999.tb00034.x PMid:10740501

14. Arda T, Arikan A. An in vitro comparison of retentive force and deformation of acetal resin and cobalt-chromium clasps. The Journal of prosthetic dentistry. 2005; 94(3):267-74. https://doi.org/10.1016/j.prosdent.2005.06.009 PMid:16126079

15. Chu CH, Chow TW. Esthetic designs of removable partial dentures. General dentistry. 2003; 51(4):322-4. PMid:15055607

16. Turner JW, Radford DR, Sherriff M. Flexural properties and

surface finishing of acetal resin denture clasps. Journal of prosthodontics. 1999; 8(3):188-95. https://doi.org/10.1111/j.1532-849X.1999.tb00034.x PMid:10740501

17. Corrente G, Vergnano L, Pascetta R, Ramadori G. A new custom-made abutment for dental implants: a technical note. International Journal of Oral & Maxillofacial Implants. 1995; 10(5). PMid:7591006

18. Singh K, Aeran H, Kumar N, Gupta N. Flexible thermoplastic denture base materials for aesthetical removable partial denture framework. Journal of clinical and diagnostic research: JCDR. 2013; 7(10):2372. https://doi.org/10.7860/JCDR/2013/5020.3527

19. Osada H, Shimpo H, Hayakawa T, Ohkubo C. Influence of thickness and undercut of thermoplastic resin clasps on retentive force. Dental materials journal. 2013; 32(3):381-9. https://doi.org/10.4012/dmj.2012-284 PMid:23718997

20. Dozic A, Voit NF, Zwartser R, Khashayar G, Aartman I. Color coverage of a newly developed system for color determination and reproduction in dentistry. Journal of dentistry. 2010; 38:e50-6. https://doi.org/10.1016/j.jdent.2010.07.004 PMid:20638437

21. Pero AC, Ignárcio J, Giro G, Mendoza-Marin DO, Paleari AG, Compagnoni MA. Surface properties and color stability of an acrylic resin combined with an antimicrobial polymer. Revista de Odontologia da UNESP. 2013; 42(4):237-42. https://doi.org/10.1590/S1807-25772013000400002

22. Koksal T, Dikbas I. Color stability of different denture teeth materials against various staining agents. Dental materials journal. 2008; 27(1):139-44. https://doi.org/10.4012/dmj.27.139 PMid:18309623

23. Faltermeier A, Behr M, Müßig D. In vitro colour stability of aesthetic brackets. The European Journal of Orthodontics. 2007; 29(4):354-8. https://doi.org/10.1093/ejo/cjm020 PMid:17702794

24. Hafezeqoran A, Ghanizadeh M, Rahbar M, Koodaryan R. Effect of Denture Cleansers on the Color Changes of Thermoplastic Denture Base Material. Journal of International Oral Health. 2016; 8(6):716.

25. Kutsch VK, Whitehouse JW, Schermerhorn K, Bowers R. The evolution and advancement of dental thermoplastics. Dental Town. 2003; 4(2):52-6.

26. Peter TP. Creating Aesthetics with Thermoplastic clasps. The Dent Liner J. 2007; 11(3):6-13.

27. Lekha K, Savitha NP, Roseline M, Nadiger RK. Acetal resin as an esthetic clasp material. Journal of interdisciplinary dentistry. 2012; 2(1):11. https://doi.org/10.4103/2229-5194.94185

28. Kuwahara K. A case of using non-metal clasp partial denture for the patient with the metal allergy. Nihon Univ J Oral Sci. 2004; 30:134-9.

29. Phoenix RD, Mansueto MA, Ackerman NA, Jones RE. Evaluation of mechanical and thermal properties of commonly used denture base resins. Journal of Prosthodontics. 2004; 13(1):17-27. https://doi.org/10.1111/j.1532-849X.2004.04002.x PMid:15032892

30. Yunus N, Rashid AA, Azmi LL, Abu-Hassan MI. Some flexural properties of a nylon denture base polymer. Journal of oral rehabilitation. 2005; 32(1):65-71. https://doi.org/10.1111/j.1365-2842.2004.01370.x PMid:15634304

31. Wataha JC, Regina LM. Dental Casting alloys. J The Dental Clinics of North America. 2004; 48:499-512. https://doi.org/10.1016/j.cden.2003.12.010 PMid:15172613

32. Bezzon OL, Pedrazzi H, Zaniquelli O, da Silva TB. Effect of casting technique on surface roughness and consequent mass loss after polishing of NiCr and CoCr base metal alloys: a comparative study with titanium. The Journal of prosthetic dentistry. 2004; 92(3):274-7. https://doi.org/10.1016/j.prosdent.2004.04.021 PMid:15343163

33. Silva JW, Sousa LL, Nakazato RZ, Codaro EN, Felipe H. Electrochemical and microstructural study of Ni-Cr-Mo alloys used in dental prostheses. Mater Sci Appl. 2011; 2:42-8. https://doi.org/10.4236/msa.2011.21006

34. Wataha JC. Biocompatibility of dental casting alloys: a review. The Journal of prosthetic dentistry. 2000; 83(2):223-34. https://doi.org/10.1016/S0022-3913(00)80016-5

35. Ardelean L, Bortun C, Motoc MA. Metal-free removable partial dentures made of a thermoplastic acetal resin and two polyamide resins. Materiale Plastice. 2007; 44(4):345-8.

36. Yu H, Huang W. Category design and clinical application of esthetic clasps. Hua xi kou qiang yi xue za zhi = Huaxi kouqiang yixue zazhi = West China journal of stomatology. 2012; 30(5):447-52.

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Dental Science

Evaluation of the Effect of Combined Low Energy Laser Application and Micro-Osteoperforations versus the Effect of Application of Each Technique Separately On the Rate of Orthodontic Tooth Movement

Ahmed Nasef Abdelhameed[1*], Wael Mohamed Mubarak Refai[2]

[1]Faculty of Dentistry, Minya University, Minya, Egypt; [2]Orthodontic Department, Faculty of Dentistry, Minya University, Minya, Egypt

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***Correspondence:** Ahmed Nasef Abdelhameed. Faculty of Dentistry, Minya University, Minya, Egypt. E-mail: ahmed.nasef@mu.edu.eg

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Abstract

AIM: The study was conducted to evaluate the effect of combined low energy laser application and Micro-Osteoperforations versus the effect of the application of each technique separately on the rate of orthodontic tooth movement.

PATIENTS AND METHODS: Three parallel groups (each group contained 10 patients) were performed; Group A: In which one side was controlled side, and the other side received micro-osteoperforations (MOPs), Group B: In which one side was controlled side, and the other side received low-level laser therapy (LLLT), Group C: In which one side was controlled side, and the other side received both MOPs and LLLT.

RESULTS: Significant statistical differences were obvious in the rate of canine retraction between each intervention and the control sides as following; the MOPs increased the rate of canine retraction by 1.6 fold more than the control side, LLLT increased the rate of canine retraction by 1.3 fold than the control side, and combination of both techniques resulted in an increase in the rate of canine retraction by 1.8 fold more than the control side.

CONCLUSION: Combination of MOPs and LLLT proved to be more efficient regarding increasing the rate of canine retraction than the application of each technique separately.

Introduction

Orthodontic treatment usually requires a long duration of about 2-3 years [1], which poses a high risk of caries [2], external root resorption [3], and decreased patient compliance [4]. Several methods are used to accelerate orthodontic tooth movement and shorten the duration of orthodontic treatment. Varieties of Surgical (corticotomy and micro-osteo perforation) and physical (electric current and LASER) methods were proposed based on our understanding of the biology of OTM [5].

Surgical corticotomy is one of the popular and widely used techniques to accelerate OTM, manipulation of anchorage, facilitating molar intrusion and molar distalization [6]. Although different surgical corticotomy techniques were attempted by many investigators [7] [8] [9], a Regional acceleratory phenomenon (RAP) is the main basic effect of corticotomy in accelerating OTM [10] [11].

In spite of all these facts, corticotomy is still an invasive surgical treatment which may cause some side effects such as, post-operative bleeding, pain, and negative impact on patient quality of life [12]. So, many other surgical less invasive techniques appeared to minimize these side effects. One of these

less invasive surgical techniques is micro-osteo perforation (MOP) [13].

Micro-osteo perforation is a surgical less invasive technique which can accelerate OTM creating predictable results. MOP can be completed chairside in a minute and does not require any advanced training [14].

Since the development of the first LASER by Maiman in 1960 [15], dental interest in lasers has been high, and research has been continuing into ways to improve dental treatment through laser application. The convenient and versatile nature of laser device has encouraged orthodontists to use it in several applications as, in diagnostic procedures, prevention of white spot lesions, bracket debonding and minor surgical procedures like gingivectomy and frenectomy.

Also, Soft laser therapy is a special category of laser application in orthodontic treatment. It is known as Low-Level energy Laser Therapy (LLLT) or as cold laser therapy. The discovery of the bio-stimulatory effect of LLLT in 1967 paved its way to be used in many indications especially in the acceleration of OTM [16], retention protocols [17], and in pain reduction [18].

From all of the previously mentioned, it was beneficial to compare between micro-osteo perforation as a less invasive surgical technique and LLLT as a non-invasive technique for acceleration of OTM. It was also a point of worthy investigation to combine both techniques aiming that there is a synergistic effect resulting from this combination.

Patients and Methods

30 patients were recruited from the Outpatient Clinic at the Department of Orthodontics, Faculty of Dentistry, Minia University with the following inclusion criteria; Age ranged from 15 to 25 years, from both sex, Healthy general medical condition, Healthy periodontal condition, Malocclusion that requires extraction of the maxillary first premolars, followed by canine retraction (dental full unit class II canine relationship or bi-maxillary protrusion), Normal shape and structure of maxillary canine, with no history of filling or root canal treatment and Normal shape and structure of maxillary first molar.

Full explanation to the patients and or parents was performed regarding the study, interventions, and possible side effects. Informed consent was submitted either by the patients and or parents. All safety precautions were followed during laser application.

At first, all patients were referred to an oral surgeon to perform extraction for the first premolars and wait for 6 weeks as a healing period followed by the beginning of orthodontic treatment till finishing the phase of levelling and alignment. Mini-screws were inserted between 1st molar and 2nd premolar which was used directly for canine retraction. Ligaturing of the upper incisors together by a ligature wire was taken into consideration.

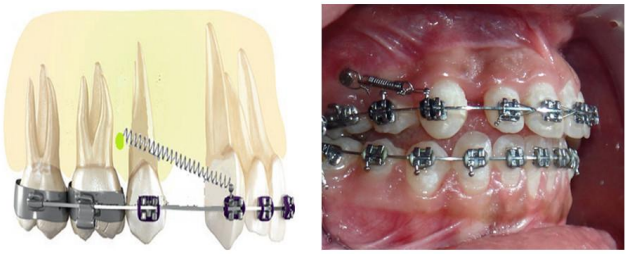

Figure 1: Lateral view of coil spring attached to canine hook and mini-screw

Group (A) contained a split-mouth study design in which one side was controlled side, and the other side received micro-osteoperforations (MOPs). Group (B) contained a split-mouth study design in which one side was controlled side, and the other side received low-level laser therapy (LLLT). Group (C) contained split-mouth study design in which one side was controlled side, and the other side received both MOPs and LLLT.

Assignment of patients and the sides of interventions were performed as following; Computer-generated random numbers was done using Microsoft Office Excel 2013 sheet. All of the 30 patients were firstly randomly assigned to one of the three groups. Then, the right sides of every 10 patients of each group were randomly assigned to be either the intervention side or control side while the left sides were automatically assigned to the choice.

Standardised canine retraction directly on the mini-screw using closing coil spring giving standard force (150 g) assured by usage of force gauge (Figure 1).

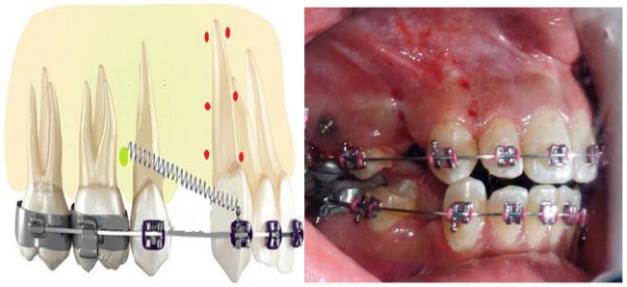

Figure 2: Lateral view showing micro-osteoperforations performed along the root of the canine

In this study, 12 MOPs were applied with a depth of 6 mm and were distributed as follows; Three MOPs were done buccally between the canine and lateral incisor roots. Three MOPs buccally were done between the canine root and the socket of the

extracted premolar. Three MOPs were done palatally between the canine and lateral incisor roots. Three MOPs were done palatally between the canine root and the socket of the extracted premolar. The technique was repeated every two weeks, so MOPs were performed 6 times as the study extended over 3 months (Figure 2).

Micro-osteoperforation tool: The aim was to create multiple pores with a certain depth (range from 3:7mm) in the alveolar bone. So, Mini-screws were used with a 1.6mm diameter and 8mm length to perform the intended perforations. That when the length of mini-screw is 8mm, and the gingival thickness is 2mm, the efficient depth of perforation in the alveolar bone will equal 6mm (Figure 3).

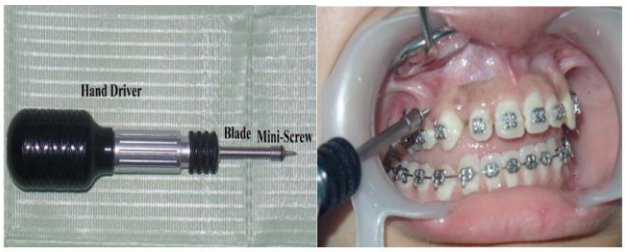

Figure 3: The used MOPs tool

The soft laser was applied using a laser machine (*DenLase-810/7*) (Figure 4) with the following specifications; Dimensions (W x H x D): 130 x 190 x 180 mm, Weight: approx. 1.5 kg, Display: LCD Touch Screen, Cooling: air-cooling, Wavelength: 810 ± 10 nm, Output power: 0.5 W/cm^2, Operation modes: continuous wave (CW).

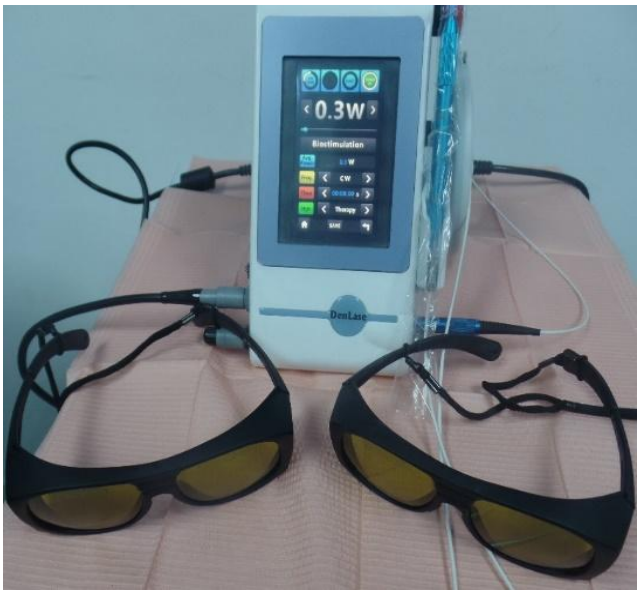

Figure 4: Laser device and the protective eyeglasses

The 1st application was at the beginning of a canine retraction, the 2nd application was after three days from the beginning of a canine retraction, the 3rd application was after one week from the beginning of a canine retraction, the 4th application was after two weeks from beginning of canine retraction, then every two weeks along three months period of the intervention.

Application of laser was carried out from buccal and palatal surfaces along the root of the canine through lens specific for low-level laser therapy and biostimulation (Figure 5).

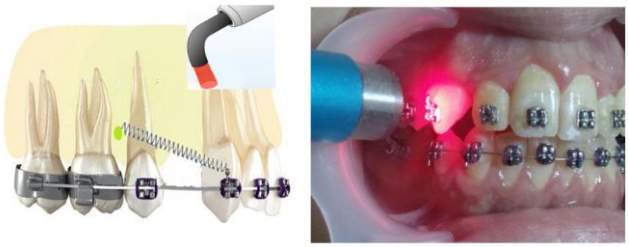

Figure 5: Lateral view showing laser application

Application of both techniques (MOPs and LLLT) was performed following the same protocols mentioned previously**.**

Data for the evaluation of each intervention were collected by direct intra-oral measurements. The measurement was taken from the canine cusp tip to the mesiobuccal cusp tip of the maxillary 1st molar using digital intra-oral calliper (Figure 6). Measurements were taken immediately before the beginning of canine retraction and every two weeks along three months following.

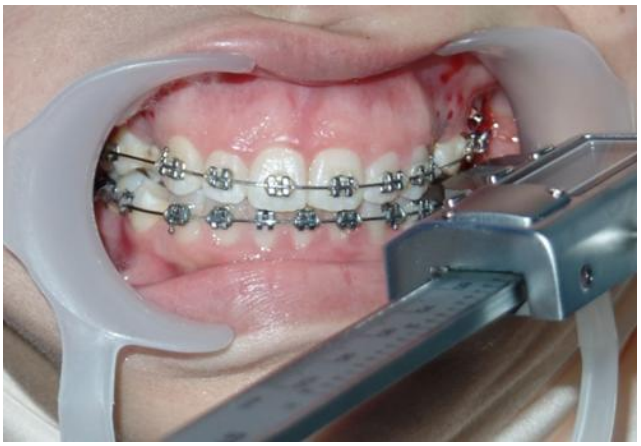

Figure 6: Intra-oral usage of a digital calliper

Results

During the study, there was one dropout patient in (Group C). Also, there was some missing appointments which were all recorded as follow; Group (A), two missing patient appointments at the 4th and 10th weeks. Group (B), one missing patient appointment at the 10th week. Group (C), no missing

patient appointments but there was one dropout patient as mentioned previously.

Measurements were taken every two weeks for three months follow up duration in all groups of the study. The measurements were taken from the canine cusp tip to the 1st molar mesiobuccal cusp tip. Outcomes of the rate of canine retraction showed a normal distribution of data. Consequently, parametric tests were chosen to evaluate the statistical significance of each group (independent sample t-test).

Table 1: Results of independent sample t-test for the mean distance (mm) travelled by the maxillary canine on both control side and MOPs side

Duration	No. of patients	Control side		Experimental side		Independent sample t-test		
		Mean	SD	Mean	SD	Mean Diff.	Std. Error Mean	P- value*
2 weeks	10	0.63 ± 0.62		1.3 ± 0.12		0.67	.00422	0.000
4 weeks	9	1.31 ± 0.23		2.16 ± 0.27		0.85	.00314	0.000
6 weeks	10	1.8 ± 0.66		2.92 ± 0.73		1.12	.00133	0.000
8 weeks	10	1.97 ± 0.76		3.43 ± 0.66		1.46	.00149	0.001
10 weeks	9	2.56 ± 0.83		3.92 ± 0.88		1.36	.00258	0.001
12 weeks	10	2.82 ± 0.39		4.33 ± 0.64		1.51	.09784	0.001

Table 1 and Figure 7 show the independent sample t-test for the mean distance travelled by the maxillary canine on both the control and the experimental (MOPs) sides indicating the highly significant difference.

Figure 7: Graphic representation of the mean distance travelled by the maxillary canine about the baseline in group A

Table 2 and Figure 8 show the independent sample t-test for the mean distance travelled by the maxillary canine on both the control and the experimental (MOPs) sides indicating the highly significant difference.

Table 2: Results of independent sample t-test for the mean distance (mm) travelled by the maxillary canine on both control side and LLLT side

Duration	No. of patients	Control side		Experimental side		Independent sample t test		
		Mean	SD	Mean	SD	Mean Diff.	Std. Error	P- value*
2 weeks	9	0.66 ± 0.55		0.98 ± 0.27		0.32	.00432	0.001
4 weeks	10	1.28 ± 0.48		1.81 ± 0.39		0.53	.003125	0.001
6 weeks	10	1.76 ± 0.83		2.38 ± 0.27		0.62	.00149	0.001
8 weeks	10	1.82 ± 0.63		2.63 ± 0.87		0.81	.02353	0.001
10 weeks	9	2.43 ± 0.23		3.26 ± 0.89		0.83	.06232	0.001
12 weeks	10	2.77 ± 0.37		3.72 ± 0.71		0.95	0.0432	0.001

Figure 8: Graphic representation of the mean distance travelled by the maxillary canine about the baseline in group B

Table 3 and Figure 9 show the independent sample t-test for the mean distance travelled by the maxillary canine on both the control and the experimental (MOPs) sides indicating the highly significant difference.

Table 3: Results of independent sample t-test for the mean distance (mm) travelled by the maxillary canine on both control side and combined MOPs & LLLT side

Duration	No. of patients	Control side		Experimental side		Independent sample t test		
		Mean	SD	Mean	SD	Mean Diff.	Std. Error	P- value*
2 weeks	10	0.66 ± 0.76		1.82 ± 0.19		1.16	.01562	0.000
4 weeks	10	1.42 ± 0.66		2.83 ± 0.12		1.41	.00223	0.000
6 weeks	10	1.73 ± 0.39		3.46 ± 0.64		1.73	.02295	0.000
8 weeks	10	1.91 ± 0.83		3.86 ± 0.27		1.95	.03549	0.000
10 weeks	9	2.46 ± 0.62		4.39 ± 0.73		1.93	.08654	0.00
12 weeks	9	2.79 ± 0.23		4.87 ± 0.88		2.08	.09853	0.000

Discussion

According to Thiruvenkatachari et al. [19] and Aboul-Ela et al., [7], titanium mini-screws provided a simple, efficient anchorage for canine retraction. Direct anchorage during canine retraction using mini-screw placed between 2nd premolar and 1st permanent molar was chosen to eliminate any molar anchorage loss which may give misleading results during measurements.

Figure 9: Graphic representation of the mean distance travelled by the maxillary canine in reference to the baseline in group C

Many techniques [20] [21] [22] are available to perform retraction of the canine in the extraction space regarding anchorage preparation and the force

used of retraction (amount, direction, and force decay). Standardization of the technique of canine retraction was a must so, the use of mini-screws was decided as a direct anchor for retraction of the canine as well as closing coil spring was used providing continuous 150 g of force for canine retraction. This force magnitude was advocated by Barlow and Kula [23], who in a systematic review, concluded that 200 g did not offer any benefit in the rate of canine retraction compared to 150 g.

Direct intra-oral measurements statistical analysis from Group A illustrated that the rate of canine retraction in the MOPs side was higher by nearly 1.6 fold in comparison to standard canine retraction in three months period.

The highest rate was observed during the 1st four weeks measuring nearly 0.9 mm every two weeks which was agreed by other clinical trials [10] [11] [12] and was explained by the accelerator effect of the MOPs procedure which is at its maximum in the 1st month. Wilcko et al., [24], theorised that the rapid orthodontic canine retraction and minimal apical root resorption that accompanied AOO/PAOO were attributable to increased regional bone turn over (the regional acceleratory phenomenon) and the associated osteopenia, i.e. calcium depletion and diminished bone density, precipitated by selective decortication. They further explained that the dynamics of the physiologic tooth movement in these patients might be more appropriately described as bone matrix transportation.

The rate of tooth movement is controlled by osteoclast recruitment and activation [6]. Therefore, regardless of the shape or the extent of the cut, bone resorption will not occur unless osteoclasts are activated. This means that similar to micro-osteoperforations, the effectiveness of corticotomy [8] or piezocision [9] can be related to the activation of cytokines that are released in response to the trauma induced during the cuts. The release of cytokines is expected to be significantly higher in corticotomy and piezocision in comparison with micro-osteoperforations due to the more invasive nature of these procedures and the extensive trauma to the bone.

Direct intra-oral measurements statistical analysis from Group B illustrated that the rate of canine retraction in the LLLT side was higher by nearly 1.3 fold in comparison to standard canine retraction in three months period. The ability of LLLT to accelerate canine retraction can be explained by the effect of LLLT on the receptor activator of the nuclear factor-KB (RANK)/RANK ligand (RANKL)/ osteoprotegerin (OPG) system which is essential for osteoclastogenesis in animals and humans [16] [17] [18].

Reviewing the literature, vast heterogeneity was found in the protocol of LLLT application to accelerate OTM. Although some authors used higher energy density ranging 5:8 J/cm^{2,} e.g. Cruz et al. [25] and Youssef et al., [26]. All previously mentioned investigators used multiple point applications which were on average five on buccal and five on palatal sides, each was applied for 10 seconds.

Regarding the frequency of laser application, Youssef et al., [26] and Cruz et al., [25] used LLLT at 0, 3,7,14 days and they repeated the same frequency of application either after 21 days or 30 days. Genc et al., [27] added two applications to the previous protocol performing 6 applications applied once before the start of anterior teeth retraction as follows: 0, 3, 7, 14, 21, and 28 days. Doshi-Mehta [17] used 4 applications in the 1st month followed by 2 applications per month until complete canine retraction.

Direct intra-oral measurements statistical analysis from Group C illustrated that the rate of canine retraction in the combined MOPs& LLLT side was higher by nearly 1.8 fold in comparison to standard canine retraction in three months period. The increased rate of canine retraction in the combined MOPs &LLLT side more than the application of each technique separately in comparison to the control side can explain the synergistic effect occurring when the two techniques were combined.

In conclusion, both MOPs and LLLT techniques are proved to accelerate the rate of canine retraction during orthodontic treatment. MOPs technique can accelerate the rate of canine retraction more than the application of LLLT as compared to the standard canine retraction technique. Combination of MOPs and LLLT proved to be more efficient regarding increasing the rate of canine retraction than the application of each technique separately.

References

1. Fisher MA, Wenger RM, Hans MG. pretreatment characteristics associated with orthodontic treatment duration. Am J Orthod Dentofac Orthop. 2010; 137:178-82. https://doi.org/10.1016/j.ajodo.2008.09.028 PMid:20152672

2. Bishara SE, Ostby AW. White spot lesions: formation, prevention and treatment. Semin Orthod. 2008; 14:174-82. https://doi.org/10.1053/j.sodo.2008.03.002

3. Pandis N, Nasika M, Polychronopoulou A, Eliades T. External apical root resorption in patients treated with conventional and self-ligating brackets. Am J Orthod Dentofac Orthop. 2008; 134:646-51. https://doi.org/10.1016/j.ajodo.2007.01.032 PMid:18984396

4. Royka A, Denes Z, Razouk G. The relationship between the length of orthodontic treatment and patient compliance. Fogorv Sz. 1999; 92:79-86.

5. Ghada Nimeri, Chung H Kau, Nadia S Abou-Kheir, and Rachel Corona. Acceleration of tooth movement during orthodontic treatment - a frontier in Orthodontics. Prog Orthod. 2013; 14:42. https://doi.org/10.1186/2196-1042-14-42 PMid:24326040 PMCid:PMC4384959

6. Cano J, Campo J, Bonilla E, Colmenero C. Corticotomy assisted orthodontics. J Clin Exp Dent. 2012; 4(1):e54-9.

https://doi.org/10.4317/jced.50642 PMid:24558526 PMCid:PMC3908811

7. Aboul-Ela SM. El-Bialy AR, El-Sayed KMF, Selim EMN, El-Mangoury NH, Mostafa YA. Miniscrew implant-supported maxillary canine retraction with and without corticotomy-facilitated orthodontics. Am J Orthod Dentofac Orthop. 2011; 139(2):252-9. https://doi.org/10.1016/j.ajodo.2009.04.028 PMid:21300255

8. Al-Naoum F, Hajeer MY, Al-Jundi A. Does alveolar corticotomy accelerate orthodontic tooth movement when retracting upper canines? A split mouth design randomized controlled trial. J Oral Maxillofac Surg. 2014; 72(10):1880-9. https://doi.org/10.1016/j.joms.2014.05.003 PMid:25128922

9. Abed S, Al Bustani A. Corticotomy assissted orthodontic canine retraction. J Bagh Coll Dentistry. 2013; 25(1):160-6. https://doi.org/10.12816/0015134

10. Mostafa YA, Mohamed Salah Fayed M, Mehanni S, El Bokle NN, Heider AM. Comparison of corticotomy-facilitayed vs standard tooth-movement techniques in dogs with miniscrews as anchor units. Am J Orthod Dentofac Orthop. 2009; 136(4):570-7. https://doi.org/10.1016/j.ajodo.2007.10.052 PMid:19815161

11. Wang L, Lee W, Lei D-L, Liu Y-P, Yamashita D-D, Yen SL-K. Tissue responses in corticotomy and osteotomy assisted tooth movement in rats: Histology and immunostaining. Am J Orthod Dentofac Orthop. 2009; 136(6): 770.el-11; discussion 770-1.

12. Cassetta M, Di CarloS, Giansanti M, Pompa G, Barbato E. The impact of osteotomy technique for corticotomy assisted orthodontic treatment (CAOT) on oral health related quality life. Eur Rev Med Pharmacol Sci. 2012; 16(12):35-40.

13. Sangsuwon C, Alansari S, Nervina J, Teixeira CC, Alikhani M. Micro-osteoperforations in accelerated orthodontics. Clin Dent Rev. 2018 (s4)1894-017-0013-1.

14. Aksakalli S, Balaban A, Nazaroglu K, Saglam E. Accelerated Tooth Movement with Orthodontic Mini-Screws. Case Rep Dent. 2017; 23(2):75-91. https://doi.org/10.1155/2017/2327591

15. Maiman T. Stimulated optical radiationin ruby lasers. Nature.1960; 187:493-4. https://doi.org/10.1038/187493a0

16. Doshi-Mehta G, Bhad-Patil W. Efficacy of low intensity laser therapy in reducing treatment time and orthodontic pain: a clinical investigation. Am J Orthod Dentofac Orthop. 2012; 141(3): 289-97. https://doi.org/10.1016/j.ajodo.2011.09.009 PMid:22381489

17. Cepera F, Torres FC, Scanavini M, Paranhos L, Capelozza Filho L, Cardoso M. Effect low level laser on bone regeneration after rapid maxillary expansion. Am J Orthod Dentofac Orthop. American Association of Orthodontics. 2012; 141(4): 444-50.

18. Ge MK, He WL, Chen J, Wen C, Yin X, Hu Z. Efficacy of low laser therapy for accelerating tooth movement during orthodontic treatment: a systematic review and meta-analysis. Lasers Med Sci. 2014.

19. Thiruvenkatachari B, Ammayappan P, Kandaswamy R. comparison of rate of canine retraction with conventional molar anchorage and titanium implant anchorage. Am J Orthod Dentofacial Orthop. 2008; 134(1)30:5.

20. Keng FY, Quick AN, Swain MV, Herbison P. A comparison of space closure rates between preactivated nickel-titanium and titanium-molybdenum alloy T-loops: a randomized controlled clinical trial. Eur J Orthod. 2012; 34(1):33-8. https://doi.org/10.1093/ejo/cjg156 PMid:21415288

21. Rohit S Kulshrestha, Ragni Tandon, and Pratik Chandra. Canine retraction: A systematic review of different methods used. J Orthod Sci. 2015; 4(1):1–8. https://doi.org/10.4103/2278-0203.149608 PMid:25657985 PMCid:PMC4314834

22. Mohammed H1, Rizk MZ, Wafaie K, Almuzian M. Effectiveness of nickel-titanium springs vs elastomeric chains in orthodontic space closure: A systematic review and meta-analysis. Orthod Craniofac Res. 2018; 21(1):12-19. https://doi.org/10.1111/ocr.12210 PMid:29265578

23. Barlow M, Kula K. Factor's influencing efficiency of sliding mechanics to close extraction space: a systematic review. Orthod Craniofac Res. 2008; 11(2):65-73. https://doi.org/10.1111/j.1601-6343.2008.00421.x PMid:18416747

24. Wilcko WM, Wilcko MT. Accelerating tooth movement: the case for corticotomy-induced orthodontics. Am J Orthod Dentofacial Ortop. 2013; 144(1):4-12. https://doi.org/10.1016/j.ajodo.2013.04.009 PMid:23810038

25. Cruz DR, Kohara EK, Ribeiro MS, Wetter NU. Effects of low intensity laser therapy on the orthodontic movement velocity of human teeth: a preliminary study. Lasers Surg Med. 2004; 35(2):117-20. https://doi.org/10.1002/lsm.20076 PMid:15334614

26. Youssef M, Ashkar S, Hamade E, Gutknecht N, Lampert F, Mir M. The effect of low level laser therapy during orthodontic movement: preliminary study. Lasers Med Sci. 2008; 23(1):27-33. https://doi.org/10.1007/s10103-007-0449-7 PMid:17361391

27. Gence G, Kocadereli I, Tasar F, Kilinc K, El S, Sarkarati B. Effect of low level laser therapy (LLLT) on orthodontic tooth movement. Lasers Med Sci. 2013; 28(1):41-7. https://doi.org/10.1007/s10103-012-1059-6 PMid:22350425

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Dental Science

Microtensile Bond Strength of Composite to Enamel Using Universal Adhesive with/without Acid Etching Compared To Etch and Rinse and Self-Etch Bonding Agents

Hoda Pouyanfar[1], Elaheh Seyed Tabaii[2], Samaneh Aghazadeh[3], Seyyed Pedram Tabatabaei Navaei Nobari[4], Mohammad Moslem Imani[5*]

[1]Department of Restorative Dentistry, Dental Faculty, Kermanshah University of Medical Sciences, Kermanshah, Iran; [2]Orthodontic Department, Qom University of Medical Sciences, Qom, Iran; [3]Private Practice, Tehran, Iran; [4]Students Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran; [5]Department of Orthodontics, Kermanshah University of Medical Sciences, Kermanshah, Iran

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Keywords: Tensile Strength; Dental Adhesives; Acid Etching, Dental; Composite Resins

***Correspondence:** Mohammad Moslem Imani. Department of Orthodontics, Kermanshah University of Medical Sciences, Kermanshah, Iran. E-mail: mmoslem.imani@yahoo.com

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Abstract

AIM: Considering the recent introduction of universal adhesives and the controversy regarding the use/no use of etchant prior to their application, this study sought to assess the microtensile bond strength of composite to enamel using universal adhesive with/without acid etching compared to three-step etch and rinse, two-step etch and rinse and two-step self-etch bonding agents.

METHODS: This in vitro, an experimental study was conducted on 80 extracted sound human molars in five groups (16 each): Scotchbond Universal adhesive (3M) with/without prior etching, Adper Scthe otchbond Multi-Purpose, Single Bond and Clearfil SE Bond. Etching was performed with 37% phosphoric acid for 20 seconds followed by rinsing and drying. The bonding agent was then applied and light-cured. The e-lite composite was bonded to surfaces and light-cured. The teeth were then mounted, sectioned and subjected to microtensile bond strength test in a universal testing machine. The mode of failure was, determined under a stereomicroscope. Data were analysed using one-way ANOVA followed by Tukey's test.

RESULTS: Universal adhesive with prior etching yielded the highest bond strength ($P = 0.03$). Pairwise comparisons showed that the bond strength of this group was significantly higher than that of universal adhesive without prior etching ($P = 0.04$). No other significant differences were noted ($P > 0.05$). The modes of failure were significantly different among the groups ($P = 0.003$).

CONCLUSION: Enamel etching with phosphoric acid can significantly increase the bond strength to universal adhesive. Universal adhesive without prior etching provided the bond strength as high as that provided by etching and rinse and self-etch bonding agents.

Introduction

At present, dental adhesive systems are highly popular among dental clinicians due to their ability to bond to enamel and dentin and enabling conservative cavity preparation. The composition of dental adhesive systems undergoes constant development, and new products are continuously introduced to the market [1] [2]. Bonding to enamel is easier, stronger and more durable than that to dentin due to higher mineral content and lower water content of the enamel compared to dentin [3]. Achieving a strong and durable bonding between the restoration and tooth structure is a prerequisite for a successful restoration [3]. On the other hand, bond failure at the tooth-restoration interface causes marginal discolouration, postoperative tooth hypersensitivity, secondary caries and pulp involvement [4] [5]. The mechanism of bonding of dental adhesive systems to the dental substrate is mainly based on the replacement of the lost minerals with resin monomers, resulting in a micromechanical interlocking of polymer in dental substrate [6]. Bonding agents mediate the

bonding of composite to enamel and dentin. Resin bond to enamel is durable and predictable and is based on penetration of resin monomers into microscopic porosities of the enamel surface caused by acid etching and subsequent formation of resin tags [7]. To increase the bond strength to the dental substrate, some modifications have been made in dental adhesives regarding their chemical composition, mechanism of bonding, number of bottles (treatment steps) and their application technique, which affect their clinical efficacy. As a result, several generations of bonding agents are now available in the dental market. The two-step etches and rinses bonding agents include two steps of acid etching and application of bonding agent. In this generation of bonding agents, the primer and bonding agent are supplied together in one bottle [8]. Demand for simplification of adhesive application resulted in the development of self-etch adhesives. The two-step self-etch adhesives do not require a separate etching step and contain advanced acidic monomers such as 4-META and 10-MDP, which confer further hydrophilicity to these adhesives compared to previous generations.

To ensure the etching capability of monomers, water is also present as an ionising agent in the composition of self-etch adhesives [9]. In two-step, self-etch adhesives, etching and priming of dentin and enamel are performed simultaneously (due to the use of acidic primers). These systems have greatly simplified the adhesive application process since they do not require rinsing and drying. As a result, the risk of over-drying or excessive moisture is no longer present [8] [10] [11]. The major superiority of the 7th generation dentin bonding agents over the 6th generation is their single-step application. These bonding agents have one-step application to minimise technical sensitivity, further facilitate the bonding process and prevent the problems related to incomplete penetration of resin tags into the porosities created by etching. As a result, these systems are extremely easy to use and save time [10].

Universal adhesives are used in dentistry in self-etch, etch and rinse or selective etching modes. They can bond to methacrylate-based restorative materials, cement, sealants, dentin, enamel, glass-ionomer and indirect restoration substrates including metals, alumina, zirconia and other ceramics. They all contain acidic monomers and have a similar application pattern as that of self-etch systems. On the other hand, nano-adhesives contain nano-fillers and can form a very strong bond to dentin and enamel. Bonding systems containing nano-fillers are known as universal systems and are applied in self-etch mode. Some studies have compared different generations of bonding agents and have reported controversial results [12] [13] [14]. The quality of bonding of coronal restoration to tooth structure depends on the type of bonding agent used.

Considering the recent introduction of universal adhesives and the controversy regarding the use/no use of etchant prior to their application with regard to their bond strength, this study aimed to assess the microtensile bond strength of enamel to composite following the application of a universal adhesive with/without prior acid etching, a three-step etch and rinse bonding agent, a two-step etch and rinse bonding agent and a two-step self-etch bonding agent.

Material and Methods

This in vitro, an experimental study was performed on 80 extracted sound human molars. The study was approved in the Ethics Committee of Kermanshah University of Medical Sciences (Code: KUMS.REC.1395.426). Minimum sample size was calculated to be 16 in each of the five groups according to a study by Joseph et al. [15], assuming the mean bond strength of 3.35 ± 1.58 MPa for two-step self-etch adhesive and 34.93 ± 2.54 MPa for universal adhesive with 95% confidence interval and 90% study power using PASS version 21.0 software (a total of 80 samples). A total of 80 permanent sound molars extracted within the past 6 months for reasons unrelated to this study were selected using convenience sampling. The teeth were immersed in 0.5% chloramine T solution at room temperature for one week for disinfection. The teeth were then stored in distilled water at room temperature until the experiment [16]. The teeth were inspected under a light microscope to ensure the absence of caries and cracks. The teeth were randomly divided into five groups (n = 16) as follows:

Group 1: The buccal/labial enamel surface was etched with 37% phosphoric acid gel (Ultradent, South Jordan, UT, USA) for 20 seconds followed by 10 seconds of rinsing with water spray and 5 seconds of drying with air spray. Adper Scotchbond Multi-Purpose (3M ESPE, St. Paul, MN, USA) primer was applied on the surface, and after gentle air spray, Adper Scotchbond Multi-Purpose bonding agent was applied. Light curing was performed using a LED light curing unit (Demetron, Kerr, Orange, CA, USA) with a light intensity of 700 mW/cm^2. Before curing, the output power of light curing unit was calibrated using a radiometer. Elite AA composite (Bisco Dental Products, Schaumburg, IL, USA) was then applied in two 2.5 mm-thick increments, and each layer was polymerized for 20 seconds. A mould measuring 9 x 9 x 5 mm was used to standardize the composite thickness in this group.

Group 2: Enamel surface was etched, rinsed and dried as explained in group 1. Single Bond (3M ESPE, St. Paul, MN, USA) bonding agent was then applied in two layers and cured using a LED light

curing unit. Elite AA composite (Bisco Dental Products, Schaumburg, IL, USA) was then applied in two 2.5 mm-thick increments, and each layer was polymerized for 20 seconds. A mould measuring 9 x 9 x 5 mm was used to standardise the composite thickness.

Group 3: Two layers of Clearfil SE Bond primer were first applied on the enamel surface and dried with gentle air spray for 5 seconds. One layer of Clearfil SE Bond bonding agent was then applied on the surface and cured using a LED light curing unit. Elite AA composite (Bisco Dental Products, Schaumburg, IL, USA) was applied in two 2.5 mm-thick increments, and each layer was polymerised for 20 seconds. A mould measuring 9 x 9 x 5 mm was used to standardise the composite thickness.

Group 4: Scotchbond Universal adhesive (3M ESPE, St. Paul, MN, USA) was applied on the surface of samples without etching and cured using a LED light curing unit. Elite AA composite (Bisco Dental Products, Schaumburg, IL, USA) was applied in two 2.5 mm-thick increments, and each layer was polymerised for 20 seconds. A mould measuring 9 x 9 x 5 mm was used to standardise the composite thickness.

Group 5: The enamel surface was first etched with 37% phosphoric acid gel, and then Scotchbond Universal adhesive (3M ESPE, St. Paul, MN, USA) was applied and cured using a LED light curing unit. Elite AA composite (Bisco Dental Products, Schaumburg, IL, USA) was applied in two 2.5 mm-thick increments, and each layer was polymerised for 20 seconds. A mould measuring 9 x 9 x 5 mm was used to standardise the composite thickness.

Table 1 shows the composition of adhesives used in this study. The teeth in each group were mounted in acrylic resin perpendicular to the horizon and parallel to each other and composite was subsequently bonded to them as described earlier. After immersion in distilled water at room temperature for 24 hours, the teeth were sectioned into slices measuring 1 x 1 mm with 8 mm height by a low-speed diamond disc of a microtome (Isomet, Buehler Ltd., Bluff, IL, USA) such that the disc was perpendicular to the tooth surface. The sections of each group were separately immersed in distilled water and incubated at 37°C and 100% humidity for 24 hours.

Table 1: Composition of adhesives used in this study

Adhesive	Composition
Clearfil SE Bond (Kuraray, Tokyo, Japan)	Primer: water, MDP, HEMA, CQ, DET, hydrophilic DMA Bond: MDP, bis-GMA, HEMA, hydrophobic DMA, CQ, DET, silanated colloidal silica
Adper Scotchbond Multi-Purpose (3M ESPE, St. Paul, MN, USA)	10-MDP methacrylate resin, HEMA, Ethanol, Water, Polyacrylic Acid Copolymer, Silane, Fillers, Initiators
Single Bond Universal Adhesive (3M ESPE, St. Paul, MN, USA)	Adhesive: MDP phosphate monomer, methacrylate resins, HEMA, silane methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators
Scotchbond Universal adhesive 3M	MDP, bis-GMA, HEMA, ethanol, water, initiators

MDPB: 12-methacryloyloxydodecylpyridinium bromide; bis-GMA: bisphenol A diglycidylmethacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: 10-methacryloyloxydecyl; dihydrogen phosphate; DMA: dimethacrylate; DET: N,N-diethanol p-toluidine; CQ: camphorquinone.

For measurement of microtensile bond strength, each sample was fixed to the plate of a universal testing machine (Z020, Zwick GmbH & Co. KG, Germany) using cyanoacrylate glue. The load was applied at a crosshead speed of 0.5 mm/minute until failure. The load at failure was recorded in Newtons (N).

The interface area of the broken piece was measured by a gauge. The load at failure in Newtons was divided by the enamel/composite interface area in square millimetres (mm^2) to obtain the bond strength in megapascals (MPa).

The samples were then inspected under a stereomicroscope (Olympus, SZX9, Iran) at (x 10) magnification to determine the mode of failure. The mode of failure was classified into three groups of cohesive within the tooth structure, cohesive within the composite and mixed (Figure 1).

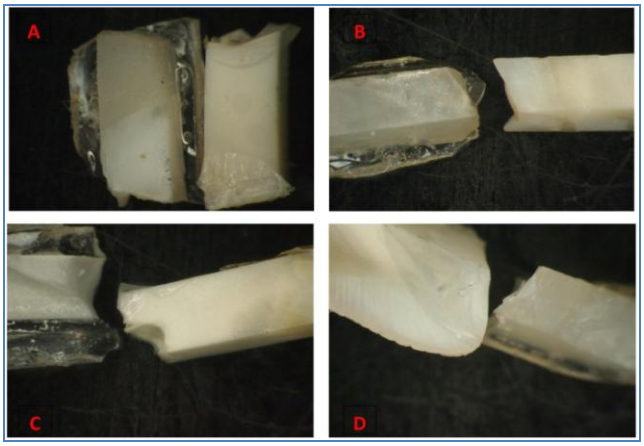

Figure 1: A) Cohesive failure in tooth structure in a universal adhesive group with an etchant; B) cohesive failure of the composite in a universal adhesive group with an etchant; C) cohesive failure of the composite in the universal adhesive group without etchant; D) cohesive failure in tooth structure in the universal adhesive group without etchant

Data were analysed using SPSS version 21 (SPSS Inc., IL, USA). Normal distribution of data was assessed using the Shapiro-Wilk test. Homogeneity of variances was assessed using the Levene's test. One-way ANOVA was applied to assess the difference in microtensile bond strength of the groups. Pairwise comparisons were carried out using Tukey's test. The modes of failure were compared among the groups using the chi-square test. $P < 0.05$ was considered significant.

Results

According to the Shapiro-Wilk test, the microtensile bond strength data were normally distributed ($P > 0.05$). The assumption of homogeneity

of variances according to the Levene's test was met (P = 0.17).

Maximum microtensile bond strength was obtained by the use of universal adhesive after etching while the minimum microtensile bond strength was noted in the use of universal adhesive without etchant (Table 2). According to one-way ANOVA, the difference in microtensile bond strength of the groups was significant (P = 0.03).

Table 2: Mean microtensile bond strength in the five groups (n=16)

Bonding agent	Mean	Standard deviation	Minimum	Maximum	Median
Scotchbond Universal 3M with etchant	65.75b	32.13	20.8	134.8	60.1
Adper Scotchbond 3M	45.81ab	19.82	15.07	84.3	41.85
Single Bond 3M	45.52ab	21.84	10.2	79.5	45.45
Clearfil SE Bond	44.91ab	15.92	23.3	76.7	46.65
Scotchbond Universal 3M without etchant	42.75a	19.79	11.16	80.0	38.6

Mean values with lowercase letters indicate no significant difference in pairwise comparisons.

Thus, Tukey's test was applied for pairwise comparisons (Table 3) and showed that only the difference in microtensile bond strength of universal adhesive with the etchant and universal adhesive without etchant was significant (P = 0.04). No other significant differences were noted in pairwise comparisons (P > 0.05).

Table 3: Pairwise comparison of groups regarding microtensile bond strength

First bonding agent	Second bonding agent	Mean difference	P-value
Single Bond 3M	Clearfil SE Bond	0.61	1.0
	Universal adhesive with an etchant	20.23	0.09
	Universal adhesive without etchant	2.77	0.99
	Adper Scotchbond	0.29	1.0
Clearfil SE Bond	Universal adhesive with an etchant	20.84	0.07
	Universal adhesive without etchant	2.16	0.99
	Adper Scotchbond	0.91	1.0
Universal adhesive with an etchant	Universal adhesive without etchant	22.99	0.04
	Adper Scotchbond	19.93	0.09
Universal adhesive without etchant	Adper Scotchbond	3.06	0.99

According to the chi-square test, the difference in modes of failure among the groups was significant (P = 0.003) such that cohesive failure within the composite had a significantly higher frequency in Clearfil SE Bond and universal adhesive without etchant groups while cohesive failure in tooth had a higher frequency in universal adhesive with etchant and Scotchbond groups (Table 4).

Table 4: Frequency of the modes of failure in the groups

Mode of failure	Universal adhesive with an etchant	Scotchbond	Single Bond	Clearfil SE Bond	Universal adhesive without etchant
In tooth	9 (56.3%)	9 (56.3%)	8 (50.0%)	0	4 (25.0%)
In composite	7 (43.8%)	7 (43.8%)	8 (50.0%)	16 (100%)	12 (75.0%)

The frequency of fractures in teeth and composite was the same in the Single Bond group (Figure 2).

Discussion

This study assessed the microtensile bond strength of enamel to composite following the use of a universal adhesive with/without prior etching, three-step etch and rinse (Scotchbond), a two-step etch and rinse (Single Bond) and two-step self-etch (Clearfil SE Bond) bonding agents. The results showed that the microtensile bond strength was significantly higher in the universal adhesive with prior etching group (65.75 MPa).

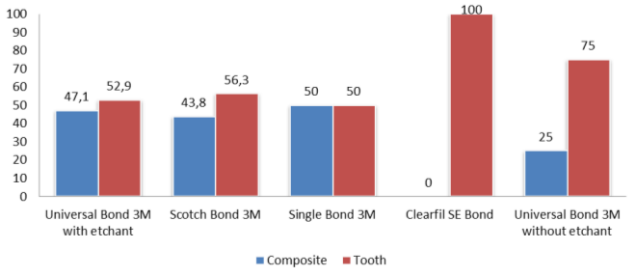

Figure 2: Frequency percentage of different modes of failure in the five groups

Pairwise comparisons showed that this group had a significant difference with universal adhesive without prior etching group. No other significant differences were noted in the bond strength among the groups. The results showed that enamel etching with phosphoric acid before the application of universal adhesive significantly increased the bond strength of enamel to composite (compared to other groups), which indicates that acid etching before the application of different bonding agents is still an acceptable method to increase the bond strength of enamel to composite. On the other hand, a universal adhesive with no prior enamel etching yielded the bond strength as high as that provided by three-step etch and rinse, a two-step etches and rinse and two-step self-etch systems. This indicates that despite the elimination of the etching step, universal adhesive yielded the bond strength as high as that of other systems.

Acid etching is a commonly used method to roughen the enamel surface and increase the bond strength of adhesive materials to enamel. Significantly higher bond strength in the universal adhesive with prior acid etching group is probably due to the formation of porosities in the enamel surface and penetration of resin into the porosities. Evidence shows that resin tags can be as long as 15-20 μ at the resin-acid etched enamel interface [17] [18]. On the

other hand, evidence shows that acid etching creates a honeycomb pattern in the enamel surfaces, which results in micromechanical retention [19]. Acid etching removes about 10 μ from the enamel surface and creates a 5-50 μm thick porous layer [19].

On the other hand, application of self-etch adhesives on the enamel is associated with some concerns [20] [21]. The superficial etching pattern of the enamel and reduction of micromechanical retention can negatively affect the bond strength and durability [22]. Also, it is not known whether application of self-etch adhesives with moderate pH on the enamel can provide adequate mechanical and chemical resistance in the oral environment as do the etch and rinse adhesives. Further investigations are warranted to answer this question.

In line with our study, van Landuyt et al., [23] indicated that enamel surface preparation by acid etching significantly increases the bond strength. However, it has been reported that acid etching decreases the bond strength to dentin [24]. New self-etch adhesives are mainly categorised as mildly acidic adhesives, and enamel preparation by acid etching before their application can significantly increase their bond strength [25]. Thus, it should be noted that simplifying the process of adhesive application by eliminating the etch and rinse steps (as in universal adhesives) although enhances the bonding process, does not necessarily result in higher clinical success rate. Further studies are required to elucidate this topic better.

Joseph et al., [15] compared the microtensile bond strength of Clearfil SE Bond two-step self-etch adhesive, Adper Easy One 7th generation bonding agent and Futurabond universal adhesive and reported that universal adhesive had the highest bond strength followed by the two-step self-etch bonding agent. The 7th generation bonding agent ranked last regarding the bond strength. This finding was in agreement with our results since universal adhesive with prior etching yielded the highest bond strength in our study.

On the other hand, Souza-Saroni et al., [26] evaluated the microtensile bond strength of Clearfil Liner Bond 2V, Prime and Bond NT/NRC, Single Bond and All Bond 2 to enamel and reported that they all provided almost similar bond strength values. In our study, all adhesives yielded equal bond strength values to enamel except for universal adhesive with prior etching. High bond strength provided by universal adhesive can be due to the presence of nano-size cross-linkers in its composition. Clearfil SE Bond had high bond strength close to that of other groups (except for universal adhesive with prior etching). This can be due to the presence of 10-MDP in its composition, which enhances its chemical bond to hydroxyapatite crystals [27].

Clearfil SE Bond is a two-step self-etch adhesive with a mildly acidic pH (1.8). Some authors consider it as the gold standard for self-etch adhesives [28]. Two-step self-etch adhesives such as Clearfil SE Bond have separate bottles for resin and primer and therefore are more hydrophobic. Thus, they can provide high bond strength in contrast to one-step self-etch adhesives. Favourable in vitro and clinical results related to the application of this adhesive can be attributed to its double bonding mechanism. Its mild pH can result in the formation of a micromechanical bond by creating a thin and uniform hybrid layer. It can resist debonding forces and shear loads applied during shear bond strength testing. It contains 10-MDP functional monomer and can, therefore, form a stable chemical bond with hydroxyapatite. It is resistant to hydrolytic degradation and seals the restoration margins for a long period [29].

Single Bond is a two-step etch and rinse adhesive that provided the bond strength as high as that of other groups in our study. It contains polymerising resin monomers dissolved in acetone or ethanol. It is reportedly suitable for bonding to enamel [30], and our results confirmed this statement.

Formulation of adhesive systems plays an important role in the performance and clinical service of dental materials. Universal and self-etch adhesives are generally less acidic and therefore have lower efficacy for demineralisation of the mineral phase of enamel and subsequent provision of micromechanical retention [31]. Type and amount of solvent and composition and percentage of monomers in adhesive systems as well as thinning agents can all affect the bond strength. The amount of filler and percentage of mass load are also variable in different bonding agents. The manufacturers do not disclose the exact composition of their products. Moreover, information regarding the rate of shrinkage and hardness of adhesives after polymerisation is limited [25].

Universal adhesive with/without prior acid etching was used in this study. Universal adhesives are self-etch and dual-core and have a pH of 2.2 to 3.2. Generally, self-etch universal adhesives with mild or very mild etching capability may not be able to provide adequately high bond strength to enamel [32]. Despite some concerns in this regard, the bond strength of universal adhesives has been reported to be acceptably high [33]. Our results suggested selective etching of enamel with phosphoric acid to increase the bond strength of the universal adhesive to enamel. This has also been suggested by another study [32]. However, some concerns exist in this respect. For example, the risk of excessive etching of dentin still exists due to the inability to precisely control the area and subsequent reduction of the bond strength to dentin [33].

Evidence shows that bond strength to enamel or dentin more than 20 MPa results in mainly cohesive failure in the dental substrate or composite [9] [34]. In our study, all failures were within the composite or

tooth. In Clearfil SE Bond, all fractures were within the composite and only in Scotchbond group, the percentage of fractures in tooth structure was slightly higher than that within the composite.

In the present study, the bond strength was measured using microtensile bond strength test. Application of microtensile load results in better stress distribution at the adhesive interface compared to conventional tensile or shear loads and yields more accurate results with less diversity [35] [36].

This test enables better stress distribution due to the smaller interface area, which was 1 mm^2 in our study. However, the bond strength tests, in general, are only suitable for ranking of adhesives because many factors such as masticatory loads, pH alterations and thermal changes are present in the oral environment and affect the bond strength of adhesives to tooth structure. Thus, the results of bond strength tests in vitro cannot well predict the performance of adhesives in the clinical setting [37]. Clinical studies are required to cast a final judgment in this regard. Also, the bond strength of different adhesives to dentin should be compared in future studies.

In conclusion, within the limitations of this study, the results showed that phosphoric acid etching of the enamel before the application of universal adhesive yielded the bond strength significantly higher than that of other groups. Universal adhesive without prior etching yielded the bond strength as high as that of two-step etch and rinse and two-step self-etch bonding agents. This finding highlights the optimal efficacy of universal adhesive in the provision of optimal bond strength with the simplified application.

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Author’s contribution

HP and MMI conceptualised this study, designed the methodology for data collection, collected and analysed the data, and wrote the manuscript. EST and SA extensively supported the development of the study concept, data analysis, and the writing, editing and finalising of the manuscript. SPTNN assisted in data entry and analysis. All authors read and approved the final manuscript.

References

1. Nunes MF, Swift EJ, Perdigao J. Effects of the adhesive composition on microtensile bond strength to human dentin. Am J Dent. 2001; 14(6):340-3. PMid:11949791

2. Frankenberger R, Predigao J, BT Rosa, M Lopes. No bottle vs multi-bottle dentin adhesives – a microtensile bond strength and morphological study. Dent Mater. 2001; 17(5):373-80. https://doi.org/10.1016/S0109-5641(00)00084-1

3. Swift EJ, Perdigao J. Bonding to enamel & dentin a brief history & state of the art. Quintessence Int. 1995; 26(2):95-110. PMid:7568728

4. Yoshikawa T, Sano H, Burrow MF, Tagami J, Pashley DH. Effects of dentin depth and cavity configuration on bond strength. J Dent Res. 1999; 78(4):898-905. https://doi.org/10.1177/00220345990780041001 PMid:10326734

5. Armstrong SR, Keller JC, Boyer DB. Mode of failure in the dentin-adhesive resin composite bonded joint as determined by strength based (MTBs) and fracture-based (CNSB) mechanical testing. Dent Mater. 2001; 17(3):201-10. https://doi.org/10.1016/S0109-5641(00)00070-1

6. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res. 1982; 16(3):265-73. https://doi.org/10.1002/jbm.820160307 PMid:7085687

7. Kimochi T, Yoshiyama M, Urayama A, Matsuo T. Adhesion of new commercial self-etching/self-priming bonding resin to human caries-infected dentin. Dent Mater. 1999; 18(4):437-43. https://doi.org/10.4012/dmj.18.437

8. Robbins JW, Hilton TJ, Schwartz RS, dos Santos Jr J. Fundamentals of operative dentistry: a contemporary approach. Summitt JB, editor. Quintessence Pub.; 2006.

9. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, Vanherle G. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent. 2003; 28(3):215-35. PMid:12760693

10. Roberson T, Heymann H, Swift E, Sturdevant C. Chap5. Sturdevant's art and science of operative dentistry. 4th Ed. Mosby Co., 2002:263-97.

11. Peumans M, Kanumilli P, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Clinical effectiveness of contemporary adhesives: A systematic review of current clinical trials. Dent Mater J. 2005; 21(9):864-81. https://doi.org/10.1016/j.dental.2005.02.003 PMid:16009415

12. Powers JM, Farah JW. Technique sensitivity in bonding to enamel and dentin. Compend Contin Educ Dent. 2010; 31 (Spec No 3):1-8; quiz 9. PMid:21053440

13. Ishikawa A, Shimada Y, Foxton RM, Tagami J. Micro-tensile and micro-shear bond strengths of current self-etch adhesives to enamel and dentin. Am J Dent. 2007; 20(3):161-6. PMid:17672257

14. McDonough WG, Antonucci JM, He J, Shimada Y, Chiang MY, Schumacher GE, et al. A micro shear test to measure bond strengths of dentin-polymer interfaces. Biomaterials. 2002; 23(17):3603-8. https://doi.org/10.1016/S0142-9612(02)00089-3

15. Joseph P, Yadav C, Satheesh K, Rahna R. Comparative evaluation of the bonding efficacy of sixth, seventh and eighth generation bonding agents: an in vitro study. Int Res J Pharm. 2013; 4(9):143-9.

16. Munoz MA, Luque I, Hass V, Reis A, Loguercio AD, Bombarda NHC. Immediate bonding properties of universal adhesive to dentine. J Dent. 2013; 41(5):404-11. https://doi.org/10.1016/j.jdent.2013.03.001 PMid:23499568

17. Gwinnett AJ, Marsui A. A study of enamel adhesives. The physical relationship between enamel and adhesive. Arch Oral Biol. 1967; 12:1615. https://doi.org/10.1016/0003-9969(67)90195-1

18. Buonocore MG, Matsui A, Gwinnett AH. Penetration of resin dental materials into enamel with reference to bonding. Arch Oral Biol. 1968; 13(1):61-70. https://doi.org/10.1016/0003-9969(68)90037-X

19. Sharpe AN. Influence of crystal orientation in human enamel on its reactivity to acid as shown by high resolution microradiography. Arch Oral Biol. 1967; 12(5):583. https://doi.org/10.1016/0003-9969(67)90077-5

20. Tay FR, Pashley DH, King NM, Carvalho RM, Tsai J, Lai SC, et al. Aggressiveness of self-etch adhesives on unground enamel. Oper Dent. 2004; 29(3):309-16. PMid:15195732

21. Pashley DH, Tay FR. Aggressiveness of contemporary self-etching adhesives. Part II: etching effects on unground enamel. Dent Mater. 2001; 17(5):430-44. https://doi.org/10.1016/S0109-5641(00)00104-4

22. Miyazaki M, Sato M, Onose H. Durability of enamel bond strength of simplified bonding systems. Oper Dent. 2000; 25(2):75-80. PMid:11203803

23. Van Landuyt KL, Kanumilli P, De Munck J, Peumans M, Lambrechts P, Van Meerbeek B. Bond strength of a mild self-etch adhesive with and without prior acid-etching. J Dent. 2006; 34(1):77-85. https://doi.org/10.1016/j.jdent.2005.04.001 PMid:15979226

24. Erhardt MC, Cavalcante LM, Pimenta LA. Influence of phosphoric acid pretreatment on self-etching bond strengths. J Esthet Restor Dent. 2004; 16(1):33-40. https://doi.org/10.1111/j.1708-8240.2004.tb00448.x PMid:15259541

25. Yaseen SM, Subba Reddy VV. Comparative evaluation of shear bond strength of two self-etching adhesives (sixth and seventh generation) on dentin of primary and permanent teeth: An in vitro study. J Indian Soc Pedod Prevent Dent. 2009; 27(1):33-8. https://doi.org/10.4103/0970-4388.50814 PMid:19414972

26. Souza-Zaroni WC, Seixas LC, Ciccone-Nogueira JC, Chimello DT, Palma-Dibb RG: Tensile bond strength of different adhesive systems to enamel and dentin. Braz Dent J. 2007; 18(2):124-8. https://doi.org/10.1590/S0103-64402007000200007

27. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, et al. Comparative study on adhesive performance of functional monomers. J Dent Res. 2004; 83(6):454-8. https://doi.org/10.1177/154405910408300604 PMid:15153451

28. Sarr M, Kane Aw, Vreven J, Mine A, Van Lanudyt KL, Peumans M. Micro-tensile bond strength and interfacial characterization of contemporary adhesives bonded to bur cut dentin. Oper Dent. 2010; 35(1):94-104. https://doi.org/10.2341/09-076-L PMid:20166416

29. Abdalla AL, El Zohairy AA, Abdel Mohsen MM, Feilzerd AJ. Bond efficacy and interface morphology of self-etching adhesives to ground enamel. J Adhes Dent. 2010; 12(1):19-25. PMid:20155226

30. Swift E, Perdigao J, Heymann H. Enamel bond strengths of one-bottle adhesives. Pediatr Dent. 1998; 20:256-262.

31. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, et al. State of the art of etch-and-rinse adhesives. Dent Mater. 2011; 27(1):1-16. https://doi.org/10.1016/j.dental.2010.10.016 PMid:21112620 PMCid:PMC3857593

32. Hanabusa M, Mine A, Kuboki T, Momoi Y, Van Ende A, Van Meerbeek B, et al. Bonding effectiveness of a new 'multi-mode'adhesive to enamel and dentine. J Dent. 2012; 40(6):475-84. https://doi.org/10.1016/j.jdent.2012.02.012 PMid:22381614

33. Mena-Serrano A, Kose C, De Paula EA, Tay LY, Reis A, Loguercio AD, et al. A new universal simplified adhesive: 6-month clinical evaluation. J Esthet Restor Dent. 2013; 25(1):55-69. https://doi.org/10.1111/jerd.12005 PMid:23374411

34. Kahveci O, Belli S. Composite bond strength to intact enamel with current simplified adhesives. J Adhes Dent. 2011; 13(1):31-7. PMid:21403934

35. Cardoso PEC, Braga RR, Carrilho MRO. Evaluation of a microtensile, shear and tensile tests determining the bond strength of three adhesive systems. Dent Mat. 1999; 14(6):394-398. https://doi.org/10.1016/S0300-5712(99)00012-3

36. Pashley DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Shono Y, et al. The microtensile bond test: a review. J Adhes Dent. 1999; 1(4):299-309. PMid:11725659

37. Braga RR, Meira JB, Boaro LC, Xavier TA. Adhesion to tooth structure: a critical review of macro test methods. Dent Mater. 2010; 26(2):e38-49. https://doi.org/10.1016/j.dental.2009.11.150 PMid:20004960

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Dental Science

The Effect of Gates-Glidden Drills on the Quality of Root Canal Treatment by Pre-Clinical Dental Students

Roohollah Sharifi[1], Amin Torabi[2], Reza Hatam[1], Nafiseh Nikkerdar[3], Hamid Reza Mozaffari[4,5], Mohsen Safaei[6*], Seyed Mojtaba Amiri[7]

[1]Department of Endodontics, School of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran; [2]Students Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran; [3]Department of Oral and Maxillofacial Radiology, School of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran; [4]Department of Oral and Maxillofacial Medicine, School of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran; [5]Medical Biology Research Centre, Kermanshah University of Medical Sciences, Kermanshah, Iran; [6]Oral and Dental Sciences Research Laboratory, School of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran; [7]Department of Biostatistics and Epidemiology, School of Health, Kermanshah University of Medical Sciences, Kermanshah, Iran

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***Correspondence:** Mohsen Safaei. Oral and Dental Sciences Research Laboratory, School of Dentistry, Kermanshah University of Medical Sciences, Kermanshah, Iran. E-mail: mohsen_safaei@yahoo.com

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Abstract

AIM: This study was conducted to investigate the effect of applying Gates-Glidden (GG) drill by pre-clinical dental students on root canal treatment quality.

METHOD: A total of 56 first molars consisting of 168 canals were selected in this study. For this purpose, 56 students who had been formerly trained by two methods of root canal preparation were randomly divided into two groups (n = 28). Group 1: the step-down method by GG and Group 2: step-back technique without GG. The prepared teeth were filled with gutta-percha/ZOE sealer using lateral condensation. Periapical radiographs were taken before and the following treatment to survey occurrence of preparation errors and CBCT images to determine residual dentine at furcation region.

RESULTS: The findings showed that among 10 error types in specimens prepared by students, the occurrence of underfilling, overfilling, inappropriate, ledge formation, and single cone was more common without GG. There were no significant differences in residual dentine amount at furcation region between preparation with and without using GG ($P > 0.05$).

CONCLUSION: Using GG for root canal preparation by dental students resulted in low errors and not an increased dentine removal risk.

Introduction

One of the most important parts in the treatment of root canal is cleaning and shaping it to eliminate the wound and maintain the original shape of the canal [1]. An ideally prepared canal should form a uniform cone between the apical third and cervical third [2].

Technical errors or the problems associated with root canal instrumentation are the factors causing root canal treatment failure [3]. Despite scientific developments and technological advances, the biomechanical instrumentation techniques and new files are still limited regarding performance and efficiency [4], [5]. Among the instruments used for canal preparation, Gates-Glidden (GG) drill is extensively used for the preparation of direct areas of root canal owing to high cutting capability, easy application, and low price [6]. Additionally, GG drills are deliberately designed to separate near the hand-piece to facilitate the removal of a fractured drill piece from the root canal [7]. GG may be necessary for access into the root canal to provide a direct path into orifices without weakening the remaining structure. Applying GG widens the cervical area, making it possible for larger files to penetrate the apical area

[8]. However, there are opposing opinions about GG. Wu et al., [9] reported that the preparation of curved canals by GG drills might reduce the thickness of dentin and increase the risk of perforation. Flores et al., [10] reported no significant differences in the four instruments of GG, Largo, LA-Axxess and Cpdrill about the removal of canal dentin. Also, GG drill was introduced as an acceptable instrument regarding preserving the thickness of root dentin during canal preparation, which did not remarkably reduce the thickness of the remaining dentin [11].

Acquiring enough skills to perform the desired RCT by undergraduate dental students has been the subject of intense research among the scholar and university instructors [12], [13], [14]. For example, at Kermanshah School of Dentistry, Iran, undergraduate students must complete a pre-clinical Endodontics course that includes both theoretical (seminars) and practical training (training of the manual files and GG drills).

This research aims to investigate the effect of using GG drill on the quality of root canal treatment by pre-clinical educational undergraduate students. In the present study, the quality of the performed treatments regarding the incidence of various technical errors was analysed by periapical radiographs, and the remaining dentin was measured by cone beam computed tomography (CBCT).

Material and Methods

In this quasi-experimental study, the samples were selected from among the extracted mandibular and maxillary molars using the simple sampling technique. Students participating in this project include 56 pre-clinical dental students in Kermanshah school of Dentistry, Iran. First, root canal treatment techniques with and without GG drills were instructed to the students theoretically and practically. Then, one tooth was randomly given to each student. The students were divided into two groups each having 28 members (n = 28). One group of students prepared one tooth by GG drills using the step-down method, and another group prepared tooth through the step-back technique without using GG drills.

A total number of 56 extracted maxillary and mandibular first molars were collected. The teeth without cracks, internal or external root resorption and the curvature of each root canal were measured according to Schneider's method [15], and those with < 25° curvature and < 22 mm length were included in the study. The teeth were kept in 0.5% chlorine solution until the initiation of the experiment. Before the treatment, the tissue residues and dental calculus were removed by a scalpel. The blocks of self-curing acrylic immediately were combined with sawdust and plaster with dimensions of 2 x 2 x 2.5 cm and a piece of wax (3 x 3 mm) was mounted at the end of the apex. The samples were placed on a flat surface by two pieces of 3 x 10 mm wax in an arch shape, and the position of the samples was marked on the plate to assimilate the teeth at the time of taking CBCT radiographs (for evaluation of the remaining dentin at the surroundings of furcation). Before treatment, CBCT radiographs were taken from the samples (10 samples in each stage), and volumetric CBCT scan was performed on each tooth with a high resolution [12 x 8] field of view, 5.4 second exposure time, 3.07 mA and 110 kV by VGI New Tom machine (Italy). Cross-sections (0.5 mm thickness and 0.1 mm interval) were prepared from each tooth. Also, a periapical radiograph was taken by an X mind machine (Italy) using the parallel technique (8 mA and 70 kV) from buccolingual direction.

Standard access cavities were prepared using diamond burs connected to a high-speed handpiece under constant water spray. The length of the canal was determined by k-files, sizes 10 and 15. To this end, the file was placed within the root canal, and a radiograph was taken to determine the length. The working length was considered to be 1 mm from the apex. Then, the instrumentation of one tooth was performed with GG drills using step-down technique and instrumentation of another tooth was carried out without GG using step-back technique. In the step-back technique, the working length was determined by file 15. Then, the files 20, 25 and 30 were used until the master apical file (MAF) was determined. After determining MAF, canal preparation was done with a working length of 1 mm short of the canal's length for each larger file, 1 mm shorter than the length of operation to file 45. In the step-down technique, the working length was determined using file 15. Next, the coronal third of the root canal was prepared by GG drills 1, 2 and 3, and files 20, 25 and 30 were applied for apical instrumentation. Continued instrumentation of the apical area was similar to the step back technique. During all the stages, the canals were rinsed with 0.5% sodium hypochlorite solution. Afterwards, they were dried with paper points. The prepared canals were filled with gutta-percha (Arya dent, Iran) and ZOE sealer (Golchay, Iran) according to the manufacturer's instructions using the lateral condensation technique.

Two calibrated and blinded endodontists evaluated the radiographs to detect different types of errors. Apical evaluation of radiographs was performed by view box. Ten possible errors detected by endodontists in periapical radiographs were recorded in separate forms, which included vertical root fracture, fractured file, zipping, and ledge underfilling, overfilling, single cone, inappropriate shaping, strip perforation and canal transportation [16]. Evaluation of CBCT radiographs was done before and after obturation by NMT Free viewer to find out the amount of initial dentin and remaining dentin in

the surroundings of furcation.

Before and after preparation of samples, the thickness of dentin at the surrounding of furcation was measured in the depths of 5 mm from the canal opening on CBCT radiographs (at axial section) using NMT Free viewer software. To determine the thickness of primary dentin, one section in a specific depth of CBCT radiograph was selected for each sample, and the thickness of primary dentin at the adjacent area of furcation in the depths of 5 mm from the opening of the canal was measured by the ruler of the software. After preparing and filling the canals, the thickness of the remaining dentin for each sample in the same section of CBCT radiograph was measured by the same method. The amount of removed dentin was calculated by subtracting the thickness of the initial dentin from the thickness of the remaining dentin.

Data were analysed by SPSS18 software using inferential statistics, including Kappa coefficient for diagnostic agreement between two endodontists and multivariate logistic regression by moderating the effect of the views of both endodontists. Independent samples t-test was applied to specify the difference between primary, removed and remaining dentin with and without application of GG drills. A p-value of less than 0.05 was considered as statistically significant.

Results

A total of 1540 errors were diagnosed through periapical evaluation, from which both endodontists agreed upon 1345 cases and disagreed on 195 cases. The diagnostic agreement between both endodontists was statistically significant about all errors in all canals of mandibular molars by periapical evaluation (Kappa coefficient: 0.6). In this section, only statistically significant difference errors between application and non-application of GG are presented (Table 1).

Table 1: Main errors observed between application and non-application of Gates Glidden drill

			With GG[5]	Without GG	
Underfilling	DB[1] (maxillary molar)	A	8.3%	40.0%	0.002
		B	8.3%	60.0%	
Overfilling	MB[2] (maxillary molar)	A	16.7%	83.3%	0.007
		B	16.7%	83.3%	
Inappropriate shaping	P[3] (maxillary molar)	A	42.9%	83.3%	0.014
		B	28.6%	100.0%	
	D[4] (mandibular molar)	A	14.3%	71.4%	0.010
		B	14.3%	57.1%	
Ledge	DB (maxillary molar)	A	8.3%	80.0%	0.001
		B	8.3%	80.0%	
Single cone	D (mandibular molar)	A	21.4%	45.0%	
		B	14.3%	28.6%	0.019

1. Distobuccal canal (DB); 2. Mesiobuccal canal (MB); 3. Palatal canal (P); 4. Distal canal (D); 5. Gates Glidden (GG).

In the distobuccal canal of maxillary molars, there was a significant difference between two groups regarding the incidence of underfilling (P = 0.002), in which the incidence of error was 7 times greater in cases where GG drill was not used. In the mesiobuccal canal of maxillary molars, there was a significant difference between two groups regarding the incidence of overfilling (P = 0.007), the incidence of error was 6 times greater in cases where GG drill was not used.

In the palatal canal of the maxillary molars, there was a significant difference between the application of GG and non-application of GG in terms of the incidence of inappropriate shaping (P = 0.014), the chance of error was 4 times greater in cases where GG was not used.

In the distobuccal canal of maxillary molars, the ledge was 10 times greater in cases where GG was not used that was statistically significant (P = 0.001). In the distal canal of mandibular molars, the incidence of the single cone was more prevalent in which the chance of error was 2.6 (P = 0.019) time greater in cases where GG drill was not used.

The evaluation of the amount of remaining dentin in the middle third of all canals by CBCT radiographs showed no significant difference between using and not using a GG drill (Independent t-test, P > 0.05) (Table 2).

Table 2: Mean and standard deviation of the volume of removed dentin

Type of Canal	The volume of remaining dentin				P-value
	With GG[6]		Without GG		
	Mean	SD	Mean	SD	
MB[1] (maxillary molar)	0.9043	0.27972	1.0067	0.30768	0.198
DB[2] (maxillary molar)	1.1000	0.38612	1.1400	0.39115	0.702
P[3] (maxillary molar)	1.1214	0.37453	1.0833	0.26394	0.662
MB (mandibular molar)	0.9133	0.31366	0.9476	0.21591	0.636
ML[4] (mandibular molar)	0.9917	0.27122	0.9500	0.29212	0.582
D[5] (mandibular molar)	0.9500	0.32046	0.9900	0.21250	0.584

1. Mesiobuccal canal (MB); 2. Distobuccal canal (DB); 3. Palatal canal (P); 4. Mesiolingual canal (ML); 5. Distal canal (D); 6. Gates Glidden (GG).

Discussion

In this study, periapical radiographs were used to evaluate the effect of applying GG by preclinical students on procedural errors [13], [16]. The results of the present research show that utilisation of the GG drills by pre-clinic students during root canal preparation significantly decreased underfilling, overfilling, inappropriate shaping, ledge and single cones (P < 0.05).

The incidences of underfilling and in the distobuccal and over filling in the mesiobuccal canal of maxillary molars were greater while GG drill was not used. Reduction of underfilling and overfilling errors in cases where GG was used might be due to a direct access to the apical area of the canal and a better control over the working length. It has been found that preparing the coronal portion of the root canal provides different benefits in irrigation efficacy, apical

control, cone fit, and compaction procedures. In this regard, apical blockage, lodging, zipping, and perforations are less likely to occur [17], [18], [19]. Mollashahi et al., [20] reported adverse effects, especially in the curved canals declined while using GG, which can be attributed to the direct access of this instrument into canals. Kfir et al., [21] showed that various techniques could differently lead to the incidence of errors. They reported the incidence rates of 5% and 2% for transportation and perforation in the 8-step technique, respectively, and frequency rates of 17%, 7% and 6% for transportation, perforation and canal obstruction in the step-back technique, respectively. To verify the effect of canal shape on the incidence of error, Yin et al., [22] demonstrated that in the curved canals, the rotary and manual systems manifested different performance regarding the incidence of error and cleaning the surface of the canal.

The results of the present research showed no significant difference between application and non-application of GG about the remaining dentin in the furcation surroundings and danger zones of the prepared canals. This result can be because in this study students were instructed how to use GG drill through anticurvature technique, as a result of which the removal rate of dentin in the area adjacent to furcation was lower. GG drill was introduced as an acceptable instrument regarding preserving the thickness of root dentin during canal preparation. This technique does not remarkably reduce the thickness of the remaining dentin and may be suitable and safe for pre-flaring [11], [23], [24], [25]. Some researchers stated that the remaining dentin during preparation with GG drills depends on the type of technique and application of GG in both step-back and crown-down techniques can yield favourable results [23]. Mahran and Abo-El-Fotouh [26] reported a different performance for three different instruments, including Pro Taper, Hero Shaper and GG about the amount of removed dentin in the root canal. Based on the contradictions reported in various studies, it seems that the amount of the remaining dentin after canal preparation depends on the shape of the canal in addition to the type and clinicians' handling of the instrument.

The results of this study also showed no significant difference between the two techniques of canal preparation with and without using GG drill about the amount of removed dentin in maxillary and mandibular molars. Akhlaghi et al., [27] reported no statistically significant difference between GG drills and rotary files regarding the amount of removed dentin in the canals of mandibular first molars with curvatures of 20-35°. Maxillary first premolars with two roots and furcation area in the middle part are sensitive to canal preparation; therefore, it is necessary to remove a limited amount of dentin in these teeth due to the little amount of dentin left after canal preparation [28]. In addition to these factors, the primary thickness of dentin is an important factor in determining the amount of dentin to be removed during canal preparation [29]. One of the noticeable limitations of this study is that the root canals morphology was not the same in the specimens. Also, the procedure was done without using head phantom during the pre-clinical endodontic course.

In conclusion, within the possible reduction of underfilling, overfilling, inappropriate shaping, ledge and single cone errors in some canals, the practical GG drill technique was found suitable by the dentistry students. Moreover, no difference was observed between the two techniques of canal preparation with and without the use of GG drill regarding the amount of remaining dentin adjacent to the furcation area.

References

1. Arora A, Taneja S, Kumar M. Comparative evaluation of shaping the ability of different rotary NiTi instruments in curved canals using CBCT. J Conserv Dent. 2014; 17(1):35-9. https://doi.org/10.4103/0972-0707.124127 PMid:24554858 PMCid:PMC3915383

2. Leonardi DP, Haragushiku GA, Tomazinho FS, Furuse AY, Volpato L, Baratto-Filho F. Undergraduate students introduction to manual and rotary root canal instrumentation. Bull Tokyo Dent Coll. 2012; 53(3):155-9. https://doi.org/10.2209/tdcpublication.53.155 PMid:23124306

3. Adebayo ET, Ahaji LE, Nnachetta RN, Nwankwo O, Akabogu-Okpeseyi N, Yaya MO, et al. the Technical quality of root canal fillings done in a Nigerian general dental clinic. BMC Oral Health. 2012; 12:42. https://doi.org/10.1186/1472-6831-12-42 PMid:23066650 PMCid:PMC3504551

4. Schmitz Mda S, Santos R, Capelli A, Jacobovitz M, Spanó JC, Pécora JD. Influence of cervical preflaring on determination of apical file size in mandibular molars: SEM analysis. Braz Dent J. 2008; 19(3):245-51. https://doi.org/10.1590/S0103-64402008000300013 PMid:18949299

5. de Alencar AH, Dummer PM, Oliveira HC, Pécora JD, Estrela C. Procedural errors during root canal preparation using rotary NiTi instruments detected by periapical radiography and cone beam computed tomography. Braz Dent J. 2010; 21(6):543-9. https://doi.org/10.1590/S0103-64402010000600011 PMid:21271046

6. Coutinho-Filho T, De-Deus G, Gurgel-Filho ED, Rocha-Lima AC, Dias KR, Barbosa CA. Evaluation of the risk of a stripping perforation with Gates-Glidden drills: serial versus crown-down sequences. Braz Oral Res. 2008; 22(1):18-24. https://doi.org/10.1590/S1806-83242008000100004 PMid:18425240

7. Al Jabbari YS, Fournelle R, Al Taweel SM, Zinelis S. Failure analysis of eleven Gates Glidden drills that fractured intraorally during post space preparation. A retrieval analysis study. Biomedical Engineering. 2018; 63(4):407-12. https://doi.org/10.1515/bmt-2016-0245 PMid:28723609

8. Dillon JS, Amita BG. To determine whether the first file to bind at the working length corresponds to the apical diameter in roots with apical curvatures both before and after preparing. J Conserv Dent. 2012; 15(4):363-366. https://doi.org/10.4103/0972-0707.101908 PMid:23112485 PMCid:PMC3482751

9. Wu MK, van der Sluis LW, Wesselink PR. The risk of furcal perforation in mandibular molars using Gates-Glidden drills with anticurvature pressure. Oral Surg Oral Med Oral Pathol Oral Radiol

Endod. 2005; 99(3):378-82. https://doi.org/10.1016/j.tripleo.2004.07.008 PMid:15716849

10. Flores CB, Montagner F, Gomes BP, Dotto GN, da Silva Schmitz M. Comparative Assessment of the Effects of Gates-Glidden, Largo, LA-Axxess, and New Brazilian Drill CPdrill on Coronal Pre-enlargement: Cone-beam Computed Tomographic Analysis. J Endod. 2014; 40(4):571-4. https://doi.org/10.1016/j.joen.2013.08.028 PMid:24666914

11. Zuckerman O, Katz A, Pilo R, Tamse A, Fuss Z. Residual dentin thickness in mesial roots of mandibular molars prepared with Lightspeed rotary instruments and Gates-Glidden reamers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003; 96(3):351-5. https://doi.org/10.1016/S1079-2104(02)91710-5

12. Fong JY, Tan VJ, Lee JR, Tong ZG, Foong YK, Tan JM, et al. Clinical audit training improves undergraduates' performance in root canal therapy. Eur J Dent Educ. 2018; 22(3):160-6. https://doi.org/10.1111/eje.12297 PMid:29266663

13. Saatchi M, Mohammadi G, Sichani AV, Moshkforoush S. Technical Quality of Root Canal Treatment Performed by Undergraduate Clinical Students of Isfahan Dental School. Iran Endod J. 2018; 13(1):88-93. PMid:29692842 PMCid:PMC5800448

14. Ribeiro DM, Reus JC, Felippe WT, Pachêco-Pereira C, Dutra KL, Santos JN, et al. Technical quality of root canal treatment performed by undergraduate students using hand instrumentation: a meta-analysis. Int Endontic J. 2018; 51(3):269-83. https://doi.org/10.1111/iej.12853 PMid:28862763

15. Schneider SW. A comparison of canal preparation in straight and curved root canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1971; 32:271-5. https://doi.org/10.1016/0030-4220(71)90230-1

16. Hulsmann M, Peters OA, Dummer PM. Mechanical preparation of root canals: shaping goals, techniques and means. Endod Topics. 2005; 10(1):30-76. https://doi.org/10.1111/j.1601-1546.2005.00152.x

17. Barbieri N, Leonardi DP, Baechtold MS, Correr GM, Gabardo MC, Zielak JC, Baratto-Filho F. Influence of cervical preflaring on apical transportation in curved root canals instrumented by reciprocating file systems. BMC oral health. 2015; 15(1):149. https://doi.org/10.1186/s12903-015-0137-0 PMid:26593244 PMCid:PMC4656179

18. Sousa K, Andrade-Junior CV, Silva JM, Duarte MA, De-Deus G, Silva EJ. Comparison of the effects of TripleGates and Gates-Glidden burs on cervical dentin thickness and root canal area by using cone beam computed tomography. J Appl Oral Sci. 2015; 23(2):164-8. https://doi.org/10.1590/1678-775720130542 PMid:26018308 PMCid:PMC4428461

19. Mollashahi NF, Sohrabi M, Mollashahi LF, Mehdizadeh M. The Efficacy of FlexMaster'sIntroFile, PreRaCe and Gates Glidden Drills in Straight-Line Access: A CBCT Assessment. Iran Endod J. 2014; 9(3):199-203.

20. Kfir A, Rosenberg E, Zuckerman O, Tamse A, Fuss Z. Comparison of procedural errors resulting during root canal preparations completed by junior dental students in patients using an '8-step method' versus 'serial step-back technique. Int Endod J. 2003; 36(1):49-53. https://doi.org/10.1046/j.1365-2591.2003.00612.x PMid:12656514

21. Yin X, Cheung GS, Zhang C, Masuda YM, Kimura Y, Matsumoto K. Micro-computed tomographic comparison of nickel-titanium rotary versus traditional instruments in C-shaped root canal system. J Endod. 2010; 36(4):708-12. https://doi.org/10.1016/j.joen.2010.01.003 PMid:20307748

22. Akhlaghi NM, Naghdi A, Bajgiran LM, Behrooz E. Computed tomography evaluation of residual root thickness after pre-flaring using gates Glidden drills: The sequence effect. Journal of conservative dentistry. J Conserv Dent. 2014; 17(2):142-145. https://doi.org/10.4103/0972-0707.128052 PMid:24778510 PMCid:PMC4001270

23. Coutinho-Filho T, De-Deus G, Gurgel-Filho ED, Rocha-Lima AC, Dias KR, Barbosa CA. Evaluation of the risk of a stripping perforation with Gates-Glidden drills: serial versus crown-down sequences. Braz Oral Res. 2008; 22(1):18-24. https://doi.org/10.1590/S1806-83242008000100004 PMid:18425240

24. Pinto SL, Marceliano-alves MF, Lins RX, Radetic EA, Lopes HP. The dentin thickness remaining in the risk zone of mandibular molars after cervical preflaring with four methods. Rev Odontol UNESP. 2017; 46(1):1-6. https://doi.org/10.1590/1807-2577.07016

25. Mahran AH, Aboel-Fotouh MM. Comparison of effects of ProTaper, HeroShaper, and Gates Glidden Burs on cervical dentin thickness and root canal volume by using multislice computed tomography. J Endod. 2008; 34(10):1219-22. https://doi.org/10.1016/j.joen.2008.06.022 PMid:18793924

26. Akhlaghi NM, Kahali R, Abtahi A, Tabatabaee S, Mehrvarzfar P, Parirokh M. Comparison of dentine removal using V-taper and K-Flexofile instruments. Int Endod J. 2010; 43(11):1029-36. https://doi.org/10.1111/j.1365-2591.2010.01769.x PMid:20636352

27. Ghoddusi J, Bagherpour A, Mahmudabadi F, Forghani M, Sarmad M. Residual dentin thickness of bifurcated maxillary premolars following two post space preparation methods. Iran Endod J. 2013; 8(3):94-8. PMid:23922568 PMCid:PMC3734522

28. Garala M, Kuttler S, Hardigan P, Steiner-Carmi R, Dorn S. A comparison of the minimum canal wall thickness remaining following preparation using two nickel-titanium rotary systems. Int Endod J. 2003; 36(9):636-42. https://doi.org/10.1046/j.1365-2591.2003.00704.x PMid:12950579

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The Correlation between Adherence and Asthma Patients Quality of Life in Medan, Indonesia

Arlinda Sari Wahyuni[1], Rozaimah Zain Hamid[2], Tamsil Syafiuddin[3], Adang Bachtiar[4], Nerdy Nerdy[5*]

[1]Department of Community Medicine, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia; [2]Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia; [3]Department of Pulmonology and Respiratory, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia; [4]Department of Health Administration and Policy, School of Public Health, Universitas Indonesia, Depok, Indonesia; [5]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia

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***Correspondence:** Nerdy Nerdy. Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. E-mail: nerdy190690@gmail.com

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Abstract

BACKGROUND: Asthma is a chronic airway disease that is based on an inflammatory process and a serious health problem around the world. Asthma is often associated with treatment management factor. Adherence is the patient's compliance towards their doctor's advice, which is accompanied by their understanding and follows the doctors' advice consistently.

AIM: This study aimed to get a valid and reliable adherence measure in asthma patients especially in Medan.

METHODS: This research used the method of quantitative done by the cross-sectional approach. The sample (200 adult asthma patients) used standard asthma medication, stable asthma patients and did not suffer from severe asthma or other accompanying diseases. Data were analysed using univariate, bivariate and multivariate analysis, which is SEM (Structural Equation Modeling) analysis.

RESULTS: The best dimension of medication adherence was the dimension of beliefs in medication 64%. The highest education adherence is at college education level which is 67.6%. Employment status with highest adherence value is medication on civil servant/military/police with the value of 67.7.

CONCLUSIONS: There is a correlation between adherence and asthma patient's quality of life in Medan.

Introduction

Asthma is a chronic airway disease which underlying pathogenesis is an inflammatory process and one of the most serious health problem around the world. The chronic inflammatory process in the respiratory tract of asthma patients involves many inflammatory cells and their elements. This condition causes the respiratory tract to become hyper-responsive, resulting in clinical symptoms that occur periodically, especially at night or early morning/dawn. This condition causes airflow limitations in the respiratory tract, which results in shortness of breath as a major clinical manifestation that greatly disrupts the activity, productivity and quality of life of asthma patients [1].

The prevalence of asthma continues to increase in both developed and developing countries, although appropriate medications for asthma management (inhalation of combination corticosteroids and agonist β2 prolonged/LABA) are available. Currently, the number of asthma patients is estimated to reach 300 million people, and total patients who died from asthma attacks reach 255,000 people. Respiratory system illness is the cause of 17.4% of deaths in the world, in the following order: lung infection (7.2%), Chronic Obstructive Pulmonary Disease (COPD) (4.8%), tuberculosis (TB) (3%), lung cancer (2.1%) and asthma (0.3%) [2].

Asthma has a wide impact on the activity, productivity, and social conditions of the community, especially among asthma patients, who will certainly

increase the burden of health financing and the economic burden of the community [3] [4]. The right management of asthma requires appropriate and adequate treatment which is right dose, appropriate duration, appropriate time, appropriate way/technique of inhalation therapy, etc. Management of asthma continues to grow, and currently, standard asthma management guidelines are outlined in the Global Initiative for Asthma (GINA 2011). Achieving and maintaining controlled asthma is the main aim in asthma management which is an optimal condition that allows asthma patients to do their activities like other healthy people. Indicators of controlled asthma are no symptoms, no activity limitations, and no symptoms at night, no reliever medication, normal lung function and no asthma attacks throughout the year [1].

Adherence is the patient's compliance towards their doctor's advice, which is accompanied by their understanding about the illness relating to the management/treatment until they follow the doctor's advice consistently [5]. The factor, adherence in the management of asthma consists of two parts: the problem of drug use such as the complexity of management, drug side effects, medical cost, and inconvenience to treatment; and the problems outside of management such as poor understanding of doctors' instructions, dissatisfaction with health personnel, not as expected, lack of supervision from doctors and families, false estimate of disease risk, cultural problems, false stigmatization, forgetting, and religion/belief problems [6].

We can draw the problem on how the instrument/model of adherence asthma patients in the management of the disease, especially in Medan and how the adherence of asthma patients to the management with asthma patients' quality of life.

This study aimed to get an instrument/model of adherence to asthma patients in the management of the disease and to know the correlation between adherence and asthma patients' quality of life.

Methods

This research used 2 stages of research. The first stage is done with the qualitative approach and the second phase with a quantitative approach. The qualitative approach aimed to develop the research instruments, and the quantitative approach aimed to analyse the research instrument, testing the hypothesis and structural model of measurement.

This research was preceded by the development stage of the research instrument. The first stage is the exploration of adherence instruments/measure tool and asthma patients' quality of life of. At this stage, the model of treatment adherence instrument is developed with indicators which are knowledge, attitude, belief, action, doctor-patient communication and family support.

The quantitative approach is made to the initial draft, which will be analysed statistically to get valid and reliable values. The validity of the constructs is tested by using Exploratory Factor Analysis and Confirmatory Factor Analysis (CFA). The following step is data validation. The process of factor analysis tries to find the relationship between independent variables [7]. CFA is essentially an exploratory statistical method, but the loading factor for variables is set based on previous studies or relevant theories. Then, CFA processed and measured the suitability of loading in a target matrix. The CFA was done by considering factor structure that was positioned. The CFA examined the suitability of a model with some specific factors and determined the specific items that measure or load on each factor [7] [8].

The testing of Hypothesis and the development of behavioural adherence model of treatment as well as its relation to asthma patients' quality of life were performed using Structural Equation Modeling (SEM) analysis. This research design is called comparative causal or explanatory research. This study explains the relationship and effect between one variable with other variables. Besides, this study uses instantaneous data (cross-sectional). The obtained data is used to predict future circumstances.

This study was done on asthma patients who live in Medan. The selection of research sites based on consideration: (1) Medan area is the capital of North Sumatera Province with the prevalence of asthma patients is estimated to be high enough that is 3% (2) standard of asthma service, especially behaviour of management/treatment of asthma patients in Indonesia and especially in Medan City, generally is not maximal [9].

The population of this study was asthma patients who live in Medan and has been treated by a general practitioner or pulmonary specialist. The inclusion criteria of this study were adult asthma patients aged 18-60 years, asthma patients who came to general practitioners or pulmonary specialists in Medan city, patients who had undergone asthma treatment with appropriate drug (inhalation of combination corticosteroids and agonist β2 prolonged/LABA) at least 6 months, stable asthma patients (not in asthma attacks), selected patients are patients who have complete address data, willing to follow this study (approved with informed consent). The exclusion criteria in this study are a history of coexisting diseases such as COPD (Chronic Obstructive Pulmonary Disease), heart disease, diabetes mellitus, hypertension, kidney disease, liver disease and a history of allergy to drugs used in this study and severe or moderate asthma patients who were hospitalised.

The sampling technique in this research with the quantitative approach is done by Consecutive Sampling which the process of sampling based on the criteria set by the researchers [10].

Asthma is a disease that is mostly influenced by season as a risk factor. Duration of data collection in this research was about 8-10 month. With this time the researcher considers the duration is long enough, so the results of this research by consecutive sampling technique can approach or resemble probability sampling results. The samples number is determined based on the hypothesis test formula one proportion and obtained a sample with the number of 200 people.

Interviews and questionnaires were completed after obtaining the patient's consent at the respondent's residence/home or at the place where the patient controls the medication. The goal is to get valid and not influenced result by other conditions (research bias).

Interviewers in this study are 6 students from Faculty of Medicine, the University of North Sumatra who has been trained by researchers. Also, the researcher's collaborate with 8 hospital nurses who have trained researchers to collect research data. Interviewers were trained to be able to guide and provide technical data filling in the prepared questionnaire. Some interviewers demonstrated the technique of filling out the questionnaire in the presence of the researcher. This was done to obtain valid data on the asthma patients study.

The Secondary data was obtained from Medan Health Care Office to determine the prevalence of asthma in Medan which originating from Basic Health Research Province of North Sumatra. The Data of asthma patients were collected from drug distributors or pharmaceutical parties who had completed patient data records of ICS drug users. Furthermore, asthma patients' data is also collected from the practice of specialist doctors and pulmonary polyclinics in a hospital or private doctors' practice.

Instruments of knowledge, attitudes, beliefs, actions, doctor-patient communication, and family support: The measurement technique of knowledge is to give a score of 1 for each correct item of the question. For attitude is to give score according to the attitude of the respondent. The assessment range is 1 to 4. Score 4 shows the patient's attitude is getting more positive. Assessment of trust based on true belief and score given is 1. Assessment of action is score 1 for corrective action. The patient doctor's communication appraisal is a score of 1 if the patient answers any or has ever been to any doctor-patient communication question. Assessment of family support variables based on the presence or absence of family support for asthma patients. A score of 1 is given when the patient answers are on the family support questionnaire.

The quality of life instruments comprise the dimensions of health, emotion, environment and activity limitations. Assessment of quality of life scores has a range of 1-5. The highest score (5) indicates the patients quality of life not disturbed.

Table 1: The Asthma Patients Demographic Characteristics in Medan

Variable	n	%
Age		
≤ 20	44	22.0
21-30	44	22.0
31-40	33	16.5
41-50	41	20.5
51-60	38	19
Mean/SD	35.7/SD 13.3	
Sex		
Male	74	37.0
Female	126	63.0
Education		
Elementry School	9	4.5
JHS	11	5.5
SHS	84	42
College	96	48
Marital status		
Married	126	63.0
Single	74	37.0
Employment		
Unemployment	11	5.5
Housewife	40	20.0
Student	40	20.0
Civil servant/Military/police	35	17.5
Private employee	28	14.0
Entrepreneur	31	15.5
Etc	15	7.5
Income		
<1 million	29	14.5
1-3 million	93	46.5
4-5 million	35	17.5
>5 million	43	21.5
Tribe		
Batak	92	46.0
Jawa	48	24.0
Melayu	11	5.5
Minang	25	12.5
Aceh	13	6.5
Etc	11	5.5

The instruments of sociodemographic characteristics are age, sex, education, marital status, employment, income, and tribe. Assessment of demographic characteristics is based on the choice determined from the patient in accordance with the criteria outlined in the operational definition in this study.

Ethical Clearance of this study was obtained in March 2011 issued by the Commission on Medical Research Ethics Faculty of Medicine, University of North Sumatra/USU Hospital.

Results

Based on the Table above, it is known that age is more prevalent in the age group < 20 years old and 21-30 years old with a proportion of 22%. Respondents of the female sex are more than male respondents that are 63%. The highest level of education is college 48%; the most marital status is married 63%, most of the employment is housewife and student which is 20% respectively. For the highest income level is 1-3 million that is 46.5%, and

most of the respondents are from the Batak tribe that is 46%.

Table 2: The Adherence Medication of Asthma Patients

Adherence	Number of Questions	Min	Max	Mean	SD
Knowledge	9	0	8	3.9	1.8
Attitudes	9	9	36	28.9	4.0
Beliefs in disease/medication	12	1	12	8.0	2.4
Actions	9	0	9	5.4	2.3
Patient-doctor communication	20	0	18	14.6	3.5
Family support	5	0	5	4.2	1.1
Total Adherence	84	45	84	66.8	8.6

The Table above contains an assessment of knowledge, attitudes, beliefs, patient-doctor communication, actions, and family support. The average knowledge is 3.9 (SD 1.8), attitudes 28.9, (SD 4) beliefs in disease/medication are 8.0 (SD 2.4), actions 5.4 (SD 2.3) and patient-doctor communication is 14.6 (SD 3.5), and family support is 4.2 (SD 1.1).

Table 3: The Distribution of Adherence Dimensions on Asthma Patients Medication

Adherence dimensions	Good		Not Good		Border*
	N	%	N	%	
Knowledge	127	63.5	73	36.5	≥ 3.9
Attitudes	101	50.5	99	49.5	≥ 28.9
Beliefs in disease/medication	128	64.0	72	36.0	≥ 8
Actions	105	52.5	95	47.5	≥ 5.4
Patient-doctor communication	100	50.0	100	50.0	≥ 14.6
Family support	113	56.5	87	43.5	≥ 4.2
Total Adherence	106	53.0	94	47.0	≥ 66.8

The results showed that the best dimension of medication adherence was the dimension of beliefs in disease/medication (64%) and the least was the patient-doctor communication dimension (50%).

Table 4: The Characteristics of Treatment Adherence Based on Sociodemographic Characteristics of Asthma Patients

Variable	n	%	SD	*p*
Age				
≤ 20	44	65.4	7.8	0.13
21-30	44	68.7	8.3	
31-40	33	68.9	8.5	
41-50	41	65.2	9.1	
51-60	38	66.1	9.2	
Sex				
Male	74	66.9	8.8	0.943
Female	126	66.8	8.6	
Education				
Elementry School	9	62.6	9.9	0.027
JHS	11	63.5	9.9	
SHS	84	65.7	7.1	
College	96	68.6	9.2	
Marital status				
Married	126	67.2	9.0	0.400
Single	74	66.2	8.0	
Employment				
Unemployment	11	60.0	7.3	0.008
Housewife	40	64.6	8.7	
Student	40	66.5	7.1	
Civil servant /Military / police	35	69.0	10.2	
Private employee	28	69.9	7.4	
Entrepreneur	31	66.1	8.7	
Etc	15	69.5	7.6	
Income				
<1 million	29	65.6	9.1	0.484
1-3 million	93	66.7	8.3	
4-5 million	35	66.1	10.9	
>5 million	43	68.5	7.0	
Tribe				
Batak	92	67.2	8.4	0.766
Jawa	48	66.7	9.9	
Melayu	11	69.6	9.5	
Minang	25	65.9	7.3	
Aceh	13	64.7	7.6	
Etc	11	65.9	8.4	

The table above shows there is no difference in the mean value of adherence based on age, sex, marital status, income and tribe with $p > 0.05$. There is an average difference in the value of adherence based on education and employment ($p < 0.05$). The highest education adherence is at college education level which is 67.6 (SD = 7.8), and the lowest level is a primary school (SD) which is 61.5 (SD = 11.3). Employment status with highest adherence value is medication on civil servant/military/police with the value of 67.7 (SD = 7.9), and the lowest is unemployment status with a value of 60.2 (SD = 5.5).

Discussion

In this study, it is found that most patients are the young adult age which is under 30 years old (44%). The average age of asthma patients is 35.7 (SD 13.3). The research from Japan, Suzuki, (2011) [11] obtained a same average age of asthma patients from the study which is 36.3 (SD = 7.9). Asthma has been known since the time of Hippocrates (2000 years ago), and until now it's still a global health problem. Epidemiologically, in people with asthma starts from childhood to adulthood. WHO shows the prevalence of asthma is about 3-5% in adults and 7-10% in children [12].

Based on sex, most patients were found are females that are 63%. Epidemiologically, asthma can affect both men and women. The prevalence of asthma in boys is greater than that of girls but after puberty asthma becomes more common in women [11]. Theoretically, it is not yet clear why the proportion of adult asthma patients are higher in women, but the population in this study are adult age group in home/residence or visiting a doctor's practice to re-control the disease. Theoretically, patients who are more concerned about the disease are women than that of a man [12], especially asthma patients in this study population are not asthma patients in attack or with severe asthma but asthma patients who are in stable conditions.

In this study, the patient's socioeconomic level is quite good. Based on the level of education in this study, it is found that highest education level is college 48%, followed by senior high school and junior high school 42% and 5.5% respectively, while the least is the level of elementary school 4.5%. Based on employment status, the highest is employee 54.5% compared to unemployed which is 45.5%. Most of the unemployed are housewives and students (40%). Meanwhile, 31.5% are from the group working as civil servants/military/police and private employees. Based on the amount of income, the largest group of income is 1-3 million/month that is 46.5%, and the least is 14.5% with income < 1 million. Meanwhile, as many as 21.5% with income > 5 million. Most of the samples

in this study were patients treated at the practice of pulmonary specialists. There is a tendency of patients who seek treatment in the practice of specialists are from good enough economic status. The results of this study differ from the results of Atmoko research (2011) [13] that got the highest level education among asthma patients is medium education 49.5% while the level of higher education is 30.8% [13]. Imelda 2007 [14] and Atmoko 2011 [13] also obtained medium and high education level that is 54.6% and 38.5% respectively. Bachtiar (2009) [15] obtained more senior high school education level patients who are 40.7% and followed by college level of 28.8%.

Based on the marital status of the results in this study, most of the patients are married that is 63%, and 37% of patients are single status. Based on the tribe, most of the asthma patients are from Batak tribe (46%), and the least is Malay and others (5.5%). It can be explained that the adult population (> 18 years) in Medan is mostly married and most of them are a population of Batak tribe by the Profile of Medan. But the Batak tribe in Medan has several variations. There are Batak Toba, Batak Mandailing and Batak Karo. Different types of Batak tribes and other tribes in Medan have variations on social-cultural conditions, including in health behaviour. In this study, it is seen a considerable variation based on tribes in asthma patients who live in Medan.

The adherence instrument medication of asthma patients in this study was developed on 6 indicators which are knowledge, attitudes, actions and doctor-patient communication, beliefs in disease/medication, and family support. 9 points were given to the number of questions for knowledge, 9 points for attitudes, 9 items for actions and 20 items for doctor-patient communication, 12 points for beliefs in disease and medication and 5 items for family support questions on treatment.

The first indicator is knowledge. Based on the results of this study, the knowledge indicator is formed based on questions of understanding symptoms, trigger factors, asthma control, during treatment of asthma, the best way for treatment, inhalation techniques, recommended examination to assess progress, and the benefits of using asthma control medication.

The patient is said to adhere to treatment if the patient understands comprehensively about all important aspects of asthma and its treatment. Asthma is a unique disease. This disease is a chronic disease that requires long-term treatment. In the mild-moderate stage, the disease is reversible which is often misunderstood by asthma patients. The patients must understand their illness from basic aspects such as understanding about illness, symptoms, trigger factors, until why they should use standard medication and the effects of the treatment given to him.

The second indicator is the attitude. The formation of treatment adherence is often associated with a person's attitude toward the disease and its treatment. A positive attitude toward disease/treatment will encourage patients including asthma patients to maintain proper treatment behaviour. Attitudes are also formed based on prior patient experience and knowledge. Often, attitudes are in line with existing knowledge. What patients should understand that asthma is an inflammatory disease and at the same time, it narrows the respiratory tract, correlated with the type of drug given, an inhaler with the combination of corticosteroids and LABA. This understanding will encourage patients to use the appropriate drug and also in an appropriate way which is by inhalation. The treatment at a stable stage must be consistent with the correct management of asthma with careful monitoring by treating doctor. Patients, in this case, should always be willing to work with doctors and follow the doctor’s instructions. Besides that, a good attitude of patients can be formed if they also want to study the disease from various sources that exist. So that, an attitude based on knowledge will certainly lead to good behaviour [16]. In this case, it is a longer adherence than just passively following the doctor’s instructions. For action indicators, the questions were formed are about the importance of asthma drug use, the use of asthma medications based on doctor's instructions, stop asthma medication by instruction, desire to cooperate with treating doctor, ask for explanation about disease treatment, consult with doctor, study asthma, and prepare asthma medication anytime.

The third indicator is beliefs. The dimensions of beliefs in disease and medication in this study are differentiated by the attitude of asthma patients to treatment. This confidence instrument was developed based on information obtained by researchers from doctors who is treating asthma patients. Many false perceptions developed among asthma patients which interfere with the success of their treatment, moreover, in terms of standard drug use by inhalation of steroid and LABA combination. Many patients consider a new standard asthma drug appropriate if it is in a state of attack, for severe illness, and for high socioeconomic groups, etc. These false perceptions must be identifiable/recognized by the doctor, so that the management of asthma patients also refers to the socio-cultural aspect. Bauman (2005) [17] mentioned one aspect that disrupts adherence treatment of asthma patients is the wrong patient's belief in the disease and its treatment. Instrument beliefs about disease/treatment that researchers successfully developed, consists of 12 questions. As for the grain of the question, it is about the perception of patients who says asthma is a mild disease and easy to cure, the absence of symptoms means asthma has healed, asthma treatment is sufficient at the time of the attack, the right treatment of asthma is by taking medicine, inhalation treatment is for severe disease and high rates of inhalation drugs will be addictive, the price of inhaled drugs is expensive and unreachable, no need

for lung function checks, no consultation with doctors, over-the-counter breathing drugs are sufficient to treat asthma, and no complication of asthma.

The fourth indicator is the action. The action is an indicator that has been done by asthma patients every day related to behaviour towards treatment. This indicator shows the level of adherence to treatment is well under way when the patient has done the aspect of regular treatment, consult with his doctor and make prevention efforts such as avoid the trigger and exercise regularly. Indicators of action in this study were formed based on 9 items of questions that is patients use asthma medication every day as recommended by treating doctors, pay attention to the schedule/time of drug administration, using reminders to use asthma medication, conduct routine consultation with doctors, perform lung function examination, doctors to assess disease progression, avoid asthma triggers, exercise routine, and read articles or papers on asthma/disease treatment.

The last indicator is family support. Family support based on Green theory is a reinforcing factor that drives factors that can lead to behaviour. Asthma patients who are undergoing treatment will continue to consume the drug if there is support from the family. Families can remind, help regarding costs, and others that can improve the success of asthma treatment. This theory is also compatible with Lewis's driving forced theory where family support is a factor that reinforces the behaviour by promoting persuasion and information. The existence of intensive family support can improve the behavior for better treatment. Adherence to the treatment of asthma will be stronger because the family always reminds, provides information, encourages and even helps the provision of necessary medical funds.

Questionnaires about Adherence of treatment of asthma patients contains comprehensive questionnaire to assess adherence of treatment with 6 dimensions of measurement that is knowledge, attitudes, beliefs, actions, doctor-patient communication, family support with a total of 84 questions. As for the researchers' knowledge, until now there is no comprehensive questionnaire to assess adherence of asthma patient treatment. The researchers cut short the questionnaire to Adherence Asthmatic Patient Questionaire Medan (AAPQ-Medan) [18]. Cronbach alpha test results in this questionnaire obtained 0.84. Azwar (2001) set this measure as considered valid and reliable if the resulting Cronbach alpha value is > 0.6. This means that the questionnaire is quite reliable for use particularly in Medan with adult asthma patients who use standard drugs. However, the weakness of this questionnaire is less practical because of too many question items (84 pieces). There should be further studies to examine and explore the questions in this study to produce a simpler measuring tool to explain the adherence of treatment in patients.

In this research, the researchers have succeeded to develop patient adherence behaviour component that is knowledge, attitudes, beliefs, actions and doctor-patient communication, and family support. Someone is said to be adherent to his treatment if he has the right knowledge, positive attitude, no beliefs in impede treatment, correct treatment, comprehensive patient-doctor communication and family support. The results of this study have proven what components form a clear adherence in the treatment of asthma patients and how strong these components in forming adherence treatment.

This study found a median adherence treatment of asthma patients was 66.8 (SD = 8.6). The score of knowledge from 9 questions is 3.9 (SD = 1.8), attitude score with 4 level of like scale start strongly in disagreeing sd very agree to get an average value of 28.9 (SD = 4.0) from 9 question, trust with average 8.0 (SD = 2.4) out of 12 questions, 5.4 (SD = 2.3) action score of 9 questions, patient-doctor communication score was 14.6 (SD = 3.5) out of 20 questions and the family support score was 4.2 (SD = 1.1) out of 5 questions.

In this study, it is found that the proportion of adherence behaviour that is still not good with only 53% have good adherence. Poor adherence is still seen in the attitude and communication components of doctor-patients with asthma-related illness and treatment that is 50.5% and 50% respectively. This indicates that the behaviour towards the treatment of asthma patients in Medan is quite low, especially for attitudes and doctor-patient communication. Recorded more than 230 million Euro in the UK and 100 billion dollars annually in the United States issued to overcome the problem due to less adherence of asthma patients to their treatment.

The results of this study also showed that the level of knowledge of asthma patients is not good in establishing the understanding of asthma, triggers and symptoms of asthma. Asthma patients' attitude towards treatment has tended to be well seen that most have shown a positive attitude, both in the perception of asthma medication as well as attitudes toward the treating doctor. But the negative attitude that is often found in patients will stop asthma medication, and control of asthma. There were 15% people not agreeing to control their asthma, and 15.5% did not agree to stop asthma medication based on instructions from doctors. Based on the belief in the disease and its treatment, it is found large wrong is in perceiving the disease of asthma. In this study, 57% of the patients believed that inhaled treatment was a treatment for severe asthma, 49% thought that asthma was a mild and easily curable disease, and 45% thought no symptoms meant that asthma had recovered. Based on treatment measures, most patients have used asthma medication. But in this study, only 24% always use reminders to use asthma medication, 38% who always communicated with their

doctors and 51.5% who regularly exercised. Based on patient-physician communication most have stated good doctor-patient communication. But in this study only 60-65% of doctors who communicate about financial and profit and loss in treatment. Based on family support, most of them are getting family support. In this study, more than 80% of patients have received family support by both moral and material aspects.

The results of this study prove that the condition of adherence of asthma patients in Medan is still very limited. Patients are not consistently attached to the disease and its treatment. This is probably because the level of interaction between doctors and patients is not so good. Though asthma is a chronic disease where the relationship between doctors and patients should be maintained maximally and lasted continuously. This study was conducted in a moment (cross-sectional), so it is not seen how the quality and continuity of doctors interactions and asthma patients.

In this study, the researchers found there is no correlation between sociodemographic factors such as age and sex with adherence treatment of asthma patients ($p > 0.05$). This is in line with Apter's study, (1998) which has no relationship between age and sex with adherence treatment in patients who are given corticosteroid inhalation twice daily [19]. This study is not in line with the research of Gamble et al., (2009) who got women more adherent compared with men ($p < 0.05$) [20].

In this study obtained adherence treatment in women almost the same as men that is 66.8 and 66.9. For the age group, treatment adherence was higher at age < 40 years, i.e. 65.4 to 68.9. This is probably because young age is a more productive age, so the conditions of asthma attacks that occur greatly disrupt the productivity and performance or achievement of learning. This must be solved immediately by the sufferer. Also at a young age, more easily remember the information which is obtained from the environment, especially from treating doctors so that this condition can increase knowledge, attitudes, beliefs, and treatment measures. But in this study, there was no significant correlation between age group with adherence treatment of asthma patients ($p > 0.05$). Meanwhile, Gillisen (2007) mentioned that pediatric and adolescent patients tend to be less adherent than adults. In this study, the study population was of adult age and did not assess adherence in children [21].

In this study also found adherence treatment is higher in patients who are still bound to marriage. This is possible because the husband or wife or other family members play a role in encouraging the adherence in asthma patients. Families can be very influential factors in determining the beliefs and health values of individuals and can also determine the treatment program they can receive. Families also provide support in making decisions about the care of sick family members.

The status of employment in this study was grouped into working groups and non-working groups. The results showed there was correlation employment with treatment adherence ($p < 0.05$). It's because the patient's working status varies greatly among the working group. In the non-working group also varied in the group of housewives, students and retirees. In this group, they have the activities that often make them forget and not adherence against their treatment

In this study, researchers found that patients with an income rate of > 5 million had the highest adherence to treatment, and the lowest treatment adherence is at the rate of < 1 million. But statistically, there is no difference in adherence treatment based on income level. This is not in line with Apter's (1998) study which found no significant association ($p = 0.002$) between income and adherence of treatment of asthma patients [15]. This is probably because not all asthma patients bear the cost of treatment, but some of the asthma patients who were studied are covered through health insurance Civil Servants as patients who received treatment at RS Pirngadi that serve civil servant health insurance.

In this study adherence of treatment is higher in Malay tribe that is 69.6 and low in Aceh tribe that is 64.7. Statistically, there is no relationship between ethnic and treatment adherence. This is not in line with Wells et al.'s investigations where there is a correlation between ethnic and treatment adherence. Wells (2008) found the difference in treatment adherence in African and American tribes [22]. Similarly, Apter's (1998) study found there is correlation adherence in the Spanish group [19]. This is probably because the tribal variations in this study are not balanced. In Indonesia, there are many different ethnic groups; especially Medan is a very heterogeneous city, where Batak tribe is more dominant in Medan.

Adherence treatment is determined by many factors. A continuous review of adherence treatment, especially aspects of the problem of treatment, psychology, and the quality of doctor-patient interactions should be undertaken. Long-term treatment often causes problems in patients. Good understanding, good attitude, adherence level and doctor-patient communication need to be developed to improve adherence of patient's treatment, especially asthma patient in Medan so that the mission of GINA 2011 which is controlled asthma can be achieved.

In conclusion, based on the analysis of the treatment adherence measurement model. The treatment adherence measurement model of asthma patients in Medan has good psychometric value (valid, reliable and fit modelling), which includes all latent variables (exogenous and endogenous) along with the indicators studied. The adherence of treatment I that

formed are aspects of knowledge, attitudes and beliefs. Adherence of treatment II which is formed from the aspects of action, doctor-patient communication and family support. Quality of life formed from aspects of health, emotions, environment and activities.

The proportion of asthma patients adherence in Medan was 53%, and the proportion of asthma patients' quality of life in Medan was 52.5%. There are differences in treatment adherence based on demographic status that is education and employment. There is no difference in treatment adherence based on sociodemographic statuses such as age, sex, marital status, income and tribe.

References

1. Global Initiative for Asthma, 2011. (http://www.ginaasthma.org accessed on Mei 2012)

2. WHO report, 2005.Asthma, (http:// www. who.int/ respiratory/ asthma/en/ index.html accessed on April 2010)

3. Indonesia PD. Tuberkulosis: pedoman diagnosis dan penatalaksanaan di Indonesia. Jakarta: PDPI. 2011:20-30.

4. Sundaru, H., 2002. Asma apa dan bagaimana pengobatannya, p 1-20 Jakarta. Balai Penerbit FK UI.

5. Bauman, A., Borland, R., Brown, C., Cockburn, J., Hill, D, Rand, 2005. Asthma adherence, a guide for health professionals. p 1-30 Australia. National Asthma Council.

6. Mangan, 2007. Enhancing patient adherence to asthma therapy (http://www.uptodate for patient, accessed on 20 Maret 2010)

7. Murti B. Desain dan ukuran sampel untuk penelitian kuantitatif dan kualitatif di bidang kesehatan. Yogyakarta: Gadjah Mada University Press. 2006; 67:113-3.

8. Wibowo, 2005. Analisis factor. p 1-10 dalam Materi Pelatihan Statistika Multivariat. Surabaya. Lembaga Penelitian dan Pengabdian Masyarakat Universitas Airlangga.

9. Marliza, 2005. Profil Pengobatan Asma di Puskesmas Kodya Medan, unpublished Thesis, Program Pendidikan Dokter Spesialis I Departemen Ilmu Penyakit Paru. FK USU. SMF Paru RSUP H Adam Malik Medan.

10. Madiyono B, Moeslichan S, Sastroasmoro S, Budiman I, Purwanto SH. Perkiraan besar sampel. Dasar-dasar metodologi penelitian klinis. Jakarta: Sagung Seto. 2002:259-86.

11. Suzuki K, Kaminuma O, Yang L, Takai T, Mori A, Umezu-Goto M, Ohtomo T, Ohmachi Y, Noda Y, Hirose S, Okumura K. Prevention of allergic asthma by vaccination with transgenic rice seed expressing mite allergen: induction of allergen-specific oral tolerance without bystander suppression. Plant biotechnology journal. 2011; 9(9):982-90. https://doi.org/10.1111/j.1467-7652.2011.00613.x PMid:21447056

12. Yunus F, Rasmin M, Sutoyo DK, Wiyono WH, Antariksa B, Fitriani F, Sahril R, Mustafa J, Zulfikar T, Alvian F. Prevalensi Asma Pada Siswa Usia 13-14 tahun Berdasarkan Kuesioner ISAAC di Jakarta. J Respir Indo. 2011; 4:31.

13. Atmoko W, Faisal HK, Bobian ET, Edisworo MW, Yunus F. prevalence of Uncontrolled Asthma and Factors Associated with the Level of Asthma Control at Asthma Clinic Persahabatan Hospital, Jakarta: pd 10–19. Respirology. 2009; 14:A223.

14. Imelda S, Yunus F, Wiyono WH. Correlation of Asthma Degree Compared to Quality of Life Measured by Asthma Quality of Life Questionnaire. Journal of the Indonesian Medical Association. 2011; 57(12).

15. Bachtiar D, Yunus F, Wiyono WH. prevalence of Controlled Asthma in Asthma Clinic Persahabatan Hospital Jakarta 2009: pd 14–20 2009–589. Respirology. 2009; 14:A247. Muzaham, (eds), 2007, Memperkenalkan sosiologi kesehatan. p 93-176 Jakarta. Penerbit Universitas Indonesia UI Press.

16. Notoatmodjo S. Promosi kesehatan dan ilmu perilaku. Jakarta: Rineka Cipta. 2007; 20.

17. Reznik M, Bauman LJ, Okelo SO, Halterman JS. Asthma identification and medication administration forms in New York City schools. Annals of Allergy, Asthma & Immunology. 2015; 114(1):67-8. https://doi.org/10.1016/j.anai.2014.10.006 PMid:25454012 PMCid:PMC4274201

18. Wahyuni AS, Hamid RZ, Syafiuddin T, Bachtiar A, Martina SJ, Amelia R. A model confirmatory of adherence behavior with standard medication treatment among patients with asthma in Medan-Indonesia. InMATEC Web of Conferences 2018 (Vol. 197, p. 07006). EDP Sciences.

19. Ledford D, Apter A, Brenner AM, Rubin K, Prestwood K, Frieri M, Lukert B. Osteoporosis in the corticosteroid-treated patient with asthma. Journal of allergy and clinical immunology. 1998; 102(3):353-62. https://doi.org/10.1016/S0091-6749(98)70120-4

20. Gamble J, Stevenson M, McClean E, Heaney LG. The prevalence of nonadherence in difficult asthma. American journal of respiratory and critical care medicine. 2009; 180(9):817-22. https://doi.org/10.1164/rccm.200902-0166OC PMid:19644048

21. Gillissen A. Patients' adherence in asthma. Journal of physiology and pharmacology. 2007; 58(5):205-22.

22. Wells K, Pladevall M, Peterson EL, Campbell J, Wang M, Lanfear DE, Williams LK. Race-ethnic differences in factors associated with inhaled steroid adherence among adults with asthma. American journal of respiratory and critical care medicine. 2008; 178(12):1194-201. https://doi.org/10.1164/rccm.200808-1233OC PMid:18849496 PMCid:PMC2599867

ID Design Press, Skopje, Republic of Macedonia
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Evaluate the Effect of Education Interventions in the Prevention of Diabetic Foot Ulcers through Knowledge of the Disease and Self-Care Practices in Saudi Arabia

Nagwa Ahmed Mohammad*, Reham Mohammad Khresheh

University of Tabuk, Tabuk, Saudi Arabia

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***Correspondence:** Nagwa Ahmed Mohammad. University of Tabuk, Tabuk, Saudi Arabia. E-mail: n-mohamed@ut.edu.sa

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Abstract

BACKGROUND: Diabetes mellitus places a substantial burden on society worldwide. Diabetic foot ulcers are a challenging problem for clinicians. Six generally accepted detriments to the healing of diabetic foot ulcers were identified: infection, glycaemic control, vascular supply, smoking, nutrition and deformity.

AIM: To evaluate the effect of educational interventions in the prevention of diabetic foot ulcers through knowledge of the disease and self-care practices.

METHODS AND DESIGN: A quasi-experimental, design was used. The study was conducted in the Internal Medicine Department and Outpatient clinic at Umulj general hospital - 2016. The study sample consists of 60 adult patients with diabetes mellitus. Approval to conduct the study obtained from the Ministry of Health and the University of Tabuk Research Ethics Committee.

RESULTS: This study shows that, a significant relationship between levels of patient's knowledge, practice and level of education.

CONCLUSION: The result of the present study concluded that implementation of the developed educational program showed significant improvement in the patients level of knowledge, patients ability to perform self-foot care and level of patient awareness after program implementation.

Introduction

The World Health Organization (WHO) has reported that Saudi Arabia ranks the second highest in the Middle East, and is seventh in the world for the rate of diabetes. It is estimated that around 7 million of the population are diabetic and almost around 3 million have pre-diabetes. Even more worrying perhaps, is the increasing pattern of diabetes noted in Saudi Arabia in the recent past. Diabetes has approximately registered a ten-fold increase in the past three eras in Saudi Arabia [1].

Diabetes mellitus is a chronic metabolic multifactorial disorder associated with altered glucose homeostasis as well as macro and microvascular complications including preventable foot problems that are common occurrences in these patients [2]. Diabetic foot problems are major causes of morbidity and premature mortality and contribute substantially to health care costs [3]. Foot ulcerations are also a major complication in diabetes patients (~25%) and infected diabetic foot ulcers are responsible for 60% of nontraumatic lower-limb amputations Previous studies have reported that early identification of people at high risk for foot problems and management of the risk factors could prevent lower extremity amputations and foot ulcerations [4] [5].

Nurses can also educate patients on the importance of controlling blood glucose levels through diet. Lastly, nurses need to educate patients about prevention and treatment of diabetic foot problems. A major problem associated with diabetes is the onset of complications that may affect the patient's health status and may become life-threatening [6]. Most importantly, the nurse should educate the patient to report foot problems to his or her doctor as soon as they are noticed. These problems include cuts or breaks in the skin, ingrown nails, pain or loss of

sensation, and changes in the colour or discolouration of the foot [7].

The study aims to evaluate the effect of educational interventions on the prevention of diabetic foot ulcers through knowledge of the disease and self-care practices. Knowledge and self-care practices about foot care will be better among the educational interventions group than the control group.

Patients and Method

The quasi-experimental study design was utilised.

The study was conducted at the Internal Medicine Department and Outpatient clinic at - Umlui General Hospital, Saudi Arabia.

Sixty adult male and female who have type 2 diabetes mellitus were included. Patients were randomly assigned into groups: group 1 (n = 30) which was the 'intervention' group and group II (n = 30) which was the control group. Group '1' participants received an educational program containing the instructions and were trained on the activities included in it, while group 'II' received standard hospital care.

Inclusion Criteria: Patients who have type 2 diabetics mellitus, both age and gender range between 18-60 years old and duration of diabetes for more than 5 years.

Exclusion criteria: Patients with gastroparesis, orthostatic hypotension and hypoglycemia unawareness (autonomic neuropathy), mentally ill patients, hearing or visual difficulties.

The work was performed by including the patients admitted on Sunday, Monday and Tuesday in the intervention group and the patients admitted during the rest of days the week in the control group.

To collect the necessary information for the study, the following tools were used:

The tool I: patients assessment sheet includes 4 items:

1. Sociodemographic data;

2. Patients' assessment related to knowledge;

3. Patients' assessment related to self-care practices;

4. Leg assessment sheet.

Tool II: A designed nursing intervention protocol includes 4 items:

1. Knowledge related to diabetes and foot care;

2. Self-care practices concerning diabetes and foot care;

3. Diabetes nutrition;

4. Insulin injection.

Tool III: Observational checklist related to foot care.

It was developed by the researcher to collect general information related to personal data and diabetic patient assessment. Content validity of the tool was tested by expertise in the medical and nursing field. This tool is divided into four parts to cover the following dimensions: Sociodemographic data, patient assessment related to knowledge, patient assessment related to self-care practices and leg assessment sheet. These data were collected, and the questionnaire sheet was filled by the researcher through, an interview, by taking a history from patients, assessment of the patient and educating them. This tool includes questions in the form of multiple choice questions and others in the form of closed questions. It compromised the following:

It includes demographic characteristics of the studied groups as regarding their age, sex, marital status, occupation, level of education, duration of diabetes and residence.

It was used to collect data as regarding: diabetes information which includes; what about diabetes, signs and symptoms of hyperglycemia and hypoglycemia, proper diabetic nutrition and complications of diabetes mellitus. Exercise information includes the following; proper diabetes exercise cautions when performing exercise and types of exercise. Diabetic foot information includes the following; what about a diabetic foot, causes of diabetic foot and complications of diabetic foot and proper foot care which categorised as; proper foot inspection, proper foot hygienic care, early detection of diabetic foot, trimming toenail, proper footwear and improving lower limb circulation.

The scoring system was rated for two levels; yes and no, each item score grade = 1, for yes answer = 1 and zero for no answer or wrong answer. Total system scores will be (19) grades. Those who obtained less than (60%) were considered having an unsatisfactory level. While those who obtained above than (60%) were considered having a satisfactory level of knowledge and practice

Reliability of the test tools was used to determine the extent to which the items in the questionnaire are related to each other. Cronbach's alpha model was used in the analysis; it is a model of internal consistency, a value greater than 0.8 denotes very good internal consistency meaning that the questionnaire is reliable. The results regarding our questionnaire were as follows 0.848 [8].

It was developed by experts in the medical and nursing field. Modifications were done based on

review related literature, the clinical learning experience of the researcher and expertise selected certain items to suit the aim of the study. Content validity of this tool was tested by expertise in the medical and nursing field. This tool was used to identify patient performance related to foot care and contain certain items were selected such as:

Foot inspection; use a mirror to see bottoms of the feet. Proper foot cleaning includes the following; washing the feet in warm water, do not soak the feet, drying the feet well and dry between toes. Nail and foot care include the following; lubricating the feet to keep skin soft and smooth, do not apply cream between the fingers and trim the nail straight across & file the edge with a nail file. Proper habits to protect the feet which categorized as; patient move ankle up and down for 5 minutes, put the feet up when setting position, don't sit for long period of time, don't cut corns and calluses, smooth corns and calluses gently, skin edges do not remove by himself, use proper footwear, check colour of feet and leg, wear slippers when getting out of bed and continuous follow up care plan.

It was used to collect data to assess the condition of the right and left leg and effect of diabetes on it. They include following categories related to foot assessment such as: Examining peripheral pulse; femoral, popliteal, posterior tibial and dorsal pedal pulse. Skin leg temperature, skin turgor and skin colour. Pain site, pain frequency and pain degree (pain degree measured by using a numeric rating scale, a pain scale measures a patient's pain intensity or other features. Pain scales are based on self-report and rated from 0 to 10): Lower limp edema, edema type (absent, mild, moderate or severe), leg edema location; (none, localized per ulcer, foot inducing ankle, to mid-calf, or to knee), perception of pain, touch and temperature. Perception to touch assessed by using Senses-Weinstein monofilament and general well-being (Satisfied and dissatisfied).

This type of data was collected through examination of patient legs pre protocol and after 3 months follow up post protocol, leg examination done to assess the effect of diabetes on foot and to prevent, early detect any complications. Confirmation was done by the researcher.

The content of protocol was developed by the researcher and revised by expertise in the medical and nursing field; the content was consistent with the related literature. This tool is divided into four parts: knowledge related to diabetes and foot care, self-care practices concerning diabetes and foot care, diabetes nutrition and insulin injection, confirmation of data was done by the researcher.

It was used to provide an increase in patients' knowledge which includes: what about diabetes, signs and symptoms of hyperglycemia and hypoglycemia, complications of diabetes mellitus. Exercise information includes the following; proper diabetes exercise cautions when performing exercise and types of exercise. Diabetic foot information includes the following; what about a diabetic foot, causes of diabetic foot and complications of the diabetic foot. Patient self-care practices based on knowledge provided to the patient, which includes: early detection of diabetic foot ulcer thorough examining the foot, prevention of diabetic foot occurrence by performing daily basis of foot care and performing the proper exercise.

This tool it was used to identify proper nutrition for patients, the content was developed based on review related literature, which includes: purpose from good nutrition, how to prepare a healthy diet, rules for proper nutrition, a component of nutrition, food pyramids and examples of models of a healthy diet.

Considering as a part of diabetes self-care that help the patient to be able to perform self-injection. This part includes: information about insulin and how to performing insulin injection

It was developed by expertise on the medical and nursing field. Modifications were done based on review related literature, the theoretical and clinical learning experience of the researchers and experts selected certain items to suit the aim of the study. Content validity of this tool was tested by expertise in the medical and nursing field. This tool was used to identify patient performance related to foot care and contain certain items were selected such as:

Washing the feet in warm water, dry between toes, do not soak the feet, lubricating the feet to keep skin soft and smooth, do not apply cream between the fingers, trim the nail straight across and file the edge with nail file, patient move ankle up and down for 5 minutes, put the feet up when setting position, don't sit for long period of time, check color of feet and leg, don't cut corns and calluses, smooth corns and calluses gently, skin edges do not remove by himself, use proper footwear, wear slippers when getting out of bed, use mirror to see bottoms of the feet and continuous follow up care plan.

Observational chick list performed and confirmed by the researcher. The scoring system was rated for two levels; done and not done, each item was observed, categorised and scored into either done correctly = 1. Don = 1, not done = 0. Total system scores for all items was (18) grades. Those who obtained less than (60%) were considered having an unsatisfactory level. While those who obtained above than (60%) were considered having a satisfactory level of practice.

The study was carried out in 3 phases:

1. The preparatory phase (first phase)

In which the study tools and the designed teaching protocol was developed, and the content was consistent with the related literature (nursing textbook,

journal and internet source) about diabetes, foot assessment and foot care, The pilot study was to test the applicability of the assessment tools, identify how data was collected effectively, also to identify the possible obstacles or problem that may hinder the data collection during the implementation phase.

2. Implementation phase (second phase)

The patient assessment was conducted pre-protocol, immediate post protocol and after 3 month follow up through: Asking patient questions to collect information that was related to sociodemographic data for example age, sex, occupation, level of education and duration of diabetes, patient knowledge assessment through asking questions related to diabetes and foot care as including, what about diabetes, proper diabetes care and proper foot care, foot assessment through examining both feet.

Total numbers of designed protocol sessions, seven sessions divided as follows: 4 patients for each session and every 4 patients group receive all seven protocol sessions reciprocally, each session time consumed about 30 minutes due to patients interests. First session: introduce myself to the patients (name and job) aim from the meeting, some sessions, orient the patients regarding the designed protocol contents, its purpose, related benefits and its impact on his/her condition.

Second session: summary about what has been discussed in the previous sessions, objectives of the new session, content of the session includes what about diabetes mellitus, definition of diabetes mellitus, risk factors of diabetes mellitus, clinical manifestation of hypoglycemia, clinical manifestation of hyperglycemia and complications of diabetes mellitus, the session ended by a summary of its contents and feedback from the patients through health education and discussion. Many patients were cooperative with the researchers; they were very interested in the given topics and asked to continue such a training program to update their knowledge.

Third session: summary about what has been discussed in the previous sessions, objectives of the new session, content of the session includes what is the diabetic foot, what are risk factors of diabetic foot ulcer and complications of diabetic foot ulcers, the session ended by a summary of its contents and feedback from the patients through discussion and asking questions.

Fourth session: summary about what has been discussed in the previous sessions, objectives of the new session, content of the session includes daily foot care, toenail care, footwear and socks, follow up care plan, the session ended by a summary of its contents and feedback from the patients through discussion and asking questions.

Fifth session: summary about what has been discussed in the previous sessions, objectives of the new session, content of the session includes purpose of nutritional planning, preparing a healthy integrated meal, general rules of dieting correctly, quality of food that suited the diabetics, times of diet, food pyramids for diabetics, models of the integrated food meals and types of food permitted without reservation, the session ended by a summary of its contents and feedback from the patients through discussion and raising of questions.

Sixth session: summary about what has been discussed in the previous sessions, objectives of the new session, the content of the session includes information about insulin, how to perform insulin injection and sites of insulin injection, the session ended by a summary of its contents and feedback from the patients through discussion and asking questions.

Last session includes: summary about what has been discussed in the previous sessions, objectives of the new session, the content of the session includes the importance of physical exercise for diabetics, what must be done when performing physical exercise, types of physical exercise and importance of physical exercise, the session ended by a summary of designed protocol contents and feedback from the patients through discussion and asking questions.

3. Evaluation phase (third phase)

Effect of the designed protocol on patient condition was done by comparing the pre and post assessment of the patients including Their knowledge, ability to foot self-care and follow up regularly.

The study approved by an institutional ethics committee, informed written consent was obtained from patients who are willing to participate in the study after the nature and purpose of the study were explained, The researchers initially introduced themselves to all patients, and they assured data confidentially. They were informed that their participation was voluntary and they have the right to withdraw any time from the study.

Data entry and statistical analysis were done using SPSS ver. 23 statistical software package. Data were presented using descriptive statistics in the form of frequencies and percentage for qualitative variables mean and standard deviations for the quantities variables the level of significance was set at ($p = 0.05$) to detect any indication of differences found in the data available.

Results

Table 1 shows that the majority of the study and control groups were females (63.3%, 53.3% respectively), and common age group category ranged from 50-59 years, regarding the duration of

diabetes more than half of the patients were affected between 5-10 years. The majorities of the patients in the study were not working (73.3%, 60% respectively) and lives in urban (76.7%, 80% respectively). Regarding social status, the majority of them were married in both groups. (Study and Control) (96.7%, 90% respectively).

Table 1: Socio-demographic characteristics distribution of the study and control groups

Socio-demographic characteristics	Study group		Control group		t-value	P-value
	No	%	No	%		
Age in years:						
20 – 29	2	6.7	2	6.7		
30- 39	1	3.3	1	3.3		
40 – 49	9	30.0	11	36.7	-0.030	0.976
	18	60.0	16	53.3		
(Mean ±SD)	48.4 ± 8.8		48.5 ± 8.2			
Duration of diabetes:						
5- 10 years	16	53.3	22	73.3		
11- 15 years	9	30.0	6	20	2.370	0.141
Above 15 years	5	16.7	2	6.7		
(Mean ±SD)	11.2 ± 4.5		8.6 ± 2.5			
	No	%	No	%	x^2-value	P-value
Gender:						
Male	11	36.7	14	46.7	0.617	0.432
Female	19	63.3	16	53.3		
Social status:						
Single	1	3.3	0	0		
Married	29	96.7	27	90.0	4.071	0.254
Divorced	0	0	1	3.3		
Widow	0	0	2	6.7		
Level of education:						
Illiterate	9	30	6	20		
Read & write	7	23.3	11	36.7	3.468	0.325
Basic education	10	33.3	12	40		
University	4	13.3	1	3.3		
Occupation:						
Employee	1	3.3	4	13.3	2.266	0.322
Worker	7	23.3	8	26.7		
No work	22	73.3	18	60		
Residence:						
Urban	23	76.7	24	80	0.096	0.757
Rural	7	23.3	6	20		

*Significant at P ≤ 0.05.

The study group indicates a significantly higher score in knowledge than the control group in both immediate post protocol and at follow up (P < 0.001). While this table also enumerates no statistically significant difference between the study and control groups pre protocol (P- 0.155), Table 2.

Table 2: Total and subtotal mean knowledge scores obtained by patient's pre, immediately and 3 months after protocol implementation

Patients knowledge	Study group	Control group	P-value
	Mean ± SD	Mean ± SD	
1-Diabetes knowledge:			
Pre protocol	0.57 ± 0.32	0.67 ± 0.24	0.203
Immediate post protocol	0.97 ± 0.09	0.67 ± 0.24	<0.001*
3 month follow up	0.91 ± 0.12	0.7 ± 0.23	<0.001*
2-Importance of exercise:			
Pre-protocol	0.06 ± 0.12	0.21 ± 0.25	0.004*
Immediate post protocol	0.76 ± 0.25	0.21 ± 0.25	<0.001*
3 month follow up	0.60 ± 0.49	0.17 ± 0.38	<0.001*
3-Diabeticfoot knowledge:			
Pre protocol	0.18 ± 0.2	0.32 ± 0.2	0.013*
Immediate post protocol	0.78 ± 0.18	0.32 ± 0.2	<0.001*
3 month follow up	0.63 ± 0.2	0.35 ± 0.2	<0.001*
4- Foot care knowledge:			
Pre protocol	0.18 ± 0.17	0.25 ± 0.2	0.148
Immediate post protocol	0.96 ± 0.06	0.25 ± 0.2	<0.001*
3 month follow up	0.87 ± 0.1	0.27 ± 0.19	<0.001*
Total mean score:			
Pre protocol	0.25 ± 0.14	0.36 ± 0.18	0.155
Immediate post protocol	0.87 ± 0.36	0.36 ± 0.18	<0.001*
3 month follow up	0.75 ± 0.27	0.37 ± 0.24	<0.001*

*Significant at P ≤ 0.05.

Table 3 shows that the study group results are significantly different from the control group in both immediate post protocol and at follow up (P < 0.001*). Also, this table shows no statistically significant difference between the study and control group pre protocol (P 0.133).

Table 3: Total mean practice scores for patient self-care practices related to foot care throughout program phases among the study and control groups

Patient practice related to self –care practices	Study group	Control group	t-value	P-value
	Mean ± SD	Mean ± SD		
Pre protocol	0.37 ± 0.22	0.29 ± 0.19	1.522	0.133
Immediate post protocol	0.79 ± 0.13	0.29 ± 0.19	11.836	< 0.001*
3 month follow up	0.76 ± 0.1	0.38 ± 0.19	9.509	< 0.001*

Table 4 shows that there was no significant difference between the level of patient's knowledge and level of patients practice pre-protocol, immediate post protocol and at follow up (P = 0.097, 0.758, 0.896 respectively).

Table 4: Comparison of the mean score of total knowledge and practice in the study group

Items	Level of patients knowledge	Level of patient practice	P-value
	Mean ± SD	Mean ± SD	
Pre protocol	0.25 ± 0.14	0.37 ± 0.22	0.097
Immediate post protocol	0.87 ± 0.36	0.79 ± 0.13	0.758
3 month follow up	0.75 ± 0.27	0.76 ± 0.1	0.896

Table 5 reveals that some patients suffer from diminished pulse was 13.3%- 36.7% while 63.3% to 83.3% were a normal pulse. Regarding skin assessment 10%- 20% show cold skin turgor and showed minimal improvement in follow up than pre protocol for the study group while in control group dry skin turgor increased in follow up than pre-protocol. As regard to skin colour, 56.7% to 80 % have red skin colour.

Table 5: Frequency and percentages of leg assessment related to pulse and skin assessment of the study and control groups

Leg assessment		Study group				Control group				x^2-value	P-value
		Pre-protocol		Follow up after 3 month		Pre-protocol		Follow up after 3 month			
		No	%	No	%	No	%	No	%		
Femoral pulse	Diminished	4	13.3	7	23.3	11	36.7	9	30		
	Normal	25	83.3	22	73.3	19	63.3	20	66.7		
	Bounding	1	3.3	1	3.3	0	0	1	3.3		
Posterior tabial pulse	Diminished	5	16.7	4	13.3	9	30	10	33.3		
	Normal	24	80	25	83.3	21	70	19	63.3		
	Bounding	1	3.3	1	3.3	0	0	1	3.3		
Dorsalis pedis pulse	Diminished	6	20	3	10	10	33.3	11	36.7		
	Normal	24	80	27	90	20	66.7	19	63.3		
Popliteal pulse	Diminished	6	20	9	30	9	30	10	33.3		
	Normal	24	80	21	70	21	70	20	66.7		
Leg temperature	Cool	4	13.3	6	20	3	10	3	10		
	Normal	26	86.7	24	80	27	90	27	90		0.942
	Dry	6	20	2	6.7	5	16.7	7	23.3		
Skin turgor	Soft	21	70	25	83.3	24	80	23	76.7		
	Normal	3	10	3	10	1	3.3	0	0		
	Pallor	7	23.3	9	30	4	13.3	7	23.3		
Skin color	Cyanosis	3	10	4	13.3	2	6.7	0	0		
	Normal	20	66.7	17	56.7	24	80	23	76.7		

*Significant at P ≤ 0.05.

Table 6 shows that the study and control group were identical in leg assessment pre protocol and in follow up. Regarding pain assessment nearly about half of patients with no pain, 46%- 53.3% with mild pain and 20%-33.3% pain increase with position dependent. Regarding oedema assessment, 83.3% to

93.3% was normal, regarding perception 36.7% to 43.3% with decreased perception.

Table 6: Frequency and percentages distribution of leg assessment related to pain, oedema and perception among the study and control groups

Leg assessment		Study group				Control group				x^2-value	P-value
		Pre-protocol		Follow up after 3 month		Pre-protocol		Follow up after 3 month			
		No	%	No	%	No	%	No	%		
Pain site	Absent	14	46.7	13	43.3	14	46.7	11	36.7		
	Foot	5	16.7	4	13.3	2	6.7	3	10		
	Foot & calf	4	13.3	5	16.7	2	6.7	2	6.7		
	Foot, calf & thigh	7	23.3	8	26.7	12	40	14	46.7		
Pain degree	Absent	14	46.7	14	46.7	14	46.7	10	33.3		
	Mild	14	46.7	14	46.7	16	53.3	16	53.3		
	Moderate	1	3.3	1	3.3	0	0	3	10		
	Severe	1	3.3	1	3.3	0	0	1	3.3		
Pain frequency	None	14	46.7	17	56.7	14	46.7	14	46.7		
	Occasional	5	16.7	3	10	2	6.7	2	6.7		
	Position dependent	6	20	6	20	10	33.3	10	33.3		
	Constant	4	13.3	3	10	4	13.3	4	13.3		
	Disturbs sleep	1	3.3	1	3.3	0	0	0	0		
Edema type and location	Absent	28	93.3	27	90	27	90	25	83.3		
	Mild (foot to mid calf)	0	0	1	3.3	3	10	4	13.3		
	Severe reach the knee	2	6.7	2	6.7	0	0	1	3.3		
Perception to touch, temperature and pain	Absent	3	10	2	6.7	0	0	1	3.3		
	Decreased	13	43.3	13	43.3	13	43.3	11	36.7		
	Increased	0	0	1	3.3	1	3.3	3	10		
	Normal	14	46.7	14	46.7	16	53.3	15	50		

In Table 7 according to the Study group results is there is a statistically positive significant correlation between duration of diabetes and perception of pain as increased duration of diabetes was associated with decreased perception of pain in the right and left legs (P-0.016*, 0.005* respectively). In the Control group, there was no statistically significant correlation between duration of diabetes and perception of pain in both legs.

Table 7: Correlation between duration of diabetes and leg perception to the pain of the study and control groups

	Study group		Control group	
	Correlation coefficient (r)	P-value	Correlation coefficient (r)	P-value
Right leg	-0.477	0.016*	-0.057	0.802
Left leg	0.543	0.005*	-0.007	0.976

*Significant at P ≤ 0.05.

Figure 1 shows a satisfactory level increase at follow up of the study group while a decrease of the control group (66.7%, 46.7%) respectively.

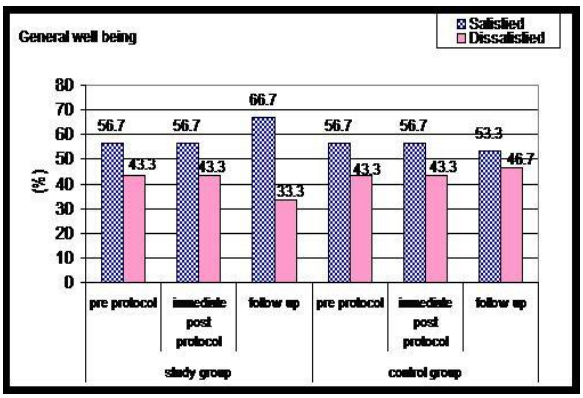

Figure 1: Satisfactory level of general well-being among the study and control groups

Figure 2 shows a direct positive correlation between the level of patient's knowledge and level of patient's education.

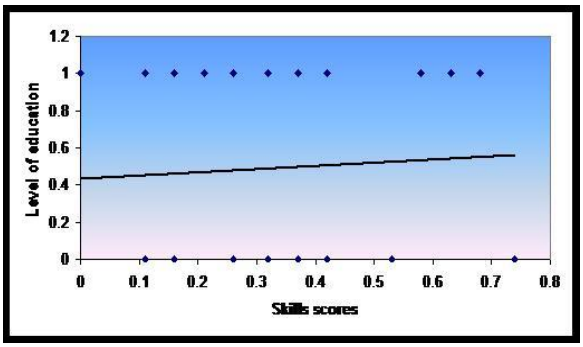

Figure 2: Correlation between level of patient's practice and level of education

Figure 3 shows a direct positive correlation between the level of patient's knowledge and level of patient's education.

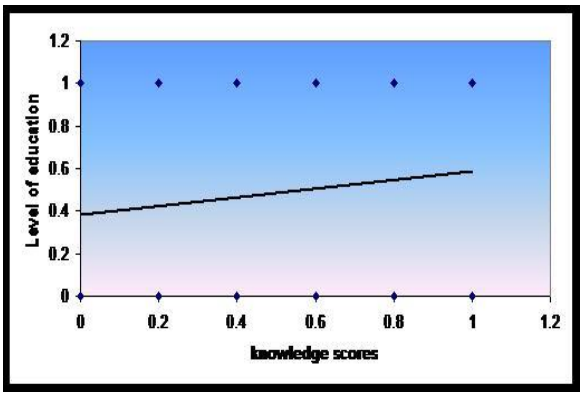

Figure 3: Correlation between level of patient's knowledge and level of education

Discussion

The purposes of this study were carried out to evaluate the effect of educational interventions on the prevention of diabetic foot ulcers through knowledge of the disease and self-care practices.

Regarding age the results indicate that more than half of patients included in this study were between 50-59 years; (60%, 53.3%) for study and control groups respectively, this finding is similar to the results of Joe (2009) which stated that the major categories of patients with diabetes were older people. Also, this result agrees with Liudmila et al. (2008) which stated that the prevalence of diabetes increase among people between 45 and 64 years old. Moreover, it has also been reported that this percentage increases significantly among older individuals.

As regarding gender, the majority of these study were female (63.3%, 53.3%) for study and control groups respectively. Results based on this study cannot provide a definite line that the female was affected more than male. This result agrees with Liudmila et al., (2008) [9] who stated that the most patient included in their study were women.

The present study reveals that regarding social status table 1 shows that most of the patients included in the present study were married at 96.7%, 90% in both groups (study and control). This result agrees with Liudmila et al. (2008) which stated that most patients were observed in the study were married [9]. Mary et al. (2008) stated that no evidence is found for the hypothesis that marriage positively associated with the prevalence of diabetes [10].

Focusing on occupation nearly about two-thirds of patients with no work 73.3%, 60%, one-quarter of patients were working 23.3%, 26.7% (driver, mechanical, circuit or plumber) these types of work might expose patients to higher risk of foot trauma especially with prolonged standing during work. This result was supported by Makota (2009) that the type of occupation can produce an adverse effect on diabetic rather than non-diabetics [11].

As regarding residence this study showed that most of patients included in the study live in urban 76.7%, 80% based on finding of the study there was no significant relationship between the two groups (study and control), this finding was also supported by Arch et al., (2008) results which stated that 88% of patients live in urban [12].

The study and control groups (Table 2) showed that there was a statistically significant improvement in patient's knowledge as regards to the items related to diabetes knowledge, the importance of exercise, diabetic foot knowledge and foot care knowledge improved after protocol implementation among the study group (< 0.001*). Control group in the assessment phase and the follow-up phase shows no statistically significant change in their knowledge, this result was supported by Abd Elateef and Mahmoud (2008) they stated that implementing of intervention protocol had a great effect in the improvement of patient’s knowledge [13].

Regarding level of patient practices there a was recognized improvement immediate post protocol and at follow up in the study group compared to the control group (p- < 0.001*), pre-test compared with the post-test show improvement in the level of patient practices 38.4% to 79.5% while compared pre-test with follow up test showed slightly decreased inpatient practices 38.4% to 75.8%.

This finding agrees with Mohamed (2008) study which stated that the patient's level of practice improved after program implementation and slightly decreased at follow up [14]. So continuing patient education is an important key in the prevention of foot complications. Also, this result was supported by Abd Elateef and Mahmoud (2008) they indicated that implementing of intervention protocol had a great effect on improving patient practices [13]. As regarding patient's leg assessment, the study shows that in both groups (Study and Control) were almost identical of leg assessment prior implementing intervention protocol but shows minimal differences of leg assessment after implementing intervention protocol of the study and control groups.

Focusing on general well being, more than half of patients in both groups with a satisfactory quality of life throughout the program of the study 66.7%, 53.3% because all patients included in this study free from foot ulcer. For example, the loss of mobility associated with foot ulcers affects patients' ability to perform simple, everyday tasks and to participate in leisure activities; these consequences often lead to depression and poor quality of life.

Concerning the relationship between the level of patients practice, level of patient's knowledge and age, there were no statistically significant differences.

As regarding relation between duration of diabetes and level of patients knowledge, the study found that no association between duration of diabetes and level of patient knowledge (p- 0.759) this finding contradicts with Van-den et al., (2010) which stated that presence of positive association between duration of diabetes and level of patient's knowledge [15].

In conclusion, providing a structured program for a patient who has type 2 diabetes mellitus was effective in improving t in the level of patients' knowledge and patients' ability to perform self-care practice.

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References

1. Al-Nozha MM, Al-Maatouq MA, Al-Mazrou YY, Al-Harthi SS, Arafah MR, Khalil MZ. Diabetes mellitus in Saudi Arabia. Saudi Med J. 2008; 25(11):1603–10.

2. Guariguata L, Whiting D, Weil C, Unwin N. The International Diabetes Federation diabetes atlas methodology for estimating global and national prevalence of diabetes in adults. Diabetes research and clinical practice. 2011; 94(3):322-32. https://doi.org/10.1016/j.diabres.2011.10.040 PMid:22100977

3. Aguiree F, Brown A, Cho NH, Dahlquist G, Dodd S, Dunning T, Hirst M, Hwang C, Magliano D, Patterson C, Scott C. IDF diabetes atlas, 2013.

4. Alqurashi Khalid A, Aljabri Khalid S, Bokhari Samia A. Prevalence of diabetes mellitus in a Saudi community. Ann Saudi Med. 2011; 31(1):19–23. https://doi.org/10.5144/0256-4947.2011.19 PMid:21245594 PMCid:PMC3101719

5. Alhowaish Abdulkarim K. Economic costs of diabetes in Saudi Arabia. J Fam Community Med. 2013; 20:1–7. https://doi.org/10.4103/2230-8229.108174 PMid:23723724 PMCid:PMC3663158

6. Bean A. Patient refusal of nutrition and hydration, the complete guide to sports nutrition. A & C black publishers, American journal hospice and palliative care. 2008; 81-83.

7. Bloomgarden Z.T. Consequences of diabetes: cardiovascular disease, diabetes care. 2004; 27(7):1825-31.

8. Revelle W, Zinbarg RE. Coefficients alpha, beta, omega, and the glb: Comments on Sijtsma. Psychometrika. 2009; 74(1):145. https://doi.org/10.1007/s11336-008-9102-z

9. Liudmila MO, Maria LZ, Carla R. Sociodemographic and clinical characteristics of a diabetic population at a primary level health care center. Annual Meeting. 2008; 7:14-18.

10. Wolfinger NH, Mason MA, Goulden M. Problems in the pipeline: Gender, marriage, and fertility in the ivory tower. The Journal of Higher Education. 2008; 79(4):388-405. https://doi.org/10.1080/00221546.2008.11772108

11. Makota S. (2009): Biological variation in predicts of retinopathy and neuropathy in type 2 diabetes, 27 (6) 1259-1264.

12. Arch JJ, Craske MG. Acceptance and commitment therapy and cognitive behavioral therapy for anxiety disorders: Different treatments, similar mechanisms?. Clinical Psychology: Science and Practice. 2008; 15(4):263-79. https://doi.org/10.1111/j.1468-2850.2008.00137.x

13. Abd-Elateef ZME, Mahmoud ST. Prevention of diabetic foot complication: Impact of implementing a designed nursing intervention protocol. Med J Cairo Univ. (2008); 27 (2): 59- 67.

14. Mohamed M. A. Improving quality of life in rheumatoid arthritis patients. Faculty of nursing. Zgazig University. 2008; 100-117.

15. Van den Arend IJ, Stolk RP, Rutten GE, Schrijvers GJ. Education integrated into structured general practice care for Type 2 diabetic patients results in sustained improvement of disease knowledge and self-care. Diabetic Medicine. 2000; 17(3):190-7. https://doi.org/10.1046/j.1464-5491.2000.00232.x PMid:10784222

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Public Health

The Freshman Weight Gain Phenomenon: Does It Apply To Lebanese Students?

Suzan A. Haidar[1,2*], Nanne K. de Vries[1], Dimitrios Papandreou[3], Rana Rizk[1,2], Mirey Karavetian[3]

[1]Maastricht University, Maastricht, Netherlands; [2]Lebanese University, Beirut, Lebanon; [3]Zayed University, Dubai, United Arab Emirates

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Keywords: Freshman 15; College students; Weight gain; Obesity; Stress

***Correspondence:** Suzan A. Haidar. Maastricht University, Maastricht, Netherlands. E-mail: suzan.haidar@liu.edu.lb

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Abstract

BACKGROUND: When transitioning from high school to university, students tend to gain weight.

AIM: The study aimed to identify whether Lebanese students experience weight changes during the transition and to identify predisposing factors.

METHODS: Eighty freshman students from four campuses in Lebanon were recruited in October 2015. Students were assessed anthropometrically and biochemically at two-time points, seven months apart. Students filled out questionnaires assessing demographic and lifestyle habits, physical activity levels, nutrition knowledge, sleep quality, and stress.

RESULTS: The students gained approximately 4.19 lbs. (1.90 kg). There was a significant increase in waist circumference, blood glucose and triglyceride levels. There were no significant changes in HDL-cholesterol, total cholesterol, knowledge score, stress level, physical activity, sleep and lifestyle habits.

CONCLUSIONS: Lebanese students do gain weight during their first year of university enrollment. Future research is necessary to assess the reasons for weight gain and develop suitable prevention programs.

Introduction

Obesity rates are at an all-time high and have reached epidemic magnitudes. According to World Health Organization data, more than 600 million adults were obese in 2014 [1]. These estimates are alarming since obesity increases the risk of a multitude of physiological and psychological chronic diseases and can lead to premature morbidity and mortality. The fight against obesity is fierce with researchers, health, and governmental organisations combining their efforts to restrict the uprise of this epidemic.

One strategy that is promising in combating obesity is to identify critical periods of weight gain; pinpoint vulnerable groups and accordingly, plan preventive interventions. One such identified critical period of weight gain is during student transition from high school to university or college [2]. This critical period could potentially dictate whether these students will live as healthy adults or possibly become overweight or obese by acquiring unhealthy habits especially since nutrition habits acquired at an early age and weight problems tend to continue in adulthood [3] [4]. Unfortunately, once weight gain occurs, it becomes hard to manage and increases vulnerability to obesity and subsequent complications [5] [6].

University students are at an increased risk of gaining weight during the freshman year; it has previously been hypothesised that the average

collegian may gain approximately 15 pounds; a phenomenon that has been coined “The Freshman 15” [7]. However, more recent empirical evidence, suggests that the 15-pound increase is exaggerated and that a 3 to 5-pound weight gain is more realistic [8] [9]. Nevertheless, even a slight weight gain is still significant and is higher than the gain experienced by the general population [9] [10] [11] which may negatively affect the future health of these students. Therefore, prevention of weight gain in such a vulnerable group during this critical period could prove to be key in fighting obesity in this population.[12] Explanations for this weight gain vary; however, some key factors that have been previously identified as culprits include increased alcohol and fast food consumption, decreased physical activity, moving away from home accompanied by lack of parental supervision and increased stress levels [13] [14]. Notably, stress may have a differential effect of on BMI [15]; Serlachius and colleagues found that students with a lower body weight tend to lose weight as a consequence of high-stress levels as opposed to students who have a higher BMI who tend to gain weight secondary to the increased stress [15].

Although, the freshman weight gain phenomena have been extensively researched and proven in the United States and Europe borders [6]; it is still not clear whether this phenomenon also applies among Middle Eastern students.

Therefore, this study aimed to investigate whether Lebanese students do gain weight during the freshman year, and determine the predisposing factors.

Methods

The study had a longitudinal design and was conducted in a private university with many campuses in Lebanon. Using two-step sampling, four campuses from different geographical locations were selected. Afterwards, all first-year students were invited to participate in the study via mass e-mail and through classroom visits made by the primary investigator. Students were informed about the objectives and methods of the study, in addition to their right to withdraw from the study at any time. Criteria for students to be included in the study were: 1) first semester of enrollment at the university, 2) free of disease, 3) not pregnant. Students were excluded from the study if they were sick at the time of the study, suffered from chronic diseases or were not fasting. Students who did not meet inclusion criteria were excluded. In total 80 students who volunteered met the inclusion criteria and consented. Ethical approval for this study was obtained from the university’s committee on Research Ethics (CRE) (case number: LIUCRE-141117-2). Participants completed the primary assessment in their first semester (T_0) and then 7 months later during enrollment in the second semester (T_1.) Only 40 students returned for follow-up out of which 27 consented to blood withdrawal. The reasons for the high dropout rate could be due to change in class schedules and fear of blood collection.

Sample Size: A statistical power analysis was performed using Power and sample size Calculations Software (version 3) to estimate the adequate number of participants needed for this study. Prior data indicate that the difference in the response of matched pairs is normally distributed with a standard deviation of 8.67. If the true difference in the mean response of matched pairs is 4.19, we would need 36 subjects to be able to reject the null hypothesis that this response difference is zero with probability (power) 0.8. Type I error probability associated with this test of this null hypothesis is 0.05. Therefore, this study was adequately powered.

Data collection took place during the first two weeks of the first semester, in the nutrition clinics of the selected campuses. The day before data collection, students were phone-called to remind them about the necessity of fasting for 12 hours before the blood draw and were given information on the location and time of meeting with the research team

Trained licensed dietitians assessed participant anthropometric which included: 1) height (cm) using a portable stadiometer, (ADE, Germany), measured to the nearest 0.1 cm, without shoes, with the subject stretching to the maximum height and the head positioned in the Frankfort plane; 2) weight (kg) using a calibrated beam scale, without shoes and while participants were wearing light clothes; waist circumference (cm) measured at the mid-point, half-way between the right iliac crest and the lower coastal region;[16] measured to the nearest 0.1 cm, using Accugirth measuring tapes. Body mass index (BMI) which is the ratio of weight (kg) and height squared (m^2) was calculated. All participants were contacted after 7 months (T_1) to recollect data using the same procedures adopted at baseline (T_0).

Weight change was calculated by subtracting weight at T_1 from weight at T_0. A one pound difference(0.45 kg) was considered to be insignificant regarding weight change and was unaccounted for.

A blood sample of 5 ml was drawn from students by a licensed phlebotomist and samples were transported to the laboratory using a thermally insulated box. Serum was analyzed for cholesterol (mg/dl), triglyceride ((TG (mg/dl)), HDL-cholesterol ((HDL-C (mg/dl)), and fasting blood glucose (FBS (mg/dl)) concentrations. LDL-cholesterol ((LDL-C mg/dl)) was calculated. The analyses were performed using a Cobas C111 automated biochemical analyser (Roche Diagnostics, Indianapolis, IN, USA) based on spectrophotometric principles. Additionally, serum cortisol (nmol/L) was measured using Cobas e411

immunoassay automated analyser (Roche Diagnostics, Indianapolis, IN, USA) based on the electrochemiluminescence (ECLIA) principle. All blood collection was done early in the morning, after an overnight fast. After blood collection, students were served a light breakfast.

The students were then asked to fill a series of questionnaires:

1) **Demographic and lifestyle habits questionnaire:** adapted from Levitsky et al., (2004) [10], included 10 open-ended questions focused on some meals consumed daily and outside the home, living arrangements, smoking status, alcohol consumption. The questionnaire included questions about perceived weight changes by the students and whether they thought their weight had increased, decreased or remained constant

2) The **International Physical Activity Questionnaire (IPAQ)** (2014) short form: a validated tool used to measure the level of exercise [17] and consisting of seven questions assessing duration and frequency of light, moderate and vigorous physical activity completed in the past week. The Metabolic equivalent of tasks (METs) was calculated by multiplying the total minutes spent in the corresponding action with the frequency (days) and the constants of 3.3, 4.0 and 8.0 for light, moderate and vigorous activity respectively. The total MET value was computed by summing up the respective MET values for all activities that were done in bouts but were longer than 10 minutes in duration.

3) The **General Nutrition Knowledge Questionnaire,** adapted from Parmenter & Wardle, (1999) [18]: includes 28 open-ended and multiple choice questions. The choices were made to be culturally sensitive and specific to the food options consumed by the sample population. Cronbach's alpha coefficient of the adapted questionnaire was 0.766, which is considered satisfactory ;

4) The **Pittsburgh Sleep Quality Index (PSQI)**, (2014), developed by Buysse et al (1989) [19]: a nine-item questionnaire where four questions assessed the duration (hours) of sleep, duration needed to fall asleep, time needed to wake up and awake time spent in bed; in addition to five other questions addressing the reasons of troubled sleep). Answers were converted to total scores using an algorithm adapted from the developers of the questionnaire, where higher scores indicated poor sleep quality.

5) The **Perceived Stress Questionnaire [20]**: a ten-item questionnaire is measuring stress levels in the last month. Answers followed a five-point scale with frequencies ranging from never to very often. The total score ranged between 0 and 40, where higher scores indicated higher stress levels.

All questionnaires were available in both English and Arabic, and respondents were able to choose their language preference. All instruments were pilot-tested on a sample of students before the study was conducted for validation, the results of which were discarded. It took students between 30 and 50 minutes to complete the questionnaires.

All statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) version 21.0, IBM. P-values < 0.05 were considered statistically significant. Descriptive analyses were performed to summarise participants' characteristics and changes in measures and laboratory data through means and standard deviations for continuous variables and frequencies and percentages for categorical ones. The normality of the data distributions was evaluated using the Shapiro-Wilk test. Within-subjects significant differences of study parameters (nutrition knowledge, number of meals eaten per day, number of out of home meals per week, stress score, sleep score, physical activity and laboratory values) were assessed using paired sample T-test, and Wilcoxon Signed Ranks Tests for normally distributed and skewed continuous variables, respectively.

Additionally, a between-subjects analysis of relevant study parameters (sub-group analysis) was conducted between participants who gained weight at T1 and those who did not use independent sample T-test for normally distributed continuous variables and Mann-Whitney U test for skewed ones. Finally, the agreement between reported and actual weight change was calculated according to Cohen (1968) [21] using the MedCalc statistical software (https://www.medcalc.org). Linear weighted Kappa (k) values and their 95% confidence intervals were presented, whereby $k < 0.2$, 0.21-0.40, 0.41-0.6, 0.61-0.80, 0.81-1.00 were interpreted as the poor, fair, moderate, good and very good strength of agreement respectively.

Results

Out of a total of 80 participants, 40 completed the study. As evident in table one, the students had a mean age of 19.3± 2.04 years and had a waist circumference of 83.16 ± 11.15 cm. On average, the participants consumed around 3 meals per day and ate approximately 5 meals out of home weekly. Their mean knowledge score was 50.14 ± 10.08 %, where 100% indicates perfect knowledge. Mean stress level was 19.28 out of a total possible 40 points and where a higher score indicates higher stress. Average sleep score was 6.55 where a score above five indicates poor sleep quality. Weekly alcohol consumption average was fairly low (0.18 ± 0.63).

More than half of the participants were

females (55%), the majority were single (97.5%), and 90% lived with their parents. Approximately, 19.5% held jobs parallel to their studies and 32.5% smoked. The mean BMI of the participants was 23.74 ± 4.49 kg/m^2, and around two-thirds (62.5%) had a normal BMI. Forty per cent of the participants had a moderately active lifestyle.

Table 1: Baseline characteristics of freshman students (n = 80)

	N	%
Gender, male	36	45.0
Social status, single	78	97.5
Live with family, yes	72	90.0
Employment, none	62	80.5
Smoker, yes	26	32.5
BMI category (kg/m²)		
Underweight	5	6.3
Normal weight	50	62.5
Overweight	18	22.5
Obese	7	8.8
Physical activity level		
Light activity	30	37.5
Moderate activity	32	40.0
High activity	18	22.5
	Mean ± SD	
Age (year)	19.30 ± 2.04	
Waist Circumference (WC) (cm)	83.16 ± 11.15	
Lifestyle Questionnaire		
Number of meals per day	2.84 ± 1.07	
Number of out of home meals per week	4.79 ± 3.82	
Alcohol consumption (drinks per week)	0.18±0.63	
Nutrition knowledge (%)	50.14 ± 10.08	
Sleep score	6.55 ± 3.11	
Stress score	19.28± 5.95	
Biochemical tests		
Fasting Blood Glucose (FBG) (mg/dl)	82.09 ±7.49	
Total cholesterol (mg/dl)	158.71 ±27.13	
LDL cholesterol (mg/dl)	93.76 ±27.91	
HDL cholesterol (mg/dl)	51.79 ±11.70	
Triglycerides (mg/dl)	76.93 ±31.76	
Cortisol (nmol/L)	533.12 ±192.38	

Data are expressed as mean ± SD. LDL: low-density lipoprotein, HDL: high-density lipoprotein; Sleep score above five indicates poor sleep quality.BMI: body mass index

During the first year of university enrollment (throughout 2 semesters), the cohort gained on average 4.19 ± 8.67 Lbs. (1.90 ± 3.93 kg), and the mean BMI of the participants significantly increased from 23.95 ± 5.38 to 24.64 ± 5.38 kg/m^2 (p = 0.002) as evident in table 2. The vast majority of the students (70%; n = 28) gained weight, and 40% of whom (n = 16) accumulated more than 5 lbs. (2.26 kg). Three students (7.5%) gained 15 lbs. (6.82 kg).

Table 2: Actual Weight change (n = 40)

Weight change	N (%)	Mean± SD	Amount (Lbs.)	N (%)
Weight loss	6 (15.0)	-7.16 ± 9.55 Lbs.	> 15	1 (2.5)
			5-15	1 (2.5)
			<5	4 (10.0)
Stable	6 (15.0)	0 Lbs.	Stable (± 1)	6 (15.0)
Weight gain	28 (70.0)	+7.55 ± 6.87 Lbs.	< 5	12 (30.0)
			5 - 15	13 (32.5)
			> 15	3 (7.5)

Overall agreement between reported and measured weight change among the participants was fair (weighted Kappa = 0.21; 95% CI: 0.05 to 0.38). The discrepancy between actual and perceived weight gain was highest among two subgroups of students which were the ones who perceived they lost weight (yet 50% had gained weight) and the ones who perceived they had maintained weight (yet 71.4% had gained weight). The only subgroup of students who had perceived weight change which was by actual weight change were the ones who had reported weight gain (Figure 1).

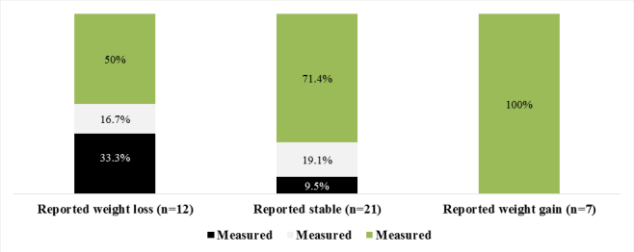

Figure 1: Weight change: Reported and actual

The effect of BMI on weight change in the first year of university is explained in Figure 2. The mean gain for all the 40 students was 4.2 ± 8.65lbs., whereby the underweight gained the most (7.2 lbs.), the normal and overweight participants gained approximately 5lbs., and the obese were the only subgroup who achieved weight loss during the 1st year of university enrollment.

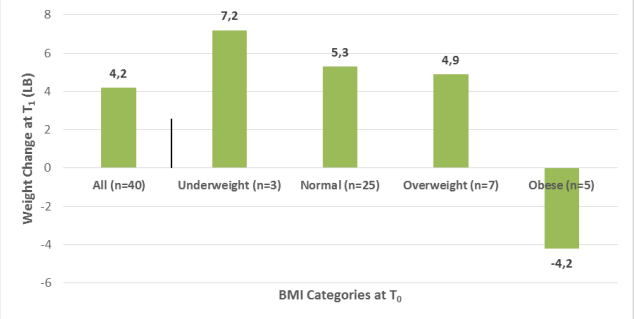

Figure 2: Effect of baseline BMI on Actual Weight Change

Lifestyle factors and, nutritional knowledge did not significantly change during the first year of university enrollment (Table 3). The absence of a pattern related to these characteristics was evident across the three weight change groups. No significant differences were noted between groups regarding the number of meals per day, the number of out of home meals consumed per week, nutrition knowledge (%), stress score, and sleep score.

Table 3: Freshman students' reported outcome measures and laboratory data

Parameter (n = 40)	T_0	T_1	P value
Nutrition knowledge (%)	49.48 ± 10.29	51.50 ± 10.82	*P* = 0.25
Number of meals eaten per day	3.03 ± 1.09	2.73 ± 0.96	*P* = 0.09
Number of out of home meals per week	5.11 ± 4.23	4.35 ± 6.74	*P* = 0.06
Stress score (max score: 40)	19.33 ± 6.09	19.90 ± 4.89	*P* = 0.64
Sleep score (> 5 = poor sleep)	6.05 ± 2.48	6.40 ± 3.19	*P* = 0.73
Physical activity (METs) per day	1880.4 ± 2289.2	2148.59 ± 3078.61	*P* = 0.62
Laboratory values (n = 27)	T_0	T_1	P value
FBG (mg/dl)	81.59 ± 6.29	85.30 ± 6.68	*P* < 0.001*
Cortisol (nmol/L)	527.9 ± 163.8	393.1 ± 133.9	*P* = 0.003*
Triglycerides (mg/dl)	71.44 ± 29.66	83.44 ± 36.10	*P* = 0.02**
LDL (mg/dl)	91.37 ± 27.32	84.19 ± 24.32	*P* = 0.01*
HDL (mg/dl)	54.33 ± 13.59	54.52 ± 13.273	*P* = 0.85
Cholesterol (mg/dl)	159.37 ± 26.77	155.41 ± 24.46	*P* = 0.15

METs: the metabolic equivalent of the task. FBG: fasting blood glucose; LDL: low-density lipoprotein, HDL: high-density lipoprotein. Data are expressed as mean ± SD. Statistically significantly difference: *P*<0.05 (*Paired Samples Test; ** Wilcoxon Signed Ranks Test).

At last, all the objective study parameters were compared between T_0 and T_1 to detect changes over time. Results showed a significant increase in

waist circumference in 40 students from 83.65 ± 12.7 cm to 86.9 ± 13.1 cm with p = 0.05. Additionally, as evident in Table 3, there was a significant increase in blood glucose and Triglyceride levels; on the other hand, there was a significant decrease in cortisol, and LDL. There were no significant changes in HDL cholesterol and total cholesterol between T_0 and T_1. Despite the changes, overall, all values were within normal limits. Furthermore, the results of the sub-analysis showed no statistically significant differences in all of the studied parameters between the weight gainers and non-weight gainers.

Discussion

This was the first study to longitudinally assess weight change during the freshman year among a sample of Lebanese university students. On average, the students gained 4.2 lbs. However there was considerable variability in weight change as 15% experienced no weight difference, 15% lost weight and 70% of the study participants put on weight. Health behaviours such as exercise, sleep, number of meals consumed and the total amount of meals eaten outside the home did not significantly differ between the first and second semester. Moreover, there were no significant changes in stress scores and nutrition knowledge throughout the study duration.

This study further supports evidence that first-year students do gain weight in the year of transition from high school to university. The changes in body weight are similar to previous literature as reported by Cooley and Turay [22], who found that students gained 4.4 pounds throughout 7 months and found no predictors for the weight gain [22]. Our results are also similar to those of Levitsky and colleagues [23] who concluded that their sample gained 4.4 lbs in 12 weeks but was also unable to determine the reasons for the weight changes [23].

There is, however, inconsistency in the literature about the average amount of weight that is typically gained during the freshman year as some studies have reported that the weight gain is typically lower than 5 lbs. For example, Vella Zarb & Elgar [13] found average weight gain to be approximately 2 lbs. (0.91 kg) whereas Butler and colleagues [24] found the increase to be 1.6 lbs. (0.72 kg). Speculation about the causes of the variability has been accounted to several factors which include the length of the study duration, intercountry variation and the socials lifestyle norms that come with them, study design and self-selection bias [8] [15] [25].

Additionally, the variation could be due to the method of how weight was reported, whether it was self-reported or measured by researchers. In the study conducted by Serlachius and colleagues [15], students gained approximately 3.4 lb (1.54 kg). However, the changes were self-reported which may have resulted in an underestimation of weight gain as students may not have realised that they had body mass [15] [25].

One potentially alarming finding in this study was the discrepancy between perceived and actual weight change, especially among weight gainers since 70% of those who gained weight were unaware of their increase. Misperception of weight status among overweight and obese adults has been associated with decreased attempts at weight loss [26], hence potentially exaggerating the obesity problem.

Many factors have been identified in the literature as possible determinants to weight gain during their freshman year which includes stress, leading a sedentary lifestyle, moving away from home and increased alcohol intake [8] [15] [27]. However, no association with any of the above factors as related to weight gain or loss was noticeable in the current study. Nutrition knowledge was very poor in our sample population at both times of data collection, and no relation with weight change was found. Nevertheless, this study did not have an educational component as participants were not exposed to any nutritional education within their curriculum that might have affected their knowledge scores or eating habits; which can explain the lack of association between these variables and weight change. Another possible explanation is that most individuals in the18-25 age category gain weight whether they attend college or not as this is a critical period, which has been named “emerging adulthood” and where individuals are at risk of gaining weight regardless of their circumstances [28].

Our results revealed that the obese lose weight. However, a sub-analysis of the data showed that only one of the students in this group had lost approximately 26 lbs and therefore skewed the results.

In parallel to the weight gain in the study participants, we also noted a significant increase in serum triglycerides, glucose and waist circumference. Although not clinically significant, the increases revealed a worsened metabolic profile which could potentially raise the risk of the future development metabolic syndrome [29]. As for the other biochemical markers, there was a significant decrease in cortisol and LDL levels. The unexpected decrease in cortisol level could be due to the intra-individual variability in cortisol response secondary to stress [30].

Our study presents numerous strengths. First, and to the best of the authors’ knowledge, this research pioneers assessment of weight changes of university students during the freshman year in a Middle Eastern country, by recruiting a sample from several Lebanese regions. The study was appropriately powered as mentioned in the methods. Additionally, weight gain was assessed objectively by

trained researchers, in parallel to blood parameters, and validated questionnaires. Furthermore, the study had a sufficient duration to assess the effect throughout the whole academic year.

However, the present study also had some limitations. The sample used was a volunteer sample, and that could bias the results as students who were are not health conscious may have been reluctant to participate in this study. Moreover, although the sample was recruited from several geographical areas, the students were recruited from only one university, which hinders the generalizability of our findings. One additional limitation was the high attrition rate (50%), yet our analysis showed that participants who dropped out had similar baseline characteristics to those who completed the study; literature has shown that a low retention rate is common in such studies [8] [9]. Furthermore, the majority of the sample did not drink alcohol and lived at home; therefore these factors could not be appropriately analysed.

In conclusion, our results provide evidence that Lebanese students do gain weight during their freshman year which could be a potential public health problem, especially since Lebanon is a country experiencing a nutrition transition and increasing obesity rates.[31] Future research should be conducted on a more representative sample and for or a longer duration to examine the predictors of weight gain. Additionally, public health interventions should be implemented by healthcare providers within universities, to raise awareness and prevent future weight gain.

References

1. World Health Organization. World Health Organization obesity and overweight fact sheet, 2003.

2. Pierce EF, Butterworth SW, Lynn TD, O'Shea J, Hammer WG. Fitness profiles and activity patterns of entering college students. J Am Coll Health. 1992; 41(2):59-62. https://doi.org/10.1080/07448481.1992.10392819 PMid:1460174

3. Craigie AM, Lake AA, Kelly SA, Adamson AJ, Mathers JC. Tracking of obesity-related behaviours from childhood to adulthood: A systematic review. Maturitas. 2011; 70(3):266-284. https://doi.org/10.1016/j.maturitas.2011.08.005 PMid:21920682

4. Guo SS, Wu W, Chumlea WC, Roche AF. Predicting overweight and obesity in adulthood from body mass index values in childhood and adolescence. The American journal of clinical nutrition. 2002; 76(3):653-8. https://doi.org/10.1093/ajcn/76.3.653 PMid:12198014

5. American College Health Association. American college health association national college health assessment spring 2006 reference group data report (abridged). Journal of American College Health. 2007; 55(4):195. https://doi.org/10.3200/JACH.55.4.195-206 PMid:17319325

6. Steptoe A, Wardle J, Cui W, et al. Trends in smoking, diet, physical exercise, and attitudes toward health in European university students from 13 countries, 1990-2000. Preventive medicine. 2002; 35(2):97-104. https://doi.org/10.1006/pmed.2002.1048 PMid:12200093

7. Brown C. The information trail of the 'Freshman 15'—a systematic review of a health myth within the research and popular literature. Health Information & Libraries Journal. 2008; 25(1):1-2. https://doi.org/10.1111/j.1471-1842.2007.00762.x PMid:18251907

8. Vella-Zarb RA, Elgar FJ. The 'freshman 5': a meta-analysis of weight gain in the freshman year of college. Journal of American College Health. 2009; 58(2):161-6. https://doi.org/10.1080/07448480903221392 PMid:19892653

9. Vadeboncoeur C, Townsend N, Foster C. A meta-analysis of weight gain in first year university students: is freshman 15 a myth? BMC Obesity. 2015; 2(1):22. https://doi.org/10.1186/s40608-015-0051-7 PMid:26217537 PMCid:PMC4511069

10. Levitsky DA, Halbmaier CA, Mrdjenovic G. The freshman weight gain: a model for the study of the epidemic of obesity. International journal of obesity and related metabolic disorders: Journal of the International Association for the Study of Obesity. 2004; 28(11):1435-1442. https://doi.org/10.1038/sj.ijo.0802776 PMid:15365585

11. Mihalopoulos NL, Auinger P, Klein JD. The Freshman 15: is it real? J Am Coll Health. 2008; 56(5):531-533. https://doi.org/10.3200/JACH.56.5.531-534 PMid:18400665 PMCid:PMC2532948

12. Girz L, Polivy J, Provencher V, et al. The four undergraduate years. Changes in weight, eating attitudes, and depression. Appetite. 2013; 69:145-150. https://doi.org/10.1016/j.appet.2013.06.002 PMid:23764239

13. Vella-Zarb RA, Elgar FJ. Predicting the 'freshman 15': Environmental and psychological predictors of weight gain in first-year university students. Health Education Journal. 2010; 69(3):321-32. https://doi.org/10.1177/0017896910369416

14. Economos CD, Hildebrandt ML, Hyatt RR. College freshman stress and weight change: differences by gender. American journal of health behavior. 2008; 32(1):16-25. https://doi.org/10.5993/AJHB.32.1.2 PMid:18021030

15. Serlachius A, Hamer M, Wardle J. Stress and weight change in university students in the United Kingdom. Physiology and Behavior. 2007; 92(4):548-553. https://doi.org/10.1016/j.physbeh.2007.04.032 PMid:17537466

16. van der Kooy K, Seidell JC. Techniques for the measurement of visceral fat: a practical guide. International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity. 1993; 17(4):187-196.

17. Craig CL, Marshall AL, Sjostrom M, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003; 35(8):1381-1395. https://doi.org/10.1249/01.MSS.0000078924.61453.FB PMid:12900694

18. Parmenter K, Wardle J. Development of a general nutrition knowledge questionnaire for adults. Eur J Clin Nutr. 1999; 53(4):298-308. https://doi.org/10.1038/sj.ejcn.1600726 PMid:10334656

19. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry research. 1989; 28(2):193-213. https://doi.org/10.1016/0165-1781(89)90047-4

20. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. Journal of health and social behavior. 1983; 24(4):385-396. https://doi.org/10.2307/2136404 PMid:6668417

21. Cohen J. Weighted kappa: nominal scale agreement with provision for scaled disagreement or partial credit. Psychological bulletin. 1968; 70(4):213-220. https://doi.org/10.1037/h0026256 PMid:19673146

22. Cooley E, Toray T. Disordered eating in college freshman women: A prospective study. Journal of American College Health. 2001; 49(5):229-35. https://doi.org/10.1080/07448480109596308 PMid:11337898

23. Levitsky DA, Garay J, Nausbaum M, Neighbors L, Dellavalle DM. Monitoring weight daily blocks the freshman weight gain: a

model for combating the epidemic of obesity. International journal of obesity. 2006; 30(6):1003. https://doi.org/10.1038/sj.ijo.0803221 PMid:16446748

24. Butler SM, Black DR, Blue CL, Gretebeck RJ. Change in diet, physical activity, and body weight in female college freshman. American journal of health behavior. 2004; 28(1):24-32. https://doi.org/10.5993/AJHB.28.1.3 PMid:14977156

25. Fedewa MV, Das BM, Evans EM, Dishman RK. Change in weight and adiposity in college students: a systematic review and meta-analysis. Am J Prev Med. 2014; 47(5):641-652. https://doi.org/10.1016/j.amepre.2014.07.035 PMid:25241201

26. Duncan DT, Wolin KY, Scharoun-Lee M, Ding EL, Warner ET, Bennett GG. Does perception equal reality? Weight misperception in relation to weight-related attitudes and behaviors among overweight and obese US adults. International Journal of Behavioral Nutrition and Physical Activity. 2011; 8(1):20. https://doi.org/10.1186/1479-5868-8-20 PMid:21426567 PMCid:PMC3073863

27. de Vos P, Hanck C, Neisingh M, Prak D, Groen H, Faas MM. Weight gain in freshman college students and perceived health. Preventive medicine reports. 2015;2:229-234. https://doi.org/10.1016/j.pmedr.2015.03.008 PMid:26844076 PMCid:PMC4721347

28. Nelson MC, Story M, Larson NI, Neumark-Sztainer D, Lytle LA. Emerging Adulthood and College-aged Youth: An Overlooked Age for Weight-related Behavior Change. Obesity. 2008; 16(10):2205-2211. https://doi.org/10.1038/oby.2008.365 PMid:18719665

29. Grundy SM. Overnutrition, ectopic lipid and the metabolic syndrome. Journal of Investigative Medicine, 2016. https://doi.org/10.1136/jim-2016-000155 PMid:27194746

30. van Eck MM, Nicolson NA, Berkhof H, Sulon J. Individual differences in cortisol responses to a laboratory speech task and their relationship to responses to stressful daily events. Biological psychology. 1996; 43(1):69-84. https://doi.org/10.1016/0301-0511(95)05159-7

31. Nasreddine L, Naja F, Chamieh MC, Adra N, Sibai A-M, Hwalla N. Trends in overweight and obesity in Lebanon: evidence from two national cross-sectional surveys (1997 and 2009). BMC Public Health. 2012; 12(1):798. https://doi.org/10.1186/1471-2458-12-798 PMid:22984791 PMCid:PMC3527186

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Rocky Road Ahead Of Nursing Presence in the Oncology Care Unit: A Qualitative Study

Fereshteh Araghian Mojarad[1], Leila Jouybari[2*], Akram Sanagoo[2]

[1]School of Nursing & Midwifery, Golestan University of Medical Sciences, Gorgan, Iran; [2]Nursing Research Center, Golestan University of Medical Sciences, Gorgan, Iran

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***Correspondence:** Leila Jouybari. Nursing Research Center, Golestan University of Medical Sciences, Gorgan, Iran. E-mail: jouybari@goums.ac.ir

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Abstract

BACKGROUND: Cancer patients need not only well-planned treatment, but also comprehensive nursing care provided with compassion, competence, and conscience. Nursing presence is an essential part of the care process in all nursing interventions.

AIM: This study aimed to identify the barriers to the nursing presence in oncology care units.

MATERIALS AND METHODS: A qualitative content analysis study was carried out with the participation of 27 nurses who were chosen by purposive sampling. The data collection instruments were semi-structured interviews and observation. The interviews were recorded and transcribed, and then coded and analysed by the Graneheim and Lundman's content analysis methodology. The criteria proposed by Guba and Lincoln were used to ensure the validity of the research.

RESULTS: From the data analysis, the researchers were able to obtain a primary theme labelled "Rocky road ahead of nursing presence" and two subthemes labelled "Difficult and stressful work environment" and "Dysfunctional rules and regulations" with several subcategories including "exposure to violence", "shortage of nursing staff", "inattention to the needs of nurses", "organizational unfairness", "excessive paperwork", and "need for detailed documentation".

CONCLUSION: There are numerous challenges ahead of achieving satisfactory nursing presence and quality care in the oncology care units. The findings highlight the key role of organisational conditions in the nursing presence and the dire need to pay further attention to the motivational factors.

Introduction

Cancer is one of the most common causes of death and health problems in the world. Cancer is the third common cause of death after cardiovascular disease and accidents in Iran [1]. Cancer patients have to endure intense therapies and also may suffer long-term sequelae after their illness and its treatment. Fatigue, lack of energy, exhaustion, and impaired physical performance are among the most common symptoms in cancer patients and can have severe physical, emotional, and social effects [1] [2]. In addition to therapy, cancer patients need comprehensive nursing care provided with compassion, competence and conscience. Such care is a fundamental right of cancer patients and their families. Nurses are the members of the healthcare team who spend the most time with patients and act as the main caregivers or active companions of patients in their life-threatening experiences. Thus, in the case of cancer patients, nurses have to overcome many obstacles to provide a quality care [3] [4]. Nurses are the members of the healthcare team who spend the most time with patients and act as the main caregivers or active companions of patients in their life-threatening experiences. Thus, in the case of cancer patients, nurses have to overcome many obstacles to provide quality care [5]. The provision of humanistic care can be impeded by factors such as technological advancement, nurse understaffing, and the replacement of nursing staff with non-nurses, which undermine the ability of nurses to attend to the patient and thus the quality of patient care [6]. One of the quality indicators of nursing care is the nursing

presence [7]. Nursing presence is an essential component of care and all nursing interventions [8] and has been described as a deliberate nursing activity that is indispensable for patient safety and the success of nursing process [9].

Paterson and Zderad have considered real presence, being or doing, cultivating goodness and more-being and experience, thinking, conceptualisation as nursing action. Presence is a conscious act grounded in the distinct personality of the nurse. This presence is a chosen human response that is freely given and can never be assigned [10]. Watson argues that there is a caring moment that forms a spiritual link between the nurse and the patient, becomes an existential turning point, and a focal point for honouring human integrity [11]. Nursing presence was first introduced to nursing texts in 1964 by Madeleine ClémenceVaillot. Nursing presence can be defined as being with the patient or being available in a reciprocal way [12]. In a study with a concept analysis approach, "nursing presence" was identified as a constructive interaction with voluntary concentration, patient-centred/task-oriented communication, responsiveness, transparency, and comprehensive engagement. This study argued that nursing presence requires clinical competence, self-actualisation, mutual cordiality, and desirable work environment, and will result in effective communication, balance/recovery, and growth and development. The nursing presence depends on a combination of personal characteristics of nurses and patients, their shared characteristics, a favourable environment for communication, and nursing decisions [13]. In a study carried out by Zamanzadeh et al., nurses of an oncology care unit stated that they give a higher priority to "monitoring and follow up" and "availability of nurses" in the care process [14].

To successfully perform professional duties regarding assuaging the physical and mental problems of patients, nurses need to have a presence when attending to their patients. Previous studies in this area are mainly focused on understanding the nature of this presentation from the nurses' viewpoint. Although nurses face many great challenges in their career, this does absolve them from their professional and moral duty toward cancer patients, who because of their pain and delicate condition, deserve more attention, monitoring, and follow up from their nurses. No study has so far investigated the barriers of nursing presence in oncology care units. Since nurses' perceptions are influenced by their care experience, one of the ways to explore this area and devising solutions at personal and professional levels is to study the experiences of nurses who work in oncology care units.

This study aimed to explore the experiences of nurses about the barriers to the nursing presence in oncology care units.

Methods

This qualitative content analysis study was carried out in 2017. As a research method, the qualitative content analysis is the subjective interpretation of the content of textual data through a systematic categorisation process to identify the meaningful pieces of content or latent and apparent patterns in the text. This process aims to produce new cognitive knowledge, enhance the researcher's perceptions of the studied phenomenon, and reveal a path toward operational solutions [15].

The research environment was the oncology care units of two teaching hospitals (in two adjacent provinces in the north of Iran). Using a purposive sampling approach, nurses with at least one year experience of working in the oncology care units were invited to participate.

Given the data saturation, 27 participants entered in this study. Data collection instrument was semi-structured and interactive interviews. In all interviews, a few primary questions such as "*Please describe a day of your work*?" and "*What prevents you from being present at the patient's bedside*?" were repeated. When needed, exploratory questions such as "*Please elaborate*", "*Please explain what you mean*", and "*Please give an example to help me better understand your experience*" were asked to deepen the interview. The average duration of interviews was 75 minutes, but they could take between 30 and 120 minutes depending on the participant.

All interviews were conducted by one researcher. The word-for-word transcript of each interview was prepared and coded within 48 hours after the interview. Data analysis was carried out simultaneously and continuously with data collection. The OneNote software was used to sort and organise the data. Data analysis was performed with the qualitative content analysis method of Graneheim and Lundman [16].

For this purpose, the transcripts of interviews were read through several times to obtain a sense of their contents. Then all interviews were considered as one analysis unit, that is, as a single text document that is intended to be analysed and coded. Paragraphs, sentences, or words were considered as meaning units, that is, the set of words and sentences that contain aspects related to each other through their content and context. Meaning units were abstracted according to their latent concepts and were coded accordingly. The codes were compared with each other based on their similarities and differences and then assigned to more abstract categories with specific labels. After a comparison of the categories and a deep reflection on their contents, the latest contents of the categories were formulated into themes.

The validity and robustness of the study were

strengthened according to the criteria proposed by Guba and Lincoln [17]. For this purpose, the researcher attempted to improve the credibility by remaining engaged with the participants and data collection process for a prolonged duration and rechecking the validity of information with percipients. The dependability of data was improved by stepwise replication as well as inquiry audit by the supervising and advising professors and other experts. The confirmability was achieved through the confirmation of university professors and with the help of their critical comments. To ensure the transferability of the study, the researcher attempted to provide an accurate report of the statements made by participants as well as a detailed description of the research process in order to facilitate future evaluation and use of findings in other contexts, so that other researchers would be able to understand the experience of interviewed nurses about the subject.

This study was approved by the ethics committee of the university as a part of larger research. To protect the rights of participants, they were asked to give written consent and received assurance that interviews will remain confidential and transcripts or comments will be published without divulging any identity.

Results

Of the 27 participants, 3 were men and the rest were women. Participants had an age range of 26-58 years and a work experience of 1-30 years. Of the 27 participants, 20 were clinical nurses, 4 were nursing managers, 1 was a head nurse, and 2 were quality improvement experts.

After deep reflection on the transcripts of interviews, 610 initial codes were extracted.

These codes were read several times, abstracted, and categorised based on their similarities and differences. After analysis and comparison, the latent meanings were identified and formulated into four themes, which were given conceptual and abstract labels according to their contents. After analysing the data regarding the experience of nurses about the barriers to the nursing presence in the oncology care units, the researchers formulated primary theme labelled “Rocky road ahead of nursing presence” and two subthemes labelled “Difficult and stressful work environment” and “Dysfunctional rules and regulations”. Each of these subthemes consists of several subcategories, which are presented in Table 1.

The oncology nurses faced numerous challenges in maintaining a presence in the patients’ bedside and fulfilling their professional care responsibilities.

Table 1: Themes, subthemes, and subcategories of barriers to the nursing presence in the oncology care units

Theme	Subtheme	Code
Rocky road ahead of nursing presence	The difficult and stressful work environment	Exposure to violence
		Shortage of nursing staff
		Inattention to the needs of nurses
		Organisational unfairness
	Dysfunctional rules and regulations	Excessive paperwork
		Need for detailed documentation

The findings suggest a work environment that is very difficult and stressful and severely undermines the prospects of achieving a nursing presence. Nurses may be exposed to violence as a result of high level of stress and negative emotions among patients and families, their poor skills (e.g. in finding patient’s veins), and their poor responsiveness to patients and their families because of understaffing an overwhelming number of patients. Some of the nurses believed they have a difficult work environment because they are constantly overworked and understaffed. In their view, the organisation views them as a tool and ignores their motivational needs (in term of wages, facilities, etc.) and expects them to work more than they are paid. This theme consists of four subcategories: “Exposure to violence”, “Shortage of nursing staff”, “Inattention to the basic needs of nurses”, and “Organizational unfairness”.

Nurses are exposed to various forms of occupational violence while attending to the patients. Physical violence is less frequent than other forms of violence and most violent acts are committed by the families of patients. Interviewed nurses stated that sometimes violence and aggression occur because of physicians giving false hope to the patient family, which result in high expectations. Nurses are less likely to stay at the patient’s bedside when they sense exposed to aggression and violence. In these cases, they attend the patient’s bedside as less often as possible or ask another nurse to do their tasks to avoid the patients or their families. Often, they end their shift with unhappiness about their job and take this feeling home to their family. One of the nurses stated:

“... We had a patient who suddenly went into arrest and expired. We did all we could for the patient. When I was coming back from the patient’s room, someone threw something toward me; it was patient’s father throwing a tea flask toward me; he then walked to me and slapped me in the face” (M.6, Male. 34, Married, Bachelor’s degree, Nurse).

Another nurse provided an example of the aggressive behaviour of patient families:

“I was in charge of the shift. A family member of a patient came to me and said that the patient is bleeding in the mouth and you must come. I sent a nurse to the patient and started calling the doctor. The same person returned and started yelling at me that why don’t you come? He is in bad shape, why are you still here?”(M.2, female. 27, Married, bachelor’s

degree).

Nurses repeatedly mentioned their physical and emotional fatigue because of working with a tight schedule and overwhelming numbers of patients, because of which they remain completely occupied for the entirety of the shift and cannot have even a short break.

One of the nurses said:

"This is the central oncology department of the province; most of the patients coming here are extremely sick or at end stages; here we have 23 patients and only three nurses; we have to work hard really" (M.15, female, 33, married, bachelor's degree).

According to the participants, the other obstacles in the way of nursing presence were the shortage of support and service staff in the care unit one of the participants said:

"... I have only two assistant nurses, and this is just enough for taking the patients to sonography, CT scan, tests... When there's no assistant, nurses have more work to do... This is the time that you should dedicate to the patients, to be with them, and you lose it doing other works" (M.3, female, 48, married, bachelor's degree, head nurse).

Some nurses believed that the lack of motivation is an obstacle to staying at the patient's side. They stated that reasons such as lack of interest in the profession, poor management, low salaries and benefits, and high mortality rates all affect nursing presence.

"Our salary is low, and there's no support to compensate for it. They say that you're an outsourced nurse. So there is little motive to put much effort for the patient since there's no money, no respect, there is practically nothing..." (M. 16, female, 29, single, bachelor's degree, nurse).

Some nurses believed there is absolutely no attention to the welfare of the staff, such as the facilities of break rooms, vacation time or fund, etc. Therefore, nurses suffer from mental and emotional fatigue, which affects the nursing presence, because they are never in the right mood and psychological state to stay with the patients.

"The system should also pay attention to the welfare of personnel. We proposed that personnel should have periodic vacations to avoid fatigue, but .....the organisation does not pay any attention" (M.26, 44, married, bachelor's degree, quality improvement expert).

Some of the participants stated that the organisation expects more work than it pays and does not understand them.

"They won't understand, they can't understand until they work here in these units. They don't see the effects of drugs on nurses; they don't see the emotional and physical problems of the guys. If you bother to look, you'll see that guys are on their feet from morning to noon" (M.22, female, 28, married, bachelor's degree, nurse)

The following is the comment of one of the head nurses about the transfer of the workforce from the oncology care unit and the organisation's negligence to retain specialist staff:

"Several times I went to the nursing office to make them retransfer a previous staff member to the oncology. I told them that I know this nurse, she's good, I approve of her work, and she has the right mentality to work with our patients. They didn't allow it and said that the other department is understaffed" (M.3, female. 48, married, bachelor's degree, head nurse).

Another theme observed in the comments of interviewees was the "dysfunctional laws and regulations" with two subcategories labelled "excessive paperwork" and "need for detailed documentation". Nurses believed the hospital accreditation program, as long as it is compulsory and poorly implemented, undermines the nursing quality. They stated that the managers of the organisation give a higher priority to the proper filling of documents than the actual results.

In Iran, the compulsory implementation of accreditation programs a method of hospital services quality assessment started in 2012. This program significantly increased the amount of paperwork that nurses have to do, as it added several hospital assessment forms to the routine forms that nurses have to complete during their work.

"There's an initial assessment form that takes all the patient information; for example, you write the entire information in one place, but you must write the entire thing again in the nursing report. ..... It's duplicate work. There's no time for the nurse to remain with the patient. In practice, the nurse starts ignoring the clinical work" (M.20, female, 43, married, bachelor's degree, nurse).

Most nurses were critical of accreditation program because of the time they had to spend on documentation. Interviewees stated that they were more interested in being with the patients than filling up forms.

"I don't like accreditation at all, because it's just paperwork" (M.24, female. 39, married, bachelor's degree, head nurse)

Nurses stated that patients expect nurses to completely focus on clinical work, but the size of non-clinical tasks practically undermines their presence beside the patient bed.

"Patients don't know what I'm writing on my papers, and won't understand; the work I'm doing on my workstation, all the paperwork, all the medication logs I put in the computer…." (M.9, female, 37, married, bachelor's degree, shift manager).

Some of the interviewed nurses stated that since the hospital evaluation system is based on the accreditation program, failing to get a good score means a reduction in the hospital credibility and grade, which results in reduced hospital income and therefore reduced income of the personnel; thus, they have to implement the hospital accreditation with great care.

"We have to do the accreditation stuff because the organisation wants us to do it. If it weren't for the mandatory accreditation, I wouldn't fill these forms" (M.24, female, 39, married, bachelor's degree, head nurse).

Participants also stated that in the event of a legal problem, the competent authorities would only accept the documents, because, from their perspective, anything that remains undocumented has not happened.

"You can find notifications one very wall and door about the complaint process and the way it'll be handled step by step; this makes you take care of your documentation because this is what saves you in the court" (M.24, male, 58, married, bachelor's degree, nurse).

Discussion

The findings of this study revealed the real perceptions and experiences of nurses about the barriers to the nursing presence in the oncology care unit. The analysis of the experiences of participants confirmed that nursing presence faces obstacles such as difficult work environment and dysfunctional laws and regulations.

One of the barriers to the nursing presence in the oncology care unit is the difficult and stressful work environment. Nurses were exposed to verbal abuse while on the patients' bedside, mostly as a result of poor technical skills of less experienced nurses. Given that nurses are the most easily accessible personnel in the hospital, the main cause of the high rate of violence and aggression against nurses is their close contact with patients and their families while they experience a stressful life-threatening situation [18]. The results of a study showed that nurses in the oncology care unit were 2.7 times more likely to be exposed to non-physical violence than those in the emergency department. This finding was attributed to the fact that oncology nurses are more prone to fatigue, reduced physical and mental health, and experiencing negative emotions.

Most interviewed nurses viewed the shortage of human resources (nurses and assistant nurses) and the intensive work shifts and consequent fatigue as a barrier to nursing presence. Nurses stated that as if a shortage of nurses is not enough, shortage of assistant nurses forces the trained nurses to do non-specialized work such as transportation of patients, and this leaves them no time to spend with patients. Various studies, including Elaraby et al., in Malaysia [19], Shinjo et al., in Japan [20], and Eygelaar et al., [21], investigated the impact of human resource imbalances and shortages on the quality of hospital services. Enforcing a minimum nurse-to-patient ratio not only improves outcomes such as patient safety [22] [23], length of stay [24], and readmission rate [25] it also increases the appeal of nursing as a career and decreases the rate of burnout among nurses [26]. The shortage of nursing staff creates overworked nurses who are unable to realise their full potential in providing quality patient care and have to spend most of their time doing basic and rudimentary tasks instead of communicating with the patients and paying attention to their needs as is theorised in the nursing presence concept. This ultimately results in job dissatisfaction, a stressful work environment, increased rate of medication error, and decreased quality of care provided to patients [27]. Most nurses believed that the organisation's lack of attention to salaries, benefits, and timely payment had crushed any motivation for effective presentations. Failure to pay attention to motivational factors in nurses can lead to low job satisfaction, and motivation reduced service quality and ultimately patient dissatisfaction with the health care services provided [28]. In a study income and benefits were found to significantly increase motivation, and discriminations in payments and the feeling of unfairness in the payment system were among the leading causes of decreased motivation and resignations [29].

The findings suggested that because of the fatigue caused by understaffing and overcrowding of the oncology care unit and its high mortality rates, nurses were inclined to be rotated out of the care unit. Job rotation, which is a labour-management technique where employees learn the skills of different care units, is known to reduce the fatigue caused by repetitive tasks. The recovery of staff motivation is one of the main tasks of care unit managers [30]. Nurses stated that the organisation asks them to be constantly available or present on the patient bedside to provide care services, but considering the amount of work that must be done in each shift, they often feel overworked and tired. Mazdaki et al. stated that there is "increasing the income gap between physicians and nurses, the crucial differences between physicians' and nurses' salaries" in Iran [31].

According to the study dysfunctional laws and regulation, and most notably the accreditation program, acted as a barrier to nursing presence. In a study by Walker, it was found that when a nurse prioritises paperwork over care services and does not converse with the patient during care, this leads to patient dissatisfaction [32]. Hospital accreditation is a

standardisation program consisting of a set of rules that hospital are required to implement, but it says nothing about how these rules should be implemented [33]. Developing policies and procedures for every activity in the hospital will only increase the volume of paperwork and reduce the actual performance by keeping the clinical staff busy with preparing documents that they believe to be inconsequential [34]. Several studies have shown that the accreditation program has had an impact on the performance and quality of services. The accreditation program is a time-consuming administrative bureaucracy and increases the workload and creates stress in employees [33] [35]. Also, no significant relationship has been found between hospital accreditation and patient satisfaction [36]. Participant of this study believed that while increasing the awareness of nurses about patient safety, the accreditation program has not been helpful overall. They argue that most of the standards required by this program have always been fulfilled even before its implementation, so the program only emphasises the documentation, which reduces the clinical presence of nurses. It was found that nurses implement this bureaucracy solely to maintain accountability toward the organisation because insufficient documentation is the first critique that is raised in academic and managerial inspections.

Another issue that interviewees identified as barriers to nursing presence were the importance of documentation for safety against organisational inquiries and legal actions, which encourage nurses to spend time on filling paperwork meticulously instead of being present for the patient to provide direct care. The provision of direct care in the study of Harrison et al. was 24%, and in the study by Kiekkas et al., 35.2%, and 32.8% in the study by Des Jardins et al., [37] [38] [39]. It is clear that complete, accurate, and on time preparation of reports is imperative for verifying that patients have received the necessary care as instructed. From the legal standpoint, the medical team has to document its activities to ensure verifiability, as only the well-documented actions will be acknowledged. Hence, given the importance of nursing reports, many nurses spend a lot of time logging events and actions and thus have little time to spend on direct interaction with patients [40]. In the present study, nurses stated that since patients and families are aware of the patient rights and thus supervisors emphasise on the meticulous documentation, they spend a lot of time and effort to fill out all documents to ensure accountability toward legal authorities as well as their employer.

In conclusion, this study found that there are several challenges and a rocky road ahead of achieving satisfactory nursing presence and providing quality care. An honest conversation with nurses is the best strategy for managers to improve their insight and ability to deal with the problems of nurses and identify the barriers to the nursing presence in oncology care units. The findings highlight the central role of the organizational conditions and the importance of attention to the motivational factors and the efficiency of laws and regulations in the nursing presence. Based on the findings of this study, it is recommended to pay further attention to the education of nursing students about the nursing presence, its association with the quality of nursing care, and how they can rely on this practice to enhance the care service provided to patients.

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References

1. Mohammadian M, Salehiniya H, Mohammadian-Hafshejani A. Some facts on incidence and mortality of cancer in Iran. Iran J Public Health. 2017; 46(10):1446-7. PMid:29308393 PMCid:PMC5750361

2. Mortazavi H. Could Art Therapy Reduce the Death Anxiety of Patients with Advanced Cancer? An Interesting Question that Deserves to be Investigated. Indian J Palliat Care. 2018; 24(3):387-388. PMid:30111962 PMCid:PMC6069628

3. Malloy P, Boit J, Tarus A, Marete J, Ferrell B, Ali Z. Providing palliative care to patients with cancer: addressing the needs in Kenya. Asia Pac J Oncol Nurs. 2017; 4(1):45. https://doi.org/10.4103/2347-5625.199073 PMid:28217729 PMCid:PMC5297231

4. Ghaljeh M, Iranmanesh S, Nayeri ND, Tirgari B, Kalantarri B. Compassion and care at the end of life: oncology nurses' experiences in South-East Iran. Int J Palliat Nurs. 2016; 22(12):588-97. https://doi.org/10.12968/ijpn.2016.22.12.588 PMid:27992279

5. Kendall S. Admiring courage: Nurses' perceptions of caring for patients with cancer. Eur J Oncol Nurs. 2006; 10(5):324-334. https://doi.org/10.1016/j.ejon.2006.01.005 PMid:16777478

6. Baljani E, Azimi N, Hosseinloo A. A survey on nurses' perception of the importance of caring behaviors and factors affecting its provision. Evidence-based Care J. 2012; 2(1):13-21.

7. Iseminger K, Levitt F, Kirk l. Healing during existential moments: the "art" of nursing presence. Nurs Clin North Am. 2009; 44(4):447. https://doi.org/10.1016/j.cnur.2009.07.001 PMid:19850181

8. Tavernier SS. An evidence-based conceptual analysis of presence. Holist Nurs Pract. 2006; 20(3):152-6. https://doi.org/10.1097/00004650-200605000-00010 PMid:16672816

9. Sandelowski M. Visible humans, vanishing bodies, and virtual nursing: complications of life, presence, place, and identity. ANS Adv Nurs Sci. 2002; 24(3):58-70. https://doi.org/10.1097/00012272-200203000-00007 PMid:11890195

10. McCamant KL. Humanistic nursing, interpersonal relations theory, and the empathy-altruism hypothesis. Nurs Sci Q. 2006; 19(4):334-8. https://doi.org/10.1177/0894318406292823 PMid:16982721

11. Watson J. Nursing: The Philosophy And Science Of Caring. University Press Of Colorado: Boulder; 2008.

12. Smith TD. The concept of nursing presence: state of the science. Sch Inq Nurs Pract. 2001; 15(4):299. PMid:11885866

13. McMahon MA, Christopher KA. Toward a mid-range theory of nursing presence. Nurs Forum. 2011; 46(2):71-82. https://doi.org/10.1111/j.1744-6198.2011.00215.x PMid:21517880

14. Zamanzadeh V, Azimzadeh R, Rahmani A, Valizadeh L. Oncology Patients' And Professional Nurses' Perceptions Of Important Nurse Caring Behaviors. BMC Nurs. 2010; 9(1):10. https://doi.org/10.1186/1472-6955-9-10 PMid:20550677 PMCid:PMC2902470

15. Hsieh HF, Shannon SE. Three approaches to qualitative content analysis. Nurse Educ Today. 2005; 15(9):1277-88.

16. Graneheim Uh, Lundman B. Qualitative content analysis in nursing research: concepts, procedures and measures to achieve trustworthiness. Nurse Educ Today. 2004; 24(2):105-12. https://doi.org/10.1016/j.nedt.2003.10.001 PMid:14769454

17. Lincoln YS, Guba EG. But is it rigorous? Trustworthiness and authenticity in naturalistic evaluation. New Directions for Program Evaluation. 1986; 1986(30):73–84. https://doi.org/10.1002/ev.1427

18. Kwok R, Law Y, Li K, Ng Y, Cheung M, V. F, et al. Prevalence of workplace violence against nurses in Hong Kong. Hong Kong Medical j. 2006; 12(1):6-9. PMid:16495582

19. Kabene SM, Orchard C, Howard JM, Soriano MA, Leduc R. The importance of human resources management in health care: a global context. Hum Resour Health. 2006; 4:20. https://doi.org/10.1186/1478-4491-4-20 PMid:16872531 PMCid:PMC1552082

20. Shinjo D, Aramaki T. Geographic distribution of healthcare resources, healthcare service provision, and patient flow in japan: A cross sectional study. Soc Sci Med. 2012; 75(11):1954-63. https://doi.org/10.1016/j.socscimed.2012.07.032 PMid:22920275

21. Eygelaar JE, Stellenberg El. Barriers to quality patient care in rural district hospitals. Curationis. 2012; 35(1):1-8. https://doi.org/10.4102/curationis.v35i1.36 PMid:23327761

22. Frith K, Anderson EF, Tseng F, Fong EA. Nurse staffing is an important strategy to prevent medication error in community hospitals. Nurs Econ. 2012; 30(5):288-94. PMid:23198612

23. Shekelle PG. Nurse–patient ratios as a patient safety strategy: a systematic review. Annals of Internal Medicine. 2013; 158(5 Part 2):404-9. https://doi.org/10.7326/0003-4819-158-5-201303051-00007 PMid:23460097

24. Spetz J, Harless Dw, Herrera CN, Mark BA. Using minimum nurse staffing regulations to measure the relationship between nursing and hospital quality of care. Med Care Res Rev. 2013; 70(4):380-99. https://doi.org/10.1177/1077558713475715 PMid:23401064

25. Frost SA, Alexandrou E. Higher nurse staffinglevels associated with reductions in unplanned readmissions to intensive care or operating theatre, and in postoperative in-hospital mortality in heart surgery patients. Evid Based Nurs. 2013; 16(2):62-3. https://doi.org/10.1136/eb-2012-100893 PMid:23144010

26. Aiken LH, Clarke SP, Sloane DM, Sochalski J, Silber JH. Hospital nurse staffing and patient mortality, nurse burnout, and job dissatisfaction. JAMA. 2002; 288(16):1987-93. https://doi.org/10.1001/jama.288.16.1987 PMid:12387650

27. Abbaszadeh A, Abdi A. Nursing shortage challenge: serious threat for health system. J Community Health. 2014; 9(1):37-47.

28. Vali L, GhorbaniNia R, Shirkhani H, ZolAla F. Comparison of the job motivation of staff worker with management emergency medical center's operational staff, kerman university of medical sciences -2013. J Hosp. 2015; 10(3):61-70.

29. Karimi Moonaghi H, Emami Zeydi A, Mirhaghi A. Patient education among nurses: bringing evidence into clinical applicability in Iran. Invest Educ Enferm. 2016; 34(1):137-151. PMid:28569983

30. Ho WH, Chang CS, Shih YL, Liang RD. Effects of job rotation and role stress among nurses on job satisfaction and organizational commitment. BMC Health Serv Res. 2009; 9:8. https://doi.org/10.1186/1472-6963-9-8 PMid:19138390 PMCid:PMC2630925

31. Mazdaki A, Rezapour A, Azari S, Harati Khaliabad T, Behzadifar M, Razi Moghadam M, et al. Comparison the earnings of specialists' physicians and nurses before and after implementing the Iran's New Tariffs Book. Med J Islam Repub Iran. 2018; 32(37):1-6. https://doi.org/10.14196/mjiri.32.37

32. Walker AC. Safety and comfort work of nurses glimpsed through patient narratives. Int J NursPract. 2002; 8(1):42-8. https://doi.org/10.1046/j.1440-172x.2002.00342.x

33. Zarifraftar M, Aryankhesal A. Challenges of Implementation of accreditation standards for health care systems and organizations: a systematic review. J Manag Sci. 2016; 2(3):191-201.

34. Salehi Z, Payravi H. Challenges in the implementation accreditation process in the hospitals: a narrative review. Iran Journal of Nursing. 2017; 30(106):23-34. https://doi.org/10.29252/ijn.30.106.23

35. Mahmoodian S, Safaei F, Meraji M, Kimiafar K, Farsinegar N, Ghasemi R. Challenges and strengths of implementing accreditation process from health information management staff perspective. J Paramed Scie Rehab. 2016; 5(2):25-33.

36. Sack C, Scherag A, Lütkes P, Günther W, Jöckel KH, Holtmann G. Is there anassociation between hospital accreditation and patient satisfaction with hospital care? A survey of 37 000 patients treated by 73 hospitals. Int J Qual Health Care. 2011; 23(3):278-83. https://doi.org/10.1093/intqhc/mzr011 PMid:21515636

37. Harrison L, Nixon G. Nursing activity in general in-tensive care. J Clin Nurs. 2002; 11:158-67. https://doi.org/10.1046/j.1365-2702.2002.00584.x PMid:11903715

38. Kiekkas P, Poulopoulou M, Papahatzi A, An- droutsopoulou C, Maliouki M, Prinou A. Nursing activities and use of time in the post anesthesia care unit. J Perianesth Nurs. 2005; 20(5):311-22. https://doi.org/10.1016/j.jopan.2005.08.004 PMid:16246808

39. Desjardinw F, Cardinal L, Belzile E, McCusker J. Reorganizing nursing work on surgical units: a time-and-motion study. Nurse Leadersh. 2008; 21(3):26-38. https://doi.org/10.12927/cjnl.2008.20057

40. Dehghan M, Dehghan D, Sheikhrabori A, Sadeghi M, Jalalian M. Quality improvement in clinical documentation: does clinical governance work? J Multidiscip Healthc. 2013; 6:441-50. https://doi.org/10.2147/JMDH.S53252 PMid:24324339 PMCid:PMC3855011

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The Relationship between Nurse's Job Stress and Patient Safety

Meysam Safi Keykaleh[1], Hamid Safarpour[1], Shiva Yousefian[1], Farshad Faghisolouk[1], Ehsan Mohammadi[2], Zohreh Ghomian[1*]

[1]Department of Health in Disasters and Emergencies, School of Public Health and Safety, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [2]Department of Nursing, School of Nursing and Midwifery, Ilam University of Medical Sciences, Ilam, Iran

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***Correspondence:** Zohreh Ghomian. Department of Health in Disasters and Emergencies, School of Health, Safety and Environment, Shahid Beheshti University of Medical Sciences, Tehran, Iran. E-mail: zghomian@gmail.com

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Abstract

BACKGROUND: Patient safety is a key element of the quality of health services. Nurses are the largest group that care for patients, observing safe in nursing care would reduce injuries, disability, morbidity and mortality. However, high stress can lead to a decline in the quality of nursing care.

AIM: This study aimed to investigate the relationship between job stress of the nurses and patient safety in a teaching hospital of Hamadan in 2017.

MATERIAL AND METHODS: This is a cross-sectional study. The data was gathered by a questionnaire of Nurse's job stress prepared by the researcher that after confirming the validity and reliability was completed by 198 nurses of three teaching hospitals of Hamadan city that were selected by simple random sampling and the checklist of patient safety that was collected by the researcher. Data analysis was done in the two levels of descriptive and analysis statistics.

RESULTS: The results showed that the job stress of the nurses and patient safety (mean = 1.75 and SD = 0.114) have been at an average level. There was no statistically significant relationship between Nurse's job stress and patient safety because the Spearman correlation coefficient showed that r = 0.007 and p = 0.919. Among the demographic factors, there was only a significant relationship between marital status and Nurse's job stress ($p < 0.05$).

CONCLUSION: Because of nursing job stress is affected by different working conditions, further studies in the many hospitals are needed. Moderate levels of patient safety are not acceptable; Therefore, heath's policymakers should focus on providing the safety of all patients at the optimal level, with more effort to reduce the stress of their nurses at the lowest level.

Introduction

Work-related stress exists among the public and in all occupation, but it is far more important in professions that deal with people's health. Job stress among health professionals and health care staff, especially nurses, is very prevalent [1], [2]. Due to the specific nature of the nursing profession, which requires high-skill, team working in stressful situations, providing 24-hour care and a great emotional burden, nurses are faced with a variety of stressors [3]. In addition to these cases, other factors such as communication with patients and their companions, communication with physicians and other nurses, high workload, long working hours, dissatisfaction with wages and benefits, working on holidays also cause stress in nurses. Stress can lead to bad consequences, and if the intensity of the stressor exceeds thresholds of tolerance, it could lead to work-related events, including increased absenteeism and leave the job, decreased job satisfaction, reduced productivity and organisational commitment, reduced quality of patient care [4], [5]. If these stresses continue, the will lead to burnout, the worst consequence of which is the reducing of quality of care patients receive because the patient was deprived of adequate care and his human rights.

Sometimes this burnout can result in care

errors and thus negative impact on patients [6]. Rising health care costs, emotional exhaustion, depersonalization and reduced personal accomplishment are the other consequences of accumulated and unmanaged job stress [7]. Of course, providing high quality and safe services to patients can lead to reduced referrals and admissions rate, increased patient satisfaction, improved health status and productivity [7], [8]. Nurses have the most direct contact with patients and are considered as the main foundation of the continuous quality improvement process. Since safe procedures are the core of nursing care to maintain and improve patient safety and if unsafe procedures are done, has not only legal consequences but also irreparable harms are incurred to patients [9], [10], which can have dire consequences for the patients and their family, including long-term accommodation, patients suffering, additional costs, dissatisfaction with the hospitals, (and thus dissatisfaction with the whole health system) and sometimes even patients death [11], [12]. It can impose a huge economic burden on the healthcare system and society [13].

Thus, according to the above discussion, this study aimed to investigate the relationship between patient safety and occupational stress in nurses.

Materials and Methods

Two instruments were developed by researchers team and used to collect data, including a Nurse's Job Stress questionnaire and Patient safety checklist, That In most of the existing checklists that have been used to measure patient safety, all safety-related factors were not fully considered, so, using work experiences, professors' and experts' comments, the researchers decided to carefully include general aspects related to patient safety that are related to nursing procedures in the checklist. Finally, a checklist of 44 items and questionnaire with 46 items and 5 detentions were designed in the following stages.

Based on literature review, we extracted many dimensions of the patient safety and the factors of the nursing job stressor, and then we selected items by taking into consideration the strengths and based on the researcher's opinion.

Instruments design performed through determining validity and reliability. To determine the content validity, we used content validity ratio (CVR) and content validity index (CVI). In this way, the 15 experts are requested to specify whether an item is necessary or not (score each item from 1 to 3). The formula of CVR = (Ne-N/2)/(N/2), in which the Ne is the number of experts indicating "essential" and N is the total number of experts. To obtain CVI, we computed the number of experts giving a rating 3 or 4 to the relevancy of each item, divided by the total number of experts. If thescore was bigger than 0.49 the item in the instrument will be accepted, and If it is less than 0.49 it is eliminated, and between two ranges were need revision [1].

To determine the face validity, we gathered viewpoints of 10 experts of nursing that they were professionals and have research experience or work in the field about sufficient appearance of our checklist and questionnaire.

To determine the Reliability of the instrument, we used the test-retest method. Therefore, in the first step the questionnaires were distributed among 30 nurses, and 10 days later we did it again. For calculating Test-Retest Reliability Coefficients, the data were analysed using SPSS 16 software that we found 0.88.

A cross-sectional study has been done on three hospitals affiliated to Hamedan University of Medical Sciences in 2017. The study population included nurses and patients that admitted to these hospitals during the study.

According to the formula ($n = \frac{(Z_{1-\alpha/2}+Z_{1-\beta})^2}{d^2} + 3$) of the correlation coefficient of two variables and based on data from previous studies, d = 0.2, Z = 1.96, the sample size was calculated 194.

However, the final sample size was 200 people by taking into account the possible loss of samples, which were studied and divided proportionally to the number of nurses in three teaching hospitals. The questionnaires distributed among these nurses and at the same time, the patient safety checklist is completed by the researcher. SPSS.Ver.16 was used to analyse the data. Data analysis was performed using descriptive statistics (frequency and percentage, mean, standard deviation) and inferential (Kolmogorov-Smirnov test for determining the normal distribution of data, Mann-Whitney U and Spearman test).

Results

Male and single participants accounted for 31.8% (n = 63) and 40.4% (n = 80) of the subjects. According to the study population, participants were divided into three age groups of 22-25, 26-34 and 35-53 years, which respectively accounted for 21.7%, 53% and 25.3% of the participants. Regarding work experience, nurses with 1-5, 5-10 and above 10 years of work experience, respectively accounted for 52%, 25.8% and 22.2% of the total samples. A total of 88.4% of the study subjects had degrees above diploma (Table 1).

Table 1: Demographic characteristics of the nurses

Sex					Age groups								
Male		Female			22-25		26-34		35-53		Total	
N	P	N	P		N	P	N	P	N	P	N	P
63	31. 8	135	68. 2		43	21.7	105	53	50	25.3	198	100
Educational level					Work experience								
Diploma		Above diploma			1-5		5-10		>10		Total	
N	P	N	P		N	P	N	P	N	P	N	P
23	11. 6	175	88.4		103	52	51	25.8	44	22.2	198	100

About the occupational stress related to job characteristics; the factors that had the greatest stress-related impact on nurses respectively included long working hours and working on holidays, high workload, lack of career advancement, the unknown importance of nursing career for others and lack of proper mindset towards nursing jobs at the society level. By the scope of nurses' job stress, their job stress is explained in the following five dimensions (Table 2).

Table 2: The status of the five dimensions of the nurses' job stress

Nurses' job stress aspect	Mean	Standard deviation	Status
Job stress related to job specifications	0.33	1.68	Average
Job stress related to management-organisational factors	0.37	1.76	Average
Job stress related to personal communication factors	0.51	2.32	Average
Job stress related to issues related to the physical environment in which a job is performed	0.46	1.85	Average
Job stress related to patient care factors	0.39	1.69	Average
Total	0.30	1.86	Average

The feeling of inadequacy for the nursing job was the least effective factor in the nurses' stress so that only 14.1% of nurses had a feeling of inadequacy towards themselves. In the study of management-organisational factors affecting job stress, the most effective factors, respectively included "lack of sufficient personnel, lack of adequate salary, lack of understanding of the needs of nurses by managers and lack of support by superiors".

Among these studied factors, "lack of coordination between different hospital units" was considered as the lowest effective factor on job stress. About the area of personal relationships affecting job stress, it was found that these factors have little effect on job stress among nurses. Among individual factors, unresolved conflicts with staffs in other units and problems with patients were the least effective factors in this regard.

Also, among physical environment factors creating stress in the workplace, lack of proper conditions such as proper ventilation, noise control, resting place, etc. was the most effective factor in the majority of nurses (52%). However, a small number of nurses (12.1%) believed that unfamiliarity with the existing equipment in the workplace was an important factor affecting stress. Below, patient-care-related factors creating stress in nurses are listed: Dealing with patients in the final moments of life and death (56.1%), non-professional expectations of patients and their relatives (53.5%), the presence of visitors in non-regulated working hours (53%). Among patient care factors, a feeling of discomfort during performing care procedures was identified as a factor in creating job stress only by 18.7% of nurses; this was the least important factor in causing stress.

Investigating patient safety status in the studied hospitals, it was found that the following factors played the most significant role in patient safety from the viewpoint of nurses (the percentage of nurses who stated that the relevant factor plays a significant role in patient safety are shown in parentheses):Preparing care equipment prior to performing procedures (62.1%), separation of infectious and non-infectious wastes (58.6%), personnel's familiarity with all five principles of drugs use (57.1%), putting the patient in a comfortable position to carry out care measures (57.1%), writing the name and surname of the patient and the related physician in boards above patients' beds (52%), rising protective railings beside the bed in case of need (51%), dealing politely and cheerfully with patients (50.5%). The overall condition of the patient safety was evaluated average with an average of 1.75% and a standard deviation of 0.114.

In general, using the Kolmogorov - Smirnov test, job stress (N = 198, Z = 0.558, P = 0.879) has normal distribution and patient safety (N = 198, Z = 1.651, P = 0.009) lacks the normal distribution. A t-test indicated that job stress score in single (P = 0.979) and married male and female groups (P = 0.001) and diploma and higher diploma (P= 0.405) had no statistically significant difference. ANOVA test indicated that job stress score was not significantly different among the three age groups and in three groups with varied work experiences with a p-value of 0.405 and 0.444, respectively.

To investigate the relationship between job stress and the patient safety that Spearman's rank correlation coefficient was used (r = 0.007 and P = 0.919). The coefficient indicates that this relationship is not significant statistically. There was also no significant relationship regarding the presence or absence of a significant relationship between job stress dimensions and patient safety (Table 3).

Table 3: Correlation between aspects of Nurses' job stress and patient safety

Nurses' job stress	Patient safety	
	R	P-value
Job stress related to job specifications	-0.05	0.486
Job stress related to management- organizational factors	0.002	0.983
Job stress related to personal communication factors	0.039	0.586
Job stress related to issues related to the physical environment in which a job is performed	0.006	0.931
Job stress related to patient care factors	-0.008	0.915

Discussion

The results of this study showed that there is no relation between demographic characteristics of

nurses and job stress. This result is consistent with the results of the study by Gandy [15]. Halvah also stated in his study that, among demographic characteristics, there is only a significant correlation between gender and job stress no significant relationship was observed in other cases [11]. In a study that was conducted by Ghasemi Mortaqi. The findings imply that among demographic variables, there was only a significant relationship between the level of education and work-related stress [12]. However, Faraji found a significant relationship between job stress and the demographic variables [13]. This difference in results may be due to differences in the working conditions of nurses, including the type of hospitals, the number of personnel providing service, number of patients etc.

In the present study, it was found that there is no significant relationship between job stress and type of employment, work experience, and work shifts. Hashemi et al. also found similar results in their study [16]. While other studies have reported different results, for example, Letvak and Buck reported that there is a negative correlation between experience and job stress [17]. Hazvehi and Zeighami relationship reported that there is a significant relationship between the number of night shifts and job stress [18].

A study by Shahraki et al. demonstrated significant differences in mean job stress for nurses regarding the type of employment [19]. Bahrami also found that there was a significant relationship between job stress and type of employment and work experience [20]. There is no compelling reason for the observed differences regarding the type of employment (permanent, temporary, contractual, project employment, etc.), but it can be expected that the higher stress level is seen in the night shift due to a more inconvenient time than other shifts(although there was no relationship in the present study).

The results of the present study indicated the average overall level of job stress among nurses. The results of the study conducted by Zeighami also suggest that 86.7 % of nurses have experienced moderate job stress [21]. The results of the study conducted by Faraji also showed that more than 70% of nurses experience medium and high job stress [22]. There was a relatively high consistency between the present study and other studies regarding the results on the most important factors creating job stress in various fields. In a study on nurses, Rahmani referred top high job stress due to a heavy workload and responsibility as well as high stress caused by the physical environment [23].

In a study, Ghasemi et al., listed the most important stressors as follows: patient's death, heavy workload, uncertainty about treatment, conflicts with colleagues, lack of personal adequacy and lack of support [12]. In a study, Hashemi also considered the followings as the most significant stressors: job nature, relationships with co-workers, management factors, workload and authority limit [16]. In a study on nurses, Flanagan also has emphasised the impact of two factors of workload and organisational support on job stress [24]. Considering that factors such as high workload, physical environmental factors, patient's death, lack of adequate salary are among the most important causes of stress in the present study, we can realise the consistency between the current study and the above studies.

In his findings, Torshizi has referred to the highest mean score of job stress factors in different areas as follows respectively: The highest score in the area of working conditions, management factors, interpersonal relations and patient's care was obtained inward congestion, insufficient salaries and benefits, lack of support from superiors and the presence of visitors during non-regulated working hours [25].

The factors mentioned above are consistent with the effective factors found in the present study. Regarding patient safety, the results of the current study show that patient safety is not affected by job stress. This result is inconsistent with results obtained by Berland. In his findings, he pointed out the impact of nurses'stress, caused by high working demands and low organisational and occupational support, on patient safety [26]. The difference between the findings of these two studies can be due to different definitions of safety in two studies or evaluation by different groups with different viewpoints towards patient safety. Thus, it can be noted that it is important for managers and health care organisations to control pressure and stress among nursing staffs.

In conclusion, the findings of our study showed that apart from individual areas, areas such as patient care, management, physical environment and working conditions are considered a source of stress for nurses. Because of nursing job stress is affected by different working conditions, further studies in the many hospitals are needed. Moderate levels of patient safety are not acceptable; therefore, heath's policymakers should focus on providing the safety of all patients at the optimal level, with more effort to reduce the stress of their nurses At the lowest level. Programs can be designed to reduce or control job stress by looking specifically at effective factors in these areas. A major step forward can be taken in promoting this valuable culture by holding workshops and training courses for health personnel to familiarise them with the culture of patient safety. And since the results of this study, which are derived from their nurses' opinions, showed that the stress in the patient's care has been overwhelming; The need to adopt measures in the process of serving patients and their relatives, as well as to control visiting hours so that stress and psychological problems of personnel are reduced as much as possible becomes clear more than ever.

References

1. Johnston DW, Jones MC, Charles K, McCann SK, McKee L. Stress in nurses: Stress-related affect and its determinants examined over the nursing day. Annals of Behavioral Medicine. 2013; 45(3):348-56. https://doi.org/10.1007/s12160-012-9458-2 PMid:23355114

2. Hebrani P, Behdani F, Mobtaker M. Evaluation of stress factors in nurses of different hospital wards. 2008.

3. Van Bogaert P, Timmermans O, Weeks SM, van Heusden D, Wouters K, Franck E. Nursing unit teams matter: Impact of unit-level nurse practice environment, nurse work characteristics, and burnout on nurse reported job outcomes, and quality of care, and patient adverse events—A cross-sectional survey. International journal of nursing studies. 2014; 51(8):1123-34. https://doi.org/10.1016/j.ijnurstu.2013.12.009 PMid:24444772

4. Sveinsdóttir H, Biering P, Ramel A. Occupational stress, job satisfaction, and working environment among Icelandic nurses: a cross-sectional questionnaire survey. International journal of nursing studies. 2006; 43(7):875-89. https://doi.org/10.1016/j.ijnurstu.2005.11.002 PMid:16360157

5. Farquharson B, Bell C, Johnston D, Jones M, Schofield P, Allan J, et al. Nursing stress and patient care: real-time investigation of the effect of nursing tasks and demands on psychological stress, physiological stress, and job performance: study protocol. Journal of advanced nursing. 2013; 69(10):2327-35. https://doi.org/10.1111/jan.12090 PMid:23387943

6. Park, Young-Mi, Kim, Souk Young. Impacts of job stress and cognitive failure on patient safety incidents among hospital nurses. 2013; 4(4):210-215.

7. Farzianpour F, Nosrati SA, Foroushani AR, Hasanpour F, Jelodar ZK, Keykale MS, et al. Relationship Between Shift Work and Personality Traits of Nurses and Their Coping Strategies. Global journal of health science. 2016; 8(5):166. https://doi.org/10.5539/gjhs.v8n5p166 PMid:26652076 PMCid:PMC4877235

8. Park Y-M, Kim SY. Impacts of job stress and cognitive failure on patient safety incidents among hospital nurses. Safety andhealth at work. 2013; 4(4):210-5. https://doi.org/10.1016/j.shaw.2013.10.003 PMid:24422177 PMCid:PMC3889080

9. Cavalheiro AM, Moura Junior DF, Lopes AC. Stress in nurses working in intensive care units. Revista Latino-Americana de Enfermagem. 2008; 16(1):29-35. https://doi.org/10.1590/S0104-11692008000100005 PMid:18392527

10. Nayomi W. Workplace stress in nursing: a literature review. 2016.

11. Benner P, Sheets V, Uris P, Malloch K, Schwed K, Jamison D. Individual, practice, and system causes of errors in nursing: a taxonomy. Journal of Nursing Administration. 2002; 32(10):509-23. https://doi.org/10.1097/00005110-200210000-00006 PMid:12394596

12. Mortaghy Ghasemi M, Ghahremani Z, Vahediane Azimi A, Ghorbani F. Nurse's job stress in a therapeutic educational center in Zanjan. Journal of Gorgan Bouyeh Faculty of Nursing and Midwifery. 2011; 8(1):42-51.

13. Faraji O, Valiee S, Moridi G, Ramazani A, Rezaei FM. Relationship between job characteristic and job stress in nurses of Kurdistan University of Medical Sciences educational hospitals. 2012.

14. Zamanzadeh V, Ghahramanian A, Rassouli M, Abbaszadeh A, Alavi-Majd H, Nikanfar A-R. Design and implementation content validity study: development of an instrument for measuring patient-centered communication. Journal of caring sciences. 2015; 4(2):165. https://doi.org/10.15171/jcs.2015.017 PMid:26161370 PMCid:PMC4484991

15. Gandi JC, Wai PS, Karick H, Dagona ZK. The role of stress and level of burnout in job performance among nurses. Mental health in family medicine. 2011; 8(3):181. PMid:22942900 PMCid:PMC3314275

16. Hashemi M, Garshad A. ASSESMENT JOB STRESS IT IN NURSING PERSONAL IN A SELECTED TEACHING HOSPITAL OF NORTH KHORASAN UNIVERSITY OF MEDICAL SCIENCES 2008. 2012.

17. Letvak S, Buck R. Factors influencing work productivity and intent to stay in nursing. Nursing Economics. 2008; 26(3):159. PMid:18616053

18. Hazavehei MM, Moghimbeigi A, Hamidi Y. Assessing stress level and stress management among Hamadan hospital nurses based on precede model. The Horizon of Medical Sciences. 2012; 18(2):78-85.

19. Shahraki Vahed A, Mardani Hamuleh M, Sanchuli J, Hamedi Shahraki S. Assessment of the relationship between mental health and job stress among nurses. J Jahrom Univ Med Sci. 2011; 8:40-3.

20. Bahrami A, Akbari HAH, Mousavi SGA, Hannani M, Ramezani Y. Job stress among the nursing staff of Kashan hospitals. Feyz Journals of Kashan University of Medical Sciences. 2011; 15(4).

21. Mohammadi SZ, Haghighi SA. Relation between job stress and burnout among nursing staff. Scientific Journal of Hamadan Nursing & Midwifery Faculty. 2011; 19(2):42-52.

22. Faraji O, Valiee S, Moridi G, Ramazani A, REZAEI FM. Relationship between job characteristic and job stress in nurses of Kurdistan University of Medical Sciences educational hospitals. 2012.

23. Rahmani F, Behshid M, Zamanzadeh V, Rahmani F. Relationship between general health, occupational stress and burnout in critical care nurses of Tabriz teaching hospitals. Iran Journal of Nursing. 2010; 23(66):54-63.

24. Flanagan NA. Testing the relationship between job stress and satisfaction in correctional nurses. Nursing Research. 2006; 55(5):316-27. https://doi.org/10.1097/00006199-200609000-00004 PMid:16980832

25. Torshizi L, Ahmadi F. Job stressors from clinical nurses'perspective. 2011.

26. Berland A, Natvig GK, Gundersen D. Patient safety and job-related stress: a focus group study. Intensive and critical care nursing. 2008; 24(2):90-7. https://doi.org/10.1016/j.iccn.2007.11.001 PMid:18096388

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Effect of the Living Environment on falls among the Elderly in Urmia

Seyed Saeed Mazloomy Mahmoodabad[1], Moradali Zareipour[1*], Mohsen Askarishahi[2], Alireza Beigomi[3]

[1]Social Determinants of Health Research Center, Department of Health Education & Promotion, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; [2]Research Center of Prevention and Epidemiology of Non-Communicable Disease, Department of Bio-Statistics and Epidemiology, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; [3]Department of Health Education & Promotion, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

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***Correspondence:** Moradali Zareipour. Social Determinants of Health Research Center, Department of Health Education&Promotion, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. E-mail: z.morad@yahoo.com

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Abstract

BACKGROUND: The living environment has an impact on the health of the elderly, and the safety of the house is one of the concerns of the elderly. Disregarding the safety concerns increases the falling.

AIM: This research was conducted with the aim of influencing the living environment on falls among elderly people in Urmia city.

METHODS: This is a cross-sectional (descriptive-analytic) study which 200 elderly people were selected by random cluster sampling. Data were collected by using a two-part questionnaire including demographic information, and home safety assessment checklist. Data were analysed by using chi-square test and logistic regression in SPSS v. 21 software.

RESULTS: The incidence of falling in the elderly was 30%. There was a significant statistical association with age, sex, marital status and history of chronic disease. Results of logistic regression showed non-safe stairs (OR = 1.1, p = 0.002), unsafe toilet/bath (OR = 1.3, p = 0.001), unsafe bedrooms (OR = 1.7, p = 0.05) unsafe living room (OR = 1.4, p = 0.02) increase the falls in the elderly, as well as male gender (OR = 1.14, p < 0.001) and living with other people (OR = 0.19, p = 0.002) reduce the falls in the elderly.

CONCLUSION: By identifying the risk factors of the physical space of the home, we can plan for implementing necessary interventions according to the risk factor or risk factors to prevent and reduce the falls in the elderly community.

Introduction

Elderly is a sensitive period in human life, in which elderly people are exposed to potential threats such as increasing of chronic illness, loneliness and isolation and lack of social support, and because of their physical and mental disabilities, their autonomy has been threatened in many cases, [1]. Considering that life expectancy has increased over the past decades in Iran, and this phenomenon has led to an increase of the population of the elderly [2], one of the most common and most serious problems is falling down in the elderly and It is the second reason for mortality of unintentional injuries in the world [3], falling is the unintentional and sudden change in physical condition, due to falling of a person at a lower level (on an object or floor) [4].

Elderly physical changes begin by entering into middle age. These changes have the greatest effect on muscle function by decreasing motor function, reducing muscle strength and muscle endurance. Thus, the physiological changes in the nervous and musculoskeletal system which occur during the ageing process and it affects on complex movements and causes the increasing of falls [5], [6], [7]. Fractures of the organs, strike on the skull and brain, fractures of the vertebrae and the rib, soft tissue and internal organs damages are common causes of falling, which they cause dependence, self-efficacy,

fear of falling, depression, immobility, daily activity limitation, hospitalization or resident in a nursing home. Falling is the result and imposing of the cost on the individual and the community [8], [9].

Falling is the result of a complex interference of several risk factors. These factors are divided into four main groups: biological risk factors (age, sex, diseases, cognitive abilities), economic social (literacy, income, habitation, public health, social isolation), behavioral (Fears of falling, lifestyle, Taking medicines at the same time, stopping sports activities, inadequate wearing) and peripheral (Building design, stairs, Corridors, carpets and Slippery floors, fences, baths, washrooms). Given that the elderly spend a long time at home, so more than half of the elderly falls are at home [10]. Hence, paying attention to the living environment is an important scope for the care of these individuals. Because of the variety of environments in different houses, it is necessary to consider the design of each component and objects in houses according to the ergonomic science [11], [12] based on the elderly living environments. Although, we usually consider our home as a safe place, many events may occur at home for the elderly. Considering the design of the house for the elderly leads to decrease the significant risks and accidents to achieve and promote the human-environment co-ordination in the elderly [13]. Several studies have shown that there is a relationship between domestic risks and falling. In the study of Chang and his colleagues, it is found that the elderly who live in a busy, dark and uncomfortable home were significantly at risk of falling [14]. Bemmel et al. showed that living at home with a high number of risks could increase the falls [15]. Thiamwong et al., found that slippery surfaces are one of the risk factors for fallings [16], but Gill et al., did not report the risk of falling and risk factors such as the laxity of the carpets, the sliding of the surfaces [17]. Due to differences in weather, culture, lifestyle, beliefs and different home environments in different societies, the falling factors of the living environment of the elderly are different.

Therefore, considering the above factors, the aim of this study was the study of the influencing of the living environment on the fallings among the elderly in Urmia.

Methods

In this descriptive-analytic study, the population of the study included the elderly people with over than 60 years old who referred to urban health centres of Urmia city in 2017. Informed consent was obtained from all individual participants included in the study. The Ethics committee of the Shahid Sadoughi University of Medical Sciences-Yazd approved this study (Ethic code: IR.SSU.SPH.REC.1395.126).

According to previous studies and estimation of elderly falls [18], [19], [20], [21], the sample size was chosen 200 elderly with 95% confidence level (d = 06, Z = 1.96, P = 0.3). The sampling method was in the following way, 10 health centres randomly selected through a lottery among the 35 urban health centres, and the elderly from each centre were randomly selected according to their population and if they were satisfied. Data collection tools in this research were a researcher-made questionnaire that included demographic characteristics including age, gender, marital status, history of falls in the last year, the place and time of falling and the home safety assessment checklist in 5 positions, bedrooms, toilets/baths, kitchens, living rooms, stairs. Content validity tests and Cronbach's alpha test were used for the validity and reliability of the home safety checklist. To determine validity, the checklist was sent to 10 health and epidemiology educators. According to the experts, the necessary corrections were applied to the checklist, and the validity of the checklist was more than 80%. In order to check the reliability of the checklist, a checklist for a pilot group of 30 elderly people were completed by using Cronbach's alpha test, which the reliability of coefficient of questions of stairs (internal and external) was 0.75, or bath/toilet was 0.72, of living room (Hall) was 0.71, of kitchen was 0.88, of bedroom, was 0.78. Home Safety Testing in the form of 37 questions that were presented as yes, no, and inapplicable options and one score were rated to Yes option and to inapplicable option, and zero scores were rated to No option. Checklist scores vary from zero to 37.

Questions of external stairs were 6 questions, for internal stairs were 8 questions, for the bathroom were 9 questions, for living room were 4 questions, for the kitchen were 5 questions, for bedroom were 5 questions. The elderly were invited to the relevant health centre by telephone, and the study objectives were explained to all participants in the study. The demographic information questionnaire was completed by the elderly. If the elderly were illiterate or unable to complete the questionnaire, the questionnaire was completed by a trained interviewer. Then the researcher, along with one Health environmental expert/professional, visited the house of the elderly, and the checklist was completed by observing 5 places, bedrooms, toilet/bathroom, kitchen, living room, stairs. The inclusion criteria were consist of being in an age group of 60 to 80 years old, non-use of anticonvulsants, able to walk without any helping tool, not being in the nursing home, and the exclusion criteria consisted of dissatisfaction, or death of the research cases or being unable to gait during the study.

Results

Sixty (30%) of the elderly had a history of falling over the last year, and the most cases of falling were at home, 36 elderly (60%) and 26 (43.3%) individuals have experienced fall in the morning. Most of the age group was from 75 to 80 (33%). Most of the elderly were housewives (42%) and married (56%). Also, the highest level of education was elementary education (33%). In economic terms, most of the elderly had a moderate economic situation (54%). The results of the Chi-square test showed that the rate of falls was higher in high age and this increase was statistically significant ($p < 0.001$).

Also, falling in alone elderly (divorced-widows) was higher than that of married elderly ($p = 0.02$). On the other hand, falling in women were more than men and were statistically meaningful ($p = 0.02$). Falling in people with chronic diseases were more than those without chronic illness ($p = 0.04$) (Table 1).

Table1: Frequency and percentage of distribution of basic characteristics of the elderly people and its relationship with falling (n = 200)

Variables		N (%)	With falls history Number (per cent)	Without falls history Number (per cent)	P-value
Age	60-65	36 (18)	4 (11.2)	32 (88.8)	P < 0.001*
	65-70	54 (27)	8 (14.9)	46 (85.1)	
	70-75	44 (22)	20(45.5)	24 (54.5)	
	80-75	66 (33)	28 (42.5)	38 (57.5)	
Sex	Male	104 (52)	28 (26.9)	76 (73.1)	P = 0.02*
	Female	96(48)	38(43.8)	58(56.2)	
Job	Housewife	84 (42)	30 (35.7)	54 (64.3)	P = 0.3
	Self-employed	40 (20)	8 (20)	32(80)	
	Retired	20(10)	6(30)	14(70)	
	Out of work	56 (28)	16 (28.6)	40 (71.4)	
Marital status	Married	112 (56)	30 (26.8)	82 (73.2)	P = 0.02*
	Divorced	26 (13)	8 (30.7)	18 (69.3)	
	Widow /widower	62(31)	26(41.9)	36(58.1)	
Education	Illiterate	58 (29)	16 (27.6)	42 (72.4)	P = 0. 2
	Elementary	66 (33)	26 (39.4)	40 (60.6)	
	Junior	42 (21)	12 (28.6)	30 (71.4)	
	Diploma and Postgraduate	36(17)	7(20)	29(80)	
History of chronic diseases	Yes	149 (74.5)	49 (32.9)	100 (67.1)	P = 0. 04
	No	51 (25.5)	11 (21.6)	40 (78.4)	
The economic situation	Weak	36 (18)	11 (30.5)	25 (69.5)	P = 0. 1
	Moderate	108 (54)	31 (28.7)	77 (71.3)	
	Good	56 (28)	12 (21.4)	44 (78.6)	

* Significant p-value.

Regarding the results of the study of the elderly living environment, the lowest level of home safety was related to the location of bathroom/toilet and hall (living room), and the highest level of home safety was related to the stairs position (internal and external) (Table 2).

Table 2: Frequency and Percentage of the Living Environment of the Elderly in Five Places of the house (n = 200)

Variables		N (%)
External stairs	Proper	162 (81)
	Not proper	38(19)
Interior stairs	Proper	158 (79)
	Not proper	42(21)
Bathroom / toilet	Proper	80 (40)
	Not Proper	120(60)
Living room	Proper	84 (42)
	Not Proper	116(58)
Kitchen	Proper	155 (77.5)
	Not Proper	45(22.5)
Bedroom	Proper	134 (67)
	Not proper	66(33)

The variables, stairs (internal/external), toilets/bathrooms, bedrooms, living room/hall, kitchen were as the main variables and gender and lifestyle were as confounding variables which are entered the logistic regression model. The results showed that variables, stairs (internal/External), toilet/bathroom, bedroom, living room/hall, gender and lifestyle affected on the rate of falling in elderly so that non-safe stairs (OR = 1.1, p = .002, 95% CI = 1.05-1.3) , Non-safe toilet / bathroom (OR = 1.3, p = .001, 95% CI = 1.1-1.5), non-safe bedroom (OR = 1.7, p = .05; 95% CI = 0.99-1.39), non-safe living room (OR = 1.4, p = .02; 95% CI = 0.53-2.9) increase the falling in elderly. Also the male gender (OR = 1.14, $p < 0.001$; 95% CI = 1.07-1.2) and living with other people (OR = 0.19, p = .002; 95% CI = 0.59-2.72) decrease the falling in elderly.

Table 3: Prognostic variables of fallings in elderly people by using logistic regression test (N = 200)

Independent variables	ß (regression coefficient)	S.E	OR odds ratio)	P-value	95% confidence Interval for odds ratio	
					Lower	Upper
Stairs (external-internal)						
Appropriate	-	-	1	-	-	-
Not Proper	1. 31	0.81	2.2	0.01	0.88	4.9
Toilet / Bathroom						
Appropriate	-	-	1	-	-	-
Not Proper	0.78	0.79	1.7	0.03	0.49	3.22
Bedroom						
Appropriate	-	-	1	-	-	-
Not Proper	0.67	0.48	1.3	0.04	0.49	2.07
Living Room / Hall						
Appropriate	-	-	1	-	-	-
Not Proper	0.81	0.61	1.4	0.02	0.53	2.9
The kitchen						
Appropriate	-	-	1	-	-	-
Not Proper	-0.07	0. 16	0.98	0.49	0.53	2.9
Gender						
Female	-	-	1	-	-	-
Male	-1.89	0.67	0.13	0.001	0.59	2.72
Living arrangements						
Alone	-	-	1	-	-	-
With a spouse, Children, Others	-1.51	0.57	0.19	0.002	0.59	2.72

Discussion

In this study, the prevalence of falls was 30% in the elderly, in Turkey was 28.8% [22], in Iran was 27% [21], in Swiss was31% [23], in Australia was 29% [24], 28.5%, in China 26/4 [19], which is similar to the result of this study. Also, the highest rate of falls was at home (60%). Considering that in Iran, elderly people often have a passive role in society and usually spend their time at home and do not work out, it can be one of the important reasons for increasing the amount of falling. In this study, the lowest rate of falls was reported at noon (26.7%), and the highest rate was in the morning (43.3%). Since the physical activity of the elderly is high during the active hours over day and night, so the probability of falling is increased during the active hours over day and night. In similar studies, the highest rate of falling of older

adults was during the active hours [25], [26], [27]. Therefore, it is recommended that older adults should be taken care during the active hours over day and night. The results of the study showed that the rate of falling is increased by increasing the age of elderly people, the incidence of falling in the elderly increased, which It could be due to chronic diseases, visual impairment, cognitive impairment, physical weakness, motion, and other factors which are confirmed in various studies [25], [28], [29].

On the other hand, fallings more likely happen in elderly people who are living alone. These results are consistent with studies by Iranfar and his colleagues, which showed that a spouse could decrease the falls [30]. It seems that Single elderly face with more preventing barriers during the employing prevention of behaviour of falls, which we can remove barriers, improve and improve their lifestyle by good planning. Having chronic diseases can lead to disabilities and weaknesses, and then cause to fall the elderly. In the study, people with chronic illness have more chance of fallings than others. In other similar studies, the incidence of falling elderly was associated with chronic diseases [18], [26], [28], [29]. Considering the results of the study, the study of elderly's living environment showed that the bath/shower room and hall (living room) had the lowest level of home safety, and the highest level of home safety was related to the location of stairs (internal and external), related to the bath/shower room and hall (living room), and the highest level of home safety was related to the location of stairs (internal and external), which was consonant by the study of Ali Zazouli and Colleagues [31]. According to the researcher, the lower level of safety of bath/toilet can be attributed to the Iranian culture, which there are not any barriers in the walls in bathrooms and their financial inability in building or repairing the safety bath/toilet. Also, the level of awareness of households is relevant to this regard. Also, the high level of the safety of stairs among different parts of the house can be related to the greater attention of households and their high awareness of having standard stairs and shielded stairs. The places where elderly spend most of their time in those environments during the day include a living room, a bedroom, a kitchen, a bathroom/toilet, and stairs. It is expected that these places will have the most occurrence of daily accidents, such as fallings.

Prevention of accidents in these places, where the elderly lives in, can be done by identifying the risk factors. Therefore, in this research, the immune status of these five parts of the house was examined, and the results of logistic regression showed that non-safe stairs in the elderly increased the chance of falling 2.2 times. According to Stevens and Abolhassani et al., stairs and aisles have been mentioned as a risk factor for the falls of the elderly [32], [33] which was consistent with the findings of the present study. The risks of the physical space of the house can be reduced by the low amount of money and fencing the slippery stairs and lighting and can reduce the risk of falling off the elderly in each home. Sophonratanapokin referred to slippery surfaces as the first reason of fallings in houses, and reported that the chance of falling in bathroom and toilet, especially in the toilets in the courtyards, are more than other physical spaces in the house [34] which was consistent with the present study which showed that the chance of fallings is 1.7 in the unsafe toilets and baths. Camilloni and colleagues introduced safety devices such as bath flooring, non-slippery bathtubs and non-slippery shower trays as pre-incident prevention [35]. Promoting the culture of using special and non-slippery flooring, installing a protective bar in the walls of the bathrooms, and the use of public media and education can have a positive effect on preventing the fall of the elderly.

Fazing of furniture in the transit of room was reviewed in this study, and the results showed that the unsafe living room cause to increase the chances of fallings the elderly about 1.4 times. Therefore, it was identified as a risk factor. Iranfar in his study described the living room as a safe place that does not endanger the elderly [30], but Camilloni introduced the living room as the place where the fall occurred [35]. Sophonratanapokin reported a significant relationship between the incidence of falls and Floor Covering and carpets in bedrooms [34], which was consistent with the findings of the study that the non-safe bedroom has increased the chance of falling 1.3 times. The use of non-clamping rugs and smooth and slippery floorings seems to be unaffected which reduces slippery floors. Sadasivam, according to the view of elderly, reported the bedroom’s light and a bright way from bed to toilets as risk factors [36], which was not consistent with the findings of the present study. Indirect electricity and lighting are used in Iranian homes, and almost all participants have enough light and brightness, especially in stairs, corridors and bathrooms in their homes.

The rate of falling off the elderly people were different in both genders, which the rate of falling in men was 0.87 times lower than that of women, which was associated with results of the study by Halil et al., [18], and the study by Jalali et al., [21], Zhang and Chen [28] in China, it is reported that falling rates of men was less than women. Iranian women have a lot to do in daily living activities such as housekeeping, shopping, taking care of grandchildren while older male after retirement tend to stay and rest, for example, in a normal day, they prefer to stay at home and perform religious duties or meet their friends. On the other hand, Elliott's study also reports that there is a great rate of falls in the number of elderly people who live alone [37], which is consistent with the findings of the study, which shows that the rate of chances of falling in elderly people living with other people is lower than alone elderly. Living with others, whether from a family or a nurse, can reduce the

number and severity of fallings regardless of emotional and psychological influences in the old days. Today, the number of elderly people who alone seem to be increasing, which provides the basis for an inconvenient event of falls at home.

In conclusion, based on the results of this research to avoid falling at home, we can use a big doorknob, Installing a protective bar on bathroom walls, toilets, hallways and stairs, non-slippery floor coverings, removing every bumpy on the floor of the rooms, like carpet weaving. Especially paying attention to the structure of the stairs, which is one of the fear causes of falling in the elderly and it is an important factor in ensuring of the elderly in safety of life, and it is recommended that not to use spiral steps with high altitudes, with bluish colours, etc. Preferably stairs should be short and small and avoid placing any slippery covers or objects such as vases or decorative items that may eliminate elderly concentration while on the stairs. Also, the electric equipment wire should be assembled in the rooms and should not be in the way of the elderly.

Reference

1. Stevens JA, Corso PS, Finkelstein EA, Miller TR. The costs of fatal and non-fatal falls among older adults. Injury prevention. 2006; 12(5):290-5. https://doi.org/10.1136/ip.2005.011015 PMid:17018668 PMCid:PMC2563445

2. Mazloomymahmoodabad S, Masoudy G, Fallahzadeh H, Jalili Z. Education based on precede-proceed on quality of life in elderly. Global journal of health science. 2014; 6(6):178. https://doi.org/10.5539/gjhs.v6n6p178 PMid:25363108 PMCid:PMC4825517

3. Pfortmueller C, Lindner G, Exadaktylos A. Reducing fall risk in the elderly: risk factors and fall prevention, a systematic review. Minerva Med. 2014; 105(4):275-81. PMid:24867188

4. Huang HC, Gau ML, Lin WC, George K. Assessing risk of falling in older adults. Public Health Nursing. 2003; 20(5):399-411. https://doi.org/10.1046/j.1525-1446.2003.20508.x PMid:12930464

5. Salminen MJ, Vahlberg TJ, Salonoja MT, Aarnio PT, Kivelä SL. Effect of a Risk-Based Multifactorial Fall Prevention Program on the Incidence of Falls. Journal of the American Geriatrics Society. 2009; 57(4):612-9. https://doi.org/10.1111/j.1532-5415.2009.02176.x PMid:19392952

6. Hornyak V, Brach JS, Wert DM, Hile E, Studenski S, VanSwearingen JM. What is the relation between fear of falling and physical activity in older adults? Archives of physical medicine and rehabilitation. 2013; 94(12):2529-34. https://doi.org/10.1016/j.apmr.2013.06.013 PMid:23816923 PMCid:PMC4878685

7. Nitz JC, Josephson DL. Enhancing functional balance and mobility among older people living in long-term care facilities. Geriatric Nursing. 2011; 32(2):106-13. https://doi.org/10.1016/j.gerinurse.2010.11.004 PMid:21237533

8. Walther L, Rogowski M, Schaaf H, Hörmann K, Löhler J. Falls and dizziness in the elderly. Elsevier. 2010; 64(6):354-357. https://doi.org/10.1016/S0030-6657(10)70586-2

9. Orces CH. Prevalence and determinants of fall-related injuries among older adults in Ecuador. Current gerontology and geriatrics research. 2014; 1(8):863473-7. https://doi.org/10.1155/2014/863473

10. Dionyssiotis Y. Analyzing the problem of falls among older people. International journal of general medicine. 2012; 5(3):805–813. https://doi.org/10.2147/IJGM.S32651 PMid:23055770 PMCid:PMC3468115

11. McCullagh MC. Home Modification: How to help patients make their homes safer and more accessible as their abilities change. AJN The American Journal of Nursing. 2006; 106(10). 54-63. https://doi.org/10.1097/00000446-200610000-00033 PMid:17016095

12. van Haastregt JC, Diederiks JP, van Rossum E, de Witte LP, Voorhoeve PM, Crebolder HF. Effects of a programme of multifactorial home visits on falls and mobility impairments in elderly people at risk: randomised controlled trial. Bmj. 2000; 321(7267):994-8. https://doi.org/10.1136/bmj.321.7267.994 PMid:11039967 PMCid:PMC27508

13 Sekiguchi T. Toward a dynamic perspective of person-environment fit. Osaka keidai ronshu. 2004; 55(1):177-90.

14. Chang N-T, Chi L-Y, Yang N-P, Chou P. The impact of falls and fear of falling on health-related quality of life in Taiwanese elderly. Journal of community health nursing. 2010; 27(2):84-95. https://doi.org/10.1080/07370011003704958 PMid:20437289

15. van Bemmel T, Vandenbroucke JP, Westendorp RG, Gussekloo J. In an observational study elderly patients had an increased risk of falling due to home hazards. Journal of clinical epidemiology. 2005; 58(1):63-7. https://doi.org/10.1016/j.jclinepi.2004.06.007 PMid:15649672

16. Thiamwong L, Thamarpirat J, Maneesriwongul W, Jitapunkul S. Thai falls risk assessment test (Thai-FRAT) developed for community-dwelling Thai elderly. Medical journal of the Medical Association of Thailand. 2008; 91(12):1823.

17. Gill TM, Williams CS, Tinetti ME. Environmental hazards and the risk of nonsyncopal falls in the homes of community-living older persons. Medical care. 2000:1174-83. https://doi.org/10.1097/00005650-200012000-00004 PMid:11186296

18. Halil M, Ulger Z, Cankurtaran M, Shorbagi A, Yavuz BB, Dede D, et al. Falls and the elderly: Is there any difference in the developing world?: A cross-sectional study from Turkey. Archives of gerontology and geriatrics. 2006; 43(3):351-9. https://doi.org/10.1016/j.archger.2005.12.005 PMid:16522334

19. Chu L-W, Chiu AY, Chi I. Falls and subsequent health service utilization in community-dwelling Chinese older adults. Archives of gerontology and geriatrics. 2008; 46(2):125-35. https://doi.org/10.1016/j.archger.2007.03.005 PMid:17467081

20. Stalenhoef P, Diederiks J, Knottnerus J, Kester A, Crebolder H. A risk model for the prediction of recurrent falls in community-dwelling elderly: a prospective cohort study. Journal of clinical epidemiology. 2002; 55(11):1088-94. https://doi.org/10.1016/S0895-4356(02)00502-4

21. Jalali MM, Gerami H, Heidarzadeh A, Soleimani R. Balance performance in older adults and its relationship with falling. Aging clinical and experimental research. 2015; 27(3):287-96. https://doi.org/10.1007/s40520-014-0273-4 PMid:25286899

22. Hartholt KA, Stevens JA, Polinder S, van der Cammen TJ, Patka P. Increase in fall-related hospitalizations in the United States, 2001–2008. Journal of Trauma and Acute Care Surgery. 2011; 71(1):255-8. https://doi.org/10.1097/TA.0b013e31821c36e7 PMid:21818033

23. Crow RS, Lohman MC, Pidgeon D, Bruce ML, Bartels SJ, Batsis JA. Frailty Versus Stopping Elderly Accidents, Deaths and Injuries Initiative Fall Risk Score: Ability to Predict Future Falls. Journal of the American Geriatrics Society. 2018. https://doi.org/10.1111/jgs.15275 PMCid:PMC5849536

24. Morris M, Osborne D, Hill K, Kendig H, Lundgren-Lindquist B, Browning C, et al. Predisposing factors for occasional and multiple falls in older Australians who live at home. Australian journal of physiotherapy. 2004; 50(3):153-9. https://doi.org/10.1016/S0004-9514(14)60153-7

25. Lehtola S, Koistinen P, Luukinen H. Falls and injurious falls late in home-dwelling life. Archives of gerontology and geriatrics. 2006; 42(2):217-24. https://doi.org/10.1016/j.archger.2005.07.002 PMid:16125808

26. Corsinovi L, Bo M, Aimonino NR, Marinello R, Gariglio F, Marchetto C, et al. Predictors of falls and hospitalization outcomes in elderly patients admitted to an acute geriatric unit. Archives of gerontology and geriatrics. 2009; 49(1):142-5. https://doi.org/10.1016/j.archger.2008.06.004 PMid:18674824

27. Bergland A, Jarnlo G-B, Laake K. Predictors of falls in the elderly by location. Aging clinical and experimental research. 2003; 15(1):43-50. https://doi.org/10.1007/BF03324479 PMid:12841418

28. Zhang Y, Chen W. Research overview and progress of the elderly falls. Chin J Gerontol. 2008; 9:929-31.

29. Coimbra AMV, Ricci NA, Coimbra IB, Costallat LTL. Falls in the elderly of the family health program. Archives of gerontology and geriatrics. 2010; 51(3):317-22. https://doi.org/10.1016/j.archger.2010.01.010 PMid:20153535

30. Iranfar M. Physical Hazards of Residences and Elderly Fall. Safety Promotion and Injury Prevention. 2018; 5(4):237-42.

31. Ali Zazouli M, Yazdany Cherati J, Ahmadnezhad A. Assessment of Safety Status of Residential Housing in Rural Families of Ramian Township (Golestan Provicine, Iran) in 2011. Journal of Mazandaran University of Medical Sciences (JMUMS). 2013; 23(2):163-75.

32. Abolhassani F, Moayyeri A, Naghavi M, Soltani A, Larijani B, Shalmani HT. Incidence and characteristics of falls leading to hip fracture in Iranian population. Bone. 2006; 39(2):408-13. https://doi.org/10.1016/j.bone.2006.01.144 PMid:16510325

33. Stevens M, Holman CAJ, Bennett N. Preventing falls in older people: impact of an intervention to reduce environmental hazards in the home. Journal of the American Geriatrics Society. 2001; 49(11):1442-7. https://doi.org/10.1046/j.1532-5415.2001.4911235.x PMid:11890581

34. Sophonratanapokin B, Sawangdee Y, Soonthorndhada K. Effect of the living environment on falls among the elderly in Thailand. Southeast Asian journal of tropical medicine and public health. 2012; 43(6):1537. PMid:23413718

35.Camilloni L, Farchi S, Rossi PG, Chini F, Di Giorgio M, Molino N, et al. A case–control study on risk factors of domestic accidents in an elderly population. International journal of injury control and safety promotion. 2011; 18(4):269-76. https://doi.org/10.1080/17457300.2011.562615 PMid:21557126

36. Sadasivam RS, Luger TM, Coley HL, Taylor BB, Padir T, Ritchie CS, et al. Robot-assisted home hazard assessment for fall prevention: A feasibility study. Journal of telemedicine and telecare. 2014; 20(1):3-10. https://doi.org/10.1177/1357633X13517350 PMid:24352900

37. Elliott S, Painter J, Hudson S. Living alone and fall risk factors in community-dwelling middle age and older adults. Journal of community health. 2009; 34(4):301-8. https://doi.org/10.1007/s10900-009-9152-x PMid:19333744

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Review Article

S100B Serum Level as a Mortality Predictor for Traumatic Brain Injury: A Meta-Analysis

Nyoman Golden[1], Tjokorda Gde Bagus Mahadewa[1*], Citra Aryanti[1], I Putu Eka Widyadharma[2]

[1]Department of Neurosurgery, Faculty of Medicine, Udayana University, Sanglah General Hospital, Bali, Indonesia; [2]Department of Neurology, Faculty of Medicine, Udayana University, Sanglah General Hospital, Bali, Indonesia

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Keywords: Traumatic brain injury; S100B serum level; Mortality

***Correspondence:** Tjokorda Gde Bagus Mahadewa. Department of Neurosurgery, Faculty of Medicine, Udayana University, Sanglah General Hospital, Bali, Indonesia. E-mail: tjokmahadewa@unud.ac.id

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Abstract

BACKGROUND: The pathogenesis of inflammatory neuronal cell damage will continue after traumatic brain injury in which contributed to subsequent mortality. Serum S100B levels were shown to be an early predictor of mortality due to traumatic brain injury.

AIM: This Meta-Analysis will analyse the mean and diagnostic strength of serum S100B levels between survived and died subjects with head injuries based on the various follow-up times of nine studies.

METHODS: We conducted a meta-anelysis in accordance with PRISMA guidelines and adhering to Cochrane Handbook for Systematic Review of Interventions. Literature search was conducted on March 16, 2018 from Medline and Scopus in the past 10 years, using various keywords related to S100, brain injury, and outcome. Duplicate journals were sorted out via EndNote. Included articles were as follows: original data from the group, clinical trials, case series, patients undergoing serum S100B levels with both short- and long-term follow-up mortality. Data were collected for mortality, serum S100B levels, and its diagnostic strength. All data were analyzed using Review Manager 5.3 (Cochrane, Denmark).

RESULTS: The results of the meta-analysis showed a significant difference in S100B levels between survived and died subjects with head injuries on overall follow-up timeline (0.91, 95.9% CI 0.7-1.12, I2 = 98%, $p < 0.001$), during treatment (1.43, 95% CI 0.97 to 1.89, I2 = 98%, $p < 0.001$), or 6 months (0.19; 95%CI 0.1-0.29, I2 = 76%, $p < 0.001$) with an average threshold value that varies according to the study method used. The mean diagnostic strength was also promising to predict early mortality (sensitivity of 77.18% and 92.33%, specificity of 78.35% and 50.6%, respectively).

CONCLUSION: S100B serum levels in the future will be potential biomarkers, and it is expected that there will be standardised guidelines for their application.

Introduction

Traumatic brain injury is a trauma that causes abnormal functioning of the brain due to impact or blows to the head. Every year, millions of people enter the ED due to head injuries, and 1.5 million of them die [1]. In Sanglah General Hospital in Denpasar, the incidence of annual head injuries averages over 2000 cases, of which 30% are moderate and severe traumatic brain injury patients [2]. Many deaths that occur before the patient arrives at the hospital or during the period of treatment [3]. However, continued inflammatory processes in secondary traumatic brain injury [4] cause molecular cell damage, changes in metabolism and cerebral blood flow, axonal disruption, and apoptosis which contribute to long-term mortality [5]. Predictors of traumatic brain injury mortality such as low Glasgow coma scores, unresponsive pupillary reactions, and hemodynamic disorders [6] are not quantitative and specific so biomarkers are needed to predict mortality in traumatic brain injury [7]. Among potential new biomarkers, S100B has high specificity for neural networks associated with mortality and a prognosis that does not benefit [8].

S100B is released after brain injury and released more as a structural factor (at high concentrations) and repair (at low concentrations), so it will be very useful in the diagnosis of traumatic brain injury [9]. The S100B ability as a predictor of traumatic brain injury mortality has been shown in studies, [10] [11] [12] some of these studies oppose these results [13]. Different results can be due to methodological differences, sample size, non-standardized tests, and different subject demographics. To further deepen the

usefulness of serum S100B levels as a predictor of traumatic brain injury mortality, this meta-analysis will present an analysis of several studies both S100B as a predictor of initial and later mortality.

This study aims to analyse the difference in mean serum S100B levels between patients who died or survived traumatic brain injury based on follow-up time.

Methods

This study presents a meta-analysis of the role of serum S100B as a predictor of mortality in traumatic brain injury. The study design follows the PRISMA guidelines for meta-analysis [13]. The steps follow the Cochrane Handbook for Systematic Review of Interventions [14] guidelines.

A comprehensive literature search was conducted by the author on March 16, 2018. Relevant studies were obtained from Medline (2008-2018) and SCOPUS (2008-2018) in the last 10 years. References from all studies are further reviewed to identify additional relevant research. The author uses a search strategy with (((((((((S100B) OR S100) OR S-100) OR S-100B) OR S100β) OR S-100β) AND ((MORTALITY) OR OUTCOME) AND (BRAIN INJURY)) AND "last 10 years" [PDat]).

Duplicate journals are managed using EndNote. The title and abstract of the search results are reviewed, and the full text is analysed for inclusion in this meta-analysis. All articles are assessed using the inclusion and exclusion criteria determined by the author. The articles are included if they contain original data from the group, clinical trials, case series, patients undergoing serum S100B levels with both short and long-term follow-up mortality. Only English language journals and full text are included in this study. Data extracted from the study included using data extraction forms. The scope of data collection is about the place of study, method, number of mortality, serum S100B levels, and the diagnostic strength of serum S100B levels. The primary parameter of this study was the difference in S100B levels in patients who died and living head injuries. Secondary parameters are the diagnostic strength of serum S100B levels.

This meta-analysis will calculate the difference in mean serum S100B levels in subjects who died and lived head injuries with follow-up during treatment, 24 hours, 3 months, 6 months, and 1 year. Data were analysed by Review Manager 5.3 (Cochrane Collaboration, Copenhagen, Denmark) and the significance limit was 0.05.

Results

The study results yielded 9 studies to be analysed [10] [12] [13] [16] [17] [18] [19] [20] [21] (Table 1) which can be included in the study. The article searching process was carried out based on the PRISMA principle (Figure 1). In this meta-analysis, we will assess the difference in S100B levels in survived and dead subjects with traumatic brain injury. Also, the diagnostic strength of serum S100B levels will also be explained.

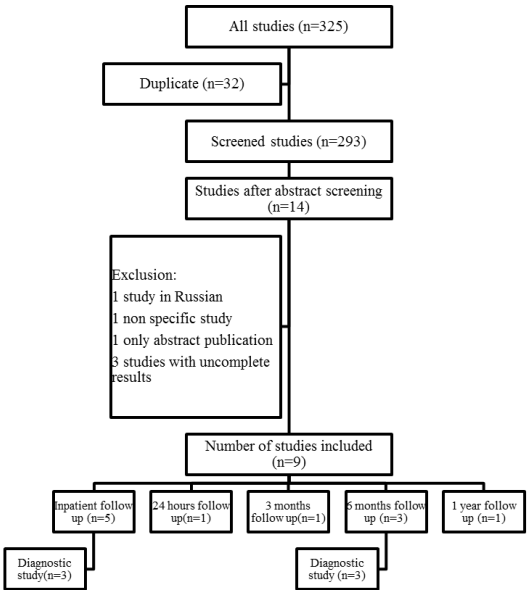

Figure 1: Flowchart PRISMA showed journal method of selection

Differences in serum S100B levels between survived and dead subjects based on follow-up time.

Overall, from the meta-analysis of the 11 studies, the mean difference calculated from random effects showed significant results ($p < 0.001$).

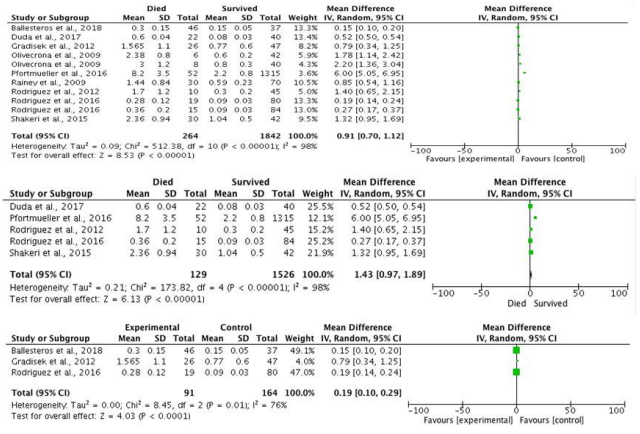

Figure 2: Forest plot difference of S100B serum levels in survived and died subjects based on follow up the timeline (a) overall, (b) within treatment, (c) 6 months

The total mean difference of serum S100B level before and after traumatic brain injury was significant statistically (0.91; 95%CI 0.7-1.12, I^2 = 98%, p < 0.001; Figure 2a). If classified based on the length of follow-up time until, during treatment, there were 5 studies reviewed. From the meta-analysis of the five studies, the random effects mean difference showed significant results (p < 0.001). The total mean difference was 1.43 (95% CI 0.97-1.89, I^2 = 98%, p < 0.001; Figure 2b). For up to 5 months of follow-up, from the three studies, the random effects mean difference also showed significant results (p < 0.001). The total mean difference was 0.19 (95% CI 0.1-0.29, I^2 = 76%, p < 0.001; Figure 2c). In the study with a follow-up period of 24 hours, 3 months, and 1 year, there was only 1 study in each study (Table 1).

Table 1: Studies included in this meta-analysis based on follow up time

Study, year	Country	Methods	Time of follow up	Mortality	Mean value of S100B
During hospital stay					
Rodriguez et al., 2012[16]	Finland	ECLIA	Inpatient	10/55	S: 0.3 ± 0.2 ug/L D: 1.7 ± 1.2 ug/L C: 0,461 ug/L AUC: 0,958 Sens: 90% Spe: 88,4%
Shakeri et al., 2015[13]	Iran	ELISA	Inpatient	30/72	S: 1.04 ± 0.5 ug/mL D: 2.36 ± 0.94 ug/mL (p<0.001)
Pfortmueller et al., 2016[10]	Austria	ELISA	Inpatient	52/1367	D: 8.2 ± 3.5 ug/L S: 2.2 ± 0.8 ug/L (p < 0.001) C: 2 ug/L
Rodriguez et al., 2016[17]	Finland	ECLIA	Inpatient	15/99	S: 0.09 ± 0.03 ug/L D: 0.36 ± 0.2 ug/L C: 0.2 ug/L (p = 0.003) AUC: 0.848 Sens: 86,7% Spe: 75%
Duda et al., 2017[18]	Polandia	ELISA	Inpatient	22/62	S: 0.08 ± 0.03 ug/L D: 0.6 ± 0.04 ug/L C: 0.12 ug/L Sens: 50% Spe: 90% 11/22 showed S100B higher than 0.12 ug/L
24 Hours					
Rainey et al., 2009[12]	UK	ELISA	24 hours	30/100	S: 0.59 ± 0.23 ug/L D: 1.44 ± 0.84 ug/L C: 0.53 ug/L AUC: 0.69 Sens: 82% Spe: 60%
3 Months					
Olivecrona et al., 2009[19]	Swedia	LIA	3 months	6/48	S: 0,6 ± 0,2 ug/L D: 2,38 ± 0,8 ug/L AUC: 0,687 Sens: 100% Spe: 38,1% C: 0.51 ug/L
6 Months					
Ballesteros et al., 2018[20]	Spain	ECLIA	6 months	46/83	S: 0.15 ± 0.05 ug/L D: 0.3 ± 0.15 ug/L AUC: 0.739
Rodriguez et al., 2016[17]	Finland	ECLIA	6 months	19/99	S: 0.09 ± 0.03 ug/L D: 0.28 ± 0.12 ug/L (p=0.002) C: 0.177 ug/L AUC: 0.855 Sens: 89.5% Spe: 76.2%
Gradisek et al., 2012[21]	Slovenia	ELISA	1 year	26/73	S: 0.77 ± 0.6 ug/L D: 1.565 ± 1.1 ug/L ug/L(p<0.001)
1 Year					
Olivecrona et al., 2009[19]	Swedia	LIA	1 year	8/48	S: 0.8 ± 0.3 ug/L D: 3 ± 1.2 ug/L AUC: 0.647 Sens: 87.5% Spe: 37.5% C: 0.51 ug/L

S: Survived, D: Died, AUC: Area Under Curve: Sens: Sensitivity, Spe: Specificity, C: Cut off.

The diagnostic strength of serum S100B levels in predicting early and late mortalities in subjects with a head injury

In this study, early mortality biomarkers were determined in samples which were taken during treatment or in the first 24 hours. The mean sensitivity of the four studies showed that the threshold value of S100B in the predicting short-term mortality was 0.328 ± 0.198 µg/L. The mean sensitivity was 77.18 ± 18.41%, and specificity was 78.35 ± 13.96%. Of the five studies, only three reported the results of AUC, with the mean AUC obtained from the four studies was 0.832 ± 0.134.

In this study, the final mortality predictions were taken with a follow-up period of more than 1 month. The mean sensitivity of the four studies showed that the threshold value of S100B in the prediction of short-term mortality was 0.399 ± 0.19 µg/L. The mean sensitivity was 92.33 ± 6.71%, and specificity was 50.6 ± 22.17%. Of the four studies, only three reported the results of AUC, so that the mean AUC obtained from the four studies was 0.732 ± 0.09.

From the graph plotted regarding the S100B threshold value that predicts mortality during long-term care and mortality in 3-6 months, it was shown that the median value of the graph was approximately 0.5 µg/L (Figure 3).

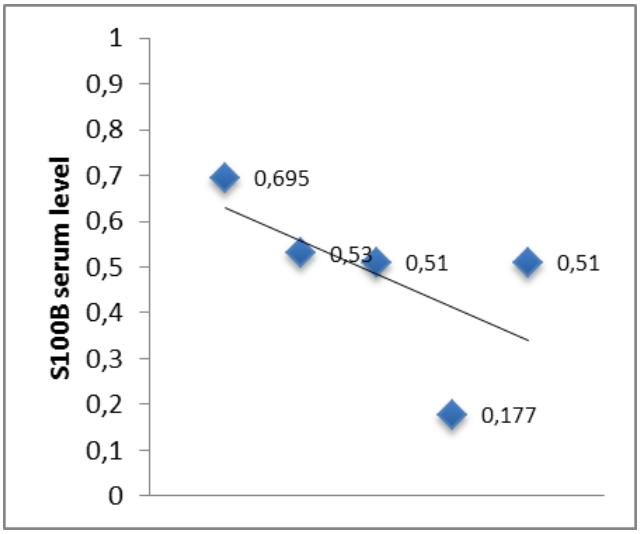

Figure 3: Cut off point of S100B serum levels which were taken during a hospital stay, 24 hours, 1 month, 3 months, 6 months, and 1 year

Discussion

Mortality rates due to TBI vary in several studies. Some studies also use different time methods and parameters to analyse this mortality. The fatality rate for TBI ranges from 13-22% [22]. After primary traumatic brain injury, secondary traumatic brain injury will continue in a few minutes, days, months, and years after primary traumatic brain injury due to excess metabolic, cellular and molecular inflammation activity [23].

S100 is a small dimeric cytosolic protein, 9-21 kDa, in the form of a homodimer with solubility in 100% ammonium sulfate liquid with a helical-loop-helical calcium bond [24]. S100B gene can be mapped on the long arm of chromosome 21q22.3. The S100B level is generally stable, not affected by storage, centrifugation, and temperature changes which will greatly facilitate sampling and reliability techniques in the analysis [25].

In TBI, S100B can be secreted into the systemic circulation along with the blood-brain barrier [26]. In the initial TBI phase, S100B is secreted as a TBI compensator with the effects of neurotrophic agents which have neuromodulating action and support the memory and thinking process [27]. In the final phase, where inflammation has been very high, and the blood-brain barrier has been disrupted, S100B acts as a destructor of neurons due to stimulation of proinflammatory cytokines and free radical activity which is often found in the pathophysiology of neurodegenerative disorders [28].

S100B as a TBI biomarker was first put forward in 1995 [29] where high S100B concentrations can be a primary and secondary TBI biomarker [30]. Researchers also found that this biomarker can be a TBI severity stratification based on GCS [31]. The ability of S100B in TBI output prediction has made some The researchers concluded that this biomarker was available as a predictor of TBI early and late diagnosis and mortality. From this meta-analysis, it was shown that high level of S100B was significantly different in the survive or died subjects with head injuries. The strength of short-term mortality predictors is also better than long-term mortality both from the significance of mean differences and diagnostic strength.

In another study, Unden in 33 patients (13%) showed that S100B could predict adverse outcomes ($p = 0.03$) but not mortality ($p = 0.182$) [32]. Shakeri et al., (2013) opposed the results that the level of S-100B could not be a predictor of mortality. The differences were due to variations in the time of collection of S100B in each study, ranging from the time of trauma to 84 hours after trauma. Research also takes heterogeneous long-term results between 1 month to several years so that the results of the study are also varied [13].

The weakness of this meta-analysis was not separating research based on varied sampling times. The optimal time for S100B sampling is still a warm discussion. Existing studies show different retrieval times [8]. In some studies, it was shown that the earlier sample was better [33]. However, some showed that the sample after 6 hours was better [34]. Some suggested 6-12 hours [3], 12 hour [35], 24 hours [36], 48 hours [37], 72 hours [11], and 84 hours [38].

The threshold value of serum S100B is still controversial. The wide range is shown by several studies. In this meta-analysis, the threshold value of S100B varies greatly depending on the method used. Although, the graph showed that the value is around 0.5 µg/L. Meta-analysis of Mercier et al., [8] on 39 studies in 1862 patients showed that serum S100B levels between 2.16-14.0 µg/L were effective for predicting adverse outcomes and mortality. Recently, a study of 3,893 patients showed a higher threshold value of 0.16-0.20 µg/L with a specificity of 51% for detecting intracranial abnormalities [11]. However, other clinicians stated that the threshold value lower than 0.1 µg/l was better for preventing false-negative cases, especially in epidural hematomas which usually indicate low S100B levels [16]. Unden et al., [32] did not find EDH subjects with S100B serum level higher than 0.14 µg/l [31]. Wolf et al., [39] have shown that the threshold value of S100B, 0.1 µg/l was found to be effective for detecting epidural hematoma at the initial presentation.

Giving the above results, it can be concluded that S100B serum level is very potential in predicting mortality due to traumatic brain injury. For further research, S100B biomarkers could be analysed in salivary and urine, which are useful as diagnostic markers. Urine and saliva samples could be obtained noninvasively so that they have the potential to be applied.

In conclusion, there is a significant difference in the mean serum S100B levels between patients who survived and died after traumatic brain injury. The diagnostic strength of serum S100B levels is promising in predicting mortality with a range of threshold values that vary according to the examination method used. S100B levels in the future will be a future potential biomarker, and it is expected that there will be a standardised guideline for its application.

References

1. Marr AL, Coronado VG. Central nervous system injury surveillance data submission standards—2002. Atlanta, GA: Centers for Disease Control and Prevention, National Center for Injury Prevention and Control, 2004.

2. Medical records derived from Sanglah Hospital. Sanglah Hospital, 2011.

3. Pentland B, Hutton LS, Jones PA. Late mortality after traumatic brain injury. J Neurol Neurosurg Psychiatry. 2005; 76:395-400. https://doi.org/10.1136/jnnp.2004.037861 PMid:15716535 PMCid:PMC1739527

4. Bloomfield SM, McKinney J, Smith L, et al. Reliability of S100B in predicting the severity of central nervous system injury. Neurocrit Care. 2007; 6:121-38. https://doi.org/10.1007/s12028-007-0008-x PMid:17522796

5. Lafrenaye AD, Krahe TE, Povlishock JT. Moderately elevated intracranial pressure after diffuse traumatic brain injury is associated with exacerbated neuronal pathology and behavioural morbidity in the rat. J Cereb Blood Flow Metab. 2014; 34(10):1628-36. https://doi.org/10.1038/jcbfm.2014.122 PMid:25027309

PMCid:PMC4269720

6. Van Beek JG, Mushkudiani NA, Steyerberg EW, Butcher I, McHugh GS, Lu J, Marmarou A, Murray GD, Maas AI. Prognostic value of admission laboratory parameters in traumatic brain injury: results from the IMPACT study. Journal of Neurotrauma. 2007; 24(2):315-28. https://doi.org/10.1089/neu.2006.0034 PMid:17375996

7. Adrian H, Marten K, Salla N, Lasse V. Biomarkers of traumatic brain injury: temporal changes in body fluids. eNeuro. 2016; 3(6):294-9. https://doi.org/10.1523/ENEURO.0294-16.2016 PMid:28032118 PMCid:PMC5175263

8. Mercier E, Boutin A, Lauzier F, Fergusson DA, Simard JF, Zarychanski R, et al. Predictive value of S-100beta protein for prognosis in patients with moderate and severe traumatic brain injury: systematic review and meta-analysis. BMJ. 2013; 346:1757-9. https://doi.org/10.1136/bmj.f1757 PMid:23558282

9. Donato R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol. 2001; 33:637-68. https://doi.org/10.1016/S1357-2725(01)00046-2

10. Pfortmueller CA, Drexel C, Krahenmann-Muller S, Leichtle AB, Fiedler GM, Lindner G, et al. S-100 B Concentrations Are a Predictor of Decreased Survival in Patients with Major Trauma, Independently of Traumatic brain injury. PloS One. 2016; 11(3):1-5. https://doi.org/10.1371/journal.pone.0152822 PMid:27031106 PMCid:PMC4816449

11. Murillo-Cabezas F, Mu-oz-Sánchez MA, Rincón-Ferrari MD, et al. The prognostic alue of the temporal course of S100B eta protein in the post-acute severe brain injury: a prospective and observational study. Brain Inj. 2010; 24:609-19. https://doi.org/10.3109/02699051003652823 PMid:20235763

12. Rainey T, Lesko M, Sacho R, Lecky F, Childs C. Predicting outcome after severe traumatic brain injury using the serum S100B biomarker: results using a single (24 h) time-point. Resuscitation. 2009; 80:341-5. https://doi.org/10.1016/j.resuscitation.2008.11.021 PMid:19150161

13. Shakeri M, Mahdkhah A, Panahi F. S100B Protein as a Post-traumatic Biomarker for Prediction of Brain Death in Association With Patient Outcomes. Arch Trauma Res. 2013; 2:76-80. https://doi.org/10.5812/atr.8549 PMid:24396798 PMCid:PMC3876553

14. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: Explanation and elaboration. BMJ. 2009; 339:b2700.12.

15. Higgins JP, Green S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.0. 0. The Cochrane Collaboration. 2008. Available from www. cochrane-handbook. org (accessed 28 January 2010). 2008.

16. Rodríguez-Rodríguez A, Egea-Guerrero JJ, Gordillo-Escobar E, Enamorado-Enamorado J, Hernández-García C, Ruiz de Azúa-López Z, Vilches-Arenas Á, Guerrero JM, Murillo-Cabezas F. S100B and Neuron-Specific Enolase as mortality predictors in patients with severe traumatic brain injury. Neurol Res. 2016; 38(2):130-7. https://doi.org/10.1080/01616412.2016.1144410 PMid:27078699

17. Rodríguez-Rodríguez A, Egea-Guerrero JJ, León-Justel A, Gordillo-Escobar E, Revuelto-Rey J, Vilches-Arenas A, Carrillo-Vico A, Domínguez-Roldán JM, Murillo-Cabezas F, Guerrero JM. Role of S100B protein in urine and serum as an early predictor of mortality after severe traumatic brain injury in adults. Clin Chim Acta. 2012; 414:228-33. https://doi.org/10.1016/j.cca.2012.09.025 PMid:23031665

18. Duda I, Krzych L, Jędrzejowska-Szypułka H, Lewin-Kowalik JL. Serum levels of the S100B protein and neuron-specific enolase are associated with mortality in critically ill patients. Biochimica Polonica. 2017; 64:16-9. https://doi.org/10.18388/abp.2017_1619

19. Olivecrona M, Rodling-Wahlström M, Naredi S, Koskinen LO. S-100B and neuron specific enolase are poor outcome predictors in severe traumatic brain injury treated by an intracranial pressure targeted therapy. J Neurol Neurosurg Psychiatry. 2009; 80(11):1241-7. https://doi.org/10.1136/jnnp.2008.158196 PMid:19602473

20. Ballesteros MA, Rubio-Lopez MI, San Martín M, Padilla A, López-Hoyos M, Llorca J, Mi-ambres E. Serum levels of S100B from jugular bulb as a biomarker of poor prognosis in patients with severe acute brain injury. J Neurol Sci. 2018; 385:109-14. https://doi.org/10.1016/j.jns.2017.12.017 PMid:29406887

21. Gradisek P, Osredkar J, Korsic M, Kremzar B. Multiple indicators model of long-term mortality in traumatic brain injury. Brain Inj. 2012; 26(12):1472-81. https://doi.org/10.3109/02699052.2012.694567 PMid:22721420

22. Gerber LM, Chiu YL, Carney N, Hartl R, Ghajar J. Marked reduction in mortality in patients with severe traumatic brain injury. J Neurosurg. 2013; 119:1583-90. https://doi.org/10.3171/2013.8.JNS13276 PMid:24098983

23. Korfias S, Stranjalis G, Boviatsis E, et al. Serum S-100B protein monitoring in patients with severe traumatic brain injury. Intensive Care Med. 2007; 33:255-60. https://doi.org/10.1007/s00134-006-0463-4 PMid:17143637

24. Raabe A, Grolms C, Sorge O, Zimmermann M, Seifert V. Serum S-100B protein in severe traumatic brain injury. Neurosurgery. 1999; 45(3):477-83. https://doi.org/10.1097/00006123-199909000-00012 PMid:10493369

25. Goyal A, Failla MD, Niyonkuru C, Amin K, Fabio A, Berger RP, Wagner AK. S100b as a Prognostic Biomarker in Outcome Prediction for Patients with Severe Traumatic Brain Injury. J Neurotrauma. 2013; 30(11):946-57. https://doi.org/10.1089/neu.2012.2579 PMid:23190274 PMCid:PMC3684103

26. Anderson RE, Hansson LO, Liska J, Settergren G, Vaage J. The effect of cardiotomy suction on the brain injury marker S100B after cardiopulmonary by-pass. Ann Thorac Surg. 2000; 69:847–50. https://doi.org/10.1016/S0003-4975(99)01526-X

27. Nishiyama H, Knopfel T, Endo S, Itohara S. Glial protein S100B modulates long-term neuronal synaptic plasticity. Proc Natl Acad Sci USA. 2002; 99:4037-42. https://doi.org/10.1073/pnas.052020999 PMid:11891290 PMCid:PMC122644

28. Herrmann M, Jost S, Kutz S, Ebert AD, Kratz T, Wunderlich MT, Synowitz H. Temporal profile of release of neurobiochemical markers of brain damage after traumatic brain injury is associated with intracranial pathology as demonstrated in cranial computerized tomography. J Neurotrauma. 2000; 17:113-22. https://doi.org/10.1089/neu.2000.17.113 PMid:10709869

29. Ingebrigtsen T, Romner B, Kongstad P, Langbakk B. Increased serum concentrations of protein S-100 after minor traumatic brain injury: a biochemical serum marker with prognostic value? J Neurol Neurosurg Psychiatry. 1995; 59:103-4. https://doi.org/10.1136/jnnp.59.1.103-a PMid:7608699 PMCid:PMC1073618

30. de Kruijk JR, Leffers JR, Menheere PP, Meerhoff S, Twijnstra A. S100B and neuron-specific enolase in serum of mild traumatic brain injury patients. A comparison with healthy controls. Acta Neurol Scand. 2001; 103:175-9. https://doi.org/10.1034/j.1600-0404.2001.103003175.x PMid:11240565

31. Kellermann I, Kleindienst A, Hore N, Buchfelder M, Brandner S. Early CSF and Serum S100B Concentrations for Outcome Prediction in Traumatic Brain Injury and Subarachnoid Hemorrhage. Clin Neurol Neurosurg. 2016; 145:79-83. https://doi.org/10.1016/j.clineuro.2016.04.005 PMid:27101088

32. Unden J, Bellner J, Astrand R, Romner B. Serum S100B levels in patients with epidural haematomas. Br J Neurosurg. 2005; 19: 43–45. https://doi.org/10.1080/02688690500089381 PMid:16147582

33. Schültke E, Sadanand V, Kelly ME, Griebel RW, Juurlink BHJ. Can admission S-100B predict the extent of brain damage in head trauma patients? Can J Neurol Sci. 2009; 36:612-6.

https://doi.org/10.1017/S031716710000812X PMid:19831131

34. Woertgen C, Rothoerl RD, Brawanski A. Early S-100B serum level correlates to quality of life in patients after severe traumatic brain injury. Brain Inj. 2002; 16:807-16. https://doi.org/10.1080/02699050210128933 PMid:12217206

35. Muller K, Townend W, Biasca N, Unden J, Waterloo K, Romner B, Ingebrigtsen T. S100B serum level predicts computed tomography findings after minor traumatic brain injury. J Trauma. 2007; 62:1452-56. https://doi.org/10.1097/TA.0b013e318047bfaa PMid:17563665

36. Murray GD, Butcher I, McHugh GS, Lu J, Mushkudiani NA, Maas AI, Marmarou A, Steyerberg EW. Multivariable prognostic analysis in traumatic brain injury: results from the IMPACT study. J Neurotrauma. 2007; 24:329-37. https://doi.org/10.1089/neu.2006.0035 PMid:17375997

37. Thelin EP, Jeppsson E, Frostell A, Svensson M, Mondello S, Bellander BM, Nelson DW. Utility of neuron-specific enolase in traumatic brain injury; relations to S100B levels, outcome, and extracranial injury severity. Crit Care. 2016; 20:285. https://doi.org/10.1186/s13054-016-1450-y PMid:27604350 PMCid:PMC5015335

38. Pelinka LE, Toegel E, Mauritz W, Redl H. Serum S 100 B: a marker of brain damage in traumatic brain injury with and without multiple trauma. Shock. 2003; 19:195–200. https://doi.org/10.1097/00024382-200303000-00001 PMid:12630517

39. Wolf H, Frantal S, Pajenda G, Leitgeb J, Sarahrudi K, Hajdu S. Analysis of S100 calcium binding protein B serum levels in different types of traumatic intracranial lesions. J Neurotrauma. 2015; 32:23–27. https://doi.org/10.1089/neu.2013.3202 PMid:25068442

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Review Article

Artificial Reproductive Technology – A Risk Factor for Retinopathy of Prematurity

Kalina Trifonova[1], Kiril Slaveykov[2*], Hristo Mumdzhiev[2], Dimitar Dzhelebov[2]

[1]Trakijski Universitet, Meditsinski Fakultet, Ophthalmology, Stara Zagora, Bulgaria; [2]Trakijski Universitet, Meditsinski Fakultet, Stara Zagora, Bulgaria

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***Correspondence:** Kiril Slaveykov. Trakijski Universitet, Meditsinski Fakultet, Stara Zagora, Bulgaria. E-mail: kirilslaveykov@gmail.com

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Abstract

BACKGROUND: Retinopathy of Prematurity (ROP) is a potentially blinding vasoproliferative disease in premature babies. The presentation and course of ROP are determined by a complex interaction of a series of risk factors, including artificial reproductive technology (ART).

AIM: To analyse and combine the information relating ART as an independent risk factor for retinopathy of prematurity.

METHODS AND MATERIAL: The article is systematic review and meta-analysis using RevMan 5. Pubmed, Scopus and Medline were searched for articles from 1990 to 2018.

RESULTS: Studies suggest that ROP is observed more frequently in ART children. They are more likely to be premature and of low birth weight than those conceived naturally. Results vary from just a tendency to a five-fold increase in risk to develop ROP in ART babies. At the same time, they might develop ROP later, and more mature newborns might be affected.

CONCLUSION: The data relating ART as a risk factor for ROP is inconclusive, but most studies show at least a tendency. The ART newborns need to be considered as a risk group for ROP and observed with greater suspicion. Even more mature ART newborns might need to be screened in order not to miss any significant pathology.

Introduction

Retinopathy of Prematurity (ROP) is a potentially blinding vasoproliferative disease in premature babies. Recent advances in neonatal care have improved the survival rates for premature infants, and this has been accompanied by an increase in the incidence of ROP [1] [2]**.** If it remains unrecognised and untreated, it can cause severe visual impairment and blindness in children. Nowadays ROP can be prevented by timely screening [3].

The presentation and course of ROP are determined by a complex interaction of several risk factors like gestational age, birth weight, as well as systemic risk factors like anaemia, sepsis, jaundice and multiple blood transfusions [4]. Other significant risk factors for developing ROP are: artificial ventilation (for more than 5 days), respiratory distress syndrome, intraventricular hemorrhage and periventricular leukomalacia, congenital heart disease, sepsis [5]**,** low Apgar score in the first and fifth minutes, longer duration of oxygen therapy [3]; intrauterine hypotrophy, bronchopulmonary dysplasia [6]**.** Poor postnatal growth in the first weeks of life [7] [8] and early postnatal hyperglycemia [9] [10] [11] are also associated with later development of ROP, while phototherapy used to treat hyperbilirubinemia is considered a protective factor [6].

This study aims to analyse and combine the information relating ART as an independent risk factor for ROP.

Methods and Materials

Systematic review and meta-analysis (using RevMan 5) were performed. Online databases: Pubmed, Scopus and Medline were searched for articles on this topic from 1990 to 2018.

Results and Discussion

Some authors report they raised the frequency of ocular abnormalities in children born after in vitro fertilisation (IVF). In the study of Anteby major ocular malformations were observed in 12 (26%) of 47 children. These malformations included Coats disease, congenital cataract, congenital glaucoma, hypoplastic optic nerve head, idiopathic optic atrophy, coloboma with microphthalmos, and retinoblastoma [12]. A probability of fixation condition and visual deficiencies in these infants was also suggested [13]**.** These adverse outcomes of assisted conception are because the gametes are exposed to a variety of drugs, physically manipulated, nurtured in potentially hazardous conditions, and perhaps placed in an inappropriate uterine environment [1]. However, according to another study which included thirty-six IVF infants with ocular malformations, the risk, compared with non-IVF children, was not increased when adjusted for maternal age, parity, smoking, and body mass index (odds ratio, 1.05; 95% confidence interval, 0.75 to 1.47) [14]**.**

ART multiple birth babies make up a considerable proportion of the ROP screening burden, and their number is likely to increase as ART is increasingly available and utilised [15]. McKibbin et al. were one of the first to investigate the workload imposed by the treatment for infertility on a ROP screening programme. They reviewed the records of all babies born between August 1991 and December 1994 in the Assisted Conception Unit and of all babies screened for ROP over the same period. Of the babies born after ART, 20% fulfilled the ROP screening criteria. ROP of any stage was present in 23% of all the assisted conception babies screened [16]. As a follow up of McKibbin et al., C. Funnell and T. Dabbs made retrospective study utilising computerised databases of ROP screening, live births, assisted conception (AC) and multiple births between April 1st 2000 and August 31st 2003 at St James's Hospital, Leeds. They concluded that the percentage of AC babies requiring ROP screening had fallen since McKibbin et al., This appeared to be at least partially due to the reduced multiple birth rates. This reduction in the multiple birth rates follows evidence that reducing the number of embryos transferred does not reduce the number of couples taking home a baby. Human Fertility and Embryology Association guidelines recommend transferring no more than two embryos in an IVF cycle. Changes in clinical practice at St James's Hospital have significantly reduced the likelihood of AC babies requiring ROP screening and developing ROP [17]. However, even singleton births resulting from AC are more likely to be premature and of low birth weight than those conceived naturally [18]. This higher rate may be attributed to various infertility cofactors, such as uterine malformations, previous operative procedures that involved cervical dilatation, and a history of pelvic infection [19].

In twin births, both babies have the same gestational age and pre-natal conditions. However, twins may develop a varied ROP course depending on birth weight and other systemic factors. The profile of 56 pairs of twins with ROP was studied and analysed for differences in zone or need for treatment while studying possible causes for the varied outcome. In 45 pairs of twins (80%) the disease progressed identically in both eyes, while in 11 pairs (20%) the ROP showed differences in zone or need for treatment. Four of these pairs were discordant. In 3 of these 4 pairs, the heavier birth weight twin had a more severe ROP course. Twins can present with asymmetric ROP course, and it is, therefore, essential to examine both twins as per screening protocols [20]. Discordant twins (discordancy is defined as a difference of 15% or more in the birth weights of the twins) may be at increased risk for ROP because of factors related to their unequal growth. 38% (10) of the lower birthweight infants had higher grades of ROP than their bigger twin [10]. Ninety-nine infants from multiple gestation births and weighing ≤ 1500 g at birth were matched with infants from single births to clarify the relationship of multiple gestations to ROP. There was no significant difference in the incidence of ROP between the twins and the singletons (relative risk [RR] = 0.84, 95% confidence intervals [CI] = 0.61, 1.16). Logistic regression analysis confirmed that very low birth weight (VLBW), despite being single or multiple gestations, was the most significant predictor of ROP occurrence in either group. These results indicate that ROP screening in VLBW twins may be conducted according to the same standard protocols as for singletons [21]. Two other studies also showed no significant difference in stage of ROP between infants of single-gestation pregnancies vs those of multiple-gestation pregnancies [22] [6]. Surprisingly, V. Chernodrinska et al. even reported a lower risk for development of ROP in infants from multiple than from single birth [23].

There is some debate regarding whether ART constitutes an independent risk factor for ROP. Studies have reported conflicting results regarding this relationship. Some of them failed to demonstrate any association between the two. Friling et al. examined routinely a study group consisting of 363 infants with a birth weight (B,W) of ≤ 1500 gm, who were hospitalised in the neonatal unit of a single tertiary-

care centre between 1998 and 2000. Data on gestational age (GA), ,BW, type of pregnancy (singleton/multiple), and type of conception (natural/assisted) were recorded, in addition to the ophthalmological results. In their sample, AC per se did not appear to be a risk factor for ROP. Singleton babies with a birth weight of ≤ 1500 g were more prone to develop ROP stages II and III than twins or triplets. GA and BW were the most significant factors associated with ROP [21]. An article of a Turkish team was published in 2016 analysing the medical records of consecutive premature triplets who had been screened for ROP in a single maternity hospital. The presence of ROP was not associated with the mode of conception (p = 0.674) [24].

Some studies show only a tendency of ART being an independent risk factor for ROP, without proving statistically significant results, so their data do not rule out a possible association. One of the first teams to study these problems [16] reviewed the records of all babies born between August 1991 and December 1994 as a result of treatment in the Assisted Conception Unit, and all babies screened for ROP over the same period. Of the babies born after AC treatment, 20% fulfilled the ROP screening criteria. ROP of any stage was present in 23% of all the assisted conception babies screened. This group also accounted for a large proportion of those reaching stage 3 disease and of those requiring treatment [16]. Watts and Adams found an association between the development of threshold severe ROP and ART (specifically IVF). They carried out a retrospective study between Dec. 1995 and Dec. 1998 of infants in a single neonatal unit serving the Brent and Harrow area of North West Thames, requiring screening and treatment of ROP. In this study, 11.7% of the group requiring screening was conceived by AC. Of all babies requiring treatment for ROP, 28.6% were born after AC. Of the AC group, 83.3% were conceived by IVF. AC using IVF rather than other techniques appeared to be the major risk factor for the development of threshold ROP. The authors advise increased vigilance when screening babies conceived by the IVF methods of AC [25]. Barker et al. performed a retrospective audit of all multiple birth babies admitted to a tertiary neonatal unit, who met the UK ROP screening criteria. A total of 205 babies met the criteria, of whom 87.3% were twins. They found no significant difference between the numbers of babies developing ROP in the ART vs non-ART groups. However, the estimated odds of developing ROP were slightly higher in the ART babies [15].

The first team to demonstrate a statistically significant association between ART and severe ROP requiring treatment in infants in the US performed their studies in Weill Cornell Medical Center Neonatal Intensive Care Unit at the New York-Presbyterian Hospital from 2002 to 2008. According to their studies AC placed infants at greater risk for treatment-requiring ROP [OR 4.5; 95% CI 1.3-15.5; p = 0.0150] [26]. Using multifactor analysis, the key finding was that, regardless of birth weight, ART was associated with a nearly five-fold increased risk of severe ROP requiring treatment, after controlling for potential confounders (OR 4.70, CI 1.52–14.57; P = 0.007). Gestational age was also a significant risk factor, and both gestational age and ART appear to be independent risk factors associated with risk of severe ROP requiring laser [27]. A few years later Yau et al., using univariate analysis showed IVF was significant independent risk factors for Type 1 ROP. A retrospective review of medical records was performed for all neonates of multiple gestations (n = 153) screened for ROP between January 2007 and December 2012 in 2 neonatal intensive care units in Hong Kong [28]. The same team a year later published an article which included all infants (n = 513) that were screened from this same period. It did not show IVF as an independent risk factor for ROP but it still showed a tendency/ odd ratio = 2.07/CI = 0.47- 6.54**/** [29].

Minasion and Fielder reported that severe ROP could affect larger and more mature babies conceived through IVF, compared with those who are not. They presented three babies conceived IVF who were close to the limits of the current screening criteria [30]. Chan et al., also observed that although the mean gestational age at birth was similar between the ART and the natural conception groups, the mean time of treatment for severe ROP was approximately one week later in the ART group (36 2/7 weeks) [27]. Watts and Adams also noted that for infants developing Stage 3 severe ROP, those conceived through IVF were born at larger gestational ages on average and with heavier birth weights than infants who received other forms of ART [25]. These differences in weight and gestational age between babies requiring treatment for severe ROP in the ART and natural conception groups may also have implications for screening criteria.

We included all the retrospective studies with newborns screened for ROP investigating the association between ART and ROP as an independent risk factor (Table 1).

Table 1: ART as an independent risk factor for ROP

Author/year	Country	Follow-up	Sample size	Natural conception	ART	Odds ratio	Significance
McKibbin, 1996	England	3 years	267	233	44	-	p>0,05 only tendency
Watts, 2000	England	3 years	179	152	27	-	p>0.05 only tendency ROP 3
Friling, 2007	Israel	2 years	363	204	159	-	P>0,05, no significance
Chan P.,2010	USA	5 years	399	253	146	4.70; [CI], 1.52–4.57	0,007 ROP 3
Şekeroğlu M., 2016	Turkey	2 years	54	18	36	-	0,674
Barker L, 2017	USA	5 years	205	125	80	-	0,837
Yau GS, 2016	China	5 years	513	---	----	2.07/CI= 0.47-6.54	0,3 ROP 1

Of all the included studies only four had sufficient data and homogeneity and were included in

the meta-analysis. We used random effect forest plot to pool the results of those studies answering if ART is an independent risk factor for ROP stage 3.

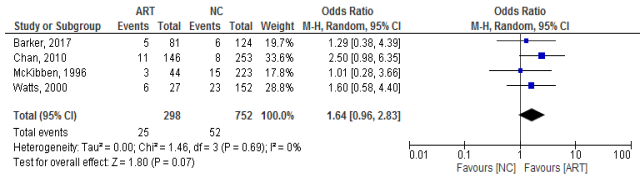

Figure 1: Odds Ratios

The results show that the studies with the biggest weight are Chan, 2010 and Wastts, 2000. All the studies are homogenous (heterogeneity Chi2 = 1.46, I = 0%). There is a positive association between ART and ROP stage 3 – Odds ratio = 1.64/0.96-2.83/ even though the test for overall effect does not show statistical significance /p = 0.07/ (Figure 1). The results of the risk ratio are also positive 1.56 [0.96, 2.52] (Figure 2).

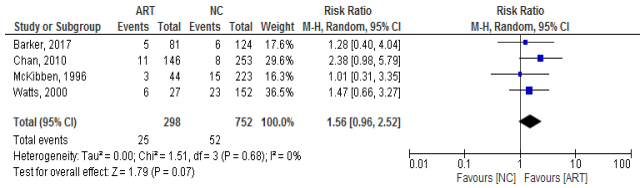

Figure 2: Risk Ratio

Conclusion

ART babies represent a large percentage of babies screened for ROP, and their number is likely to increase. Research on ART as a risk factor for ROP is present, though the subject became a popular study topic in recent years. Although the data relating artificial reproduction as an independent risk factor ROP is inconclusive, it still raises several questions which need further examination:

- Multiple gestations are rejected as an independent risk factor, though it indirectly leads to earlier birth and lower weight. Another thing to consider is discordancy between twins, which increases the risk of ROP.

- Even though results from the literature review are inconsistent, meta-analysis shows at least an increased tendency to develop ROP stage 3. Combined with the fact that ROP in ART infants develops later and affects heavier newborns, a change of screening criteria might be required in the future.

Further studies on this topic are needed, to fully recognise the potential differences in the ocular development of ART babies compared to naturally conceived ones.

References

1. Gibson DL, Sheps SB, Uh SH, Schechter MT, McCormick AQ. Retinopathy of prematurity-induced blindness: birth weight-specific survival and the new epidemic. Pediatrics. 1990; 6:405-12.

2. Valentine PH, Jackson JC, Kalina RE, Woodrum DE. Increased survival of low birth weight infants: impact on the incidence of retinopathy of prematurity. Pediatrics. 1989; 84(3):442-5. PMid:2788864

3. Alajbegovic-Halimic J, Zvizdic D, Alimanovic-Halilovic E, Dodik I, Duvnjak S. Risk factors for retinopathy of prematurity in prematurely born children. Medical Archives. 2015; 69(6):409. https://doi.org/10.5455/medarh.2015.69.409-413 PMid:26843736 PMCid:PMC4720470

4. Dutta S, Narang S, Narang A, Dogra M, Gupta A, Risk factors of threshold retinopathy of prematurity. Indian Pediatr. 2004; 41(7):665-71. PMid:15297681

5. Yang CS, Chen SJ, Lee FL, Hsu WM, Liu JH. Retinopathy of Prematurity: Screening, Incidence and Risk Factors Analysis, 201.

6. Mladenov O, Chernodrinska V, Petkova I, Dimitrova G, Kemilev P, et al. Retinopathy of Prematurity – Incidence and Risk Factors in Bulgaria. International Journal of Pharmaceutical Science Invention. 2017; 6(7):18-23.

7. Hellstrom A, Hard AL, Engstrom E, Niklasson A, Andersson E, Smith L, et al. Early weight gain predicts retinopathy in preterm infants: new, simple, efficient approach to screening. Pediatrics. 2009; 123:e638–45. https://doi.org/10.1542/peds.2008-2697 PMid:19289449

8. Lofqvist C, Andersson E, Sigurdsson J, Engstrom E, Hard AL, Niklasson A, et al. Longitudinal postnatal weight and insulin-like growth factor I measurements in the prediction of retinopathy of prematurity. Arch Ophthalmol. 2006; 124:1711–8. https://doi.org/10.1001/archopht.124.12.1711 PMid:17159030

9. Blanco CL, Baillargeon JG, Morrison RL, Gong AK. Hyperglycemia in extremely low birth weight infants in a predominantly Hispanic population and related morbidities. J Perinatol. 2006; 26:737–41. https://doi.org/10.1038/sj.jp.7211594 PMid:16929343

10. Fellows RR1, McGregor ML, Bremer DL, Rogers GL, Miller D., Retinopathy of prematurity in discordant twins. J Pediatr Ophthalmol Strabismus. 1995; 32(2):86-8. PMid:7629675

11. Garg R, Agthe AG, Donohue PK, Lehmann CU. Hyperglycemia and retinopathy of prematurity in very low birth weight infants. J Perinatol. 2003; 23:186–94. https://doi.org/10.1038/sj.jp.7210879 PMid:12732854

12. Anteby I, Cohen E, Anteby E, Ben Ezra D. Ocular manifestations in children born after in vitro fertilisation, Arch Ophthalmol. 2001; 119(10):1525-9. https://doi.org/10.1001/archopht.119.10.1525 PMid:11594955

13. Jafarzadehpur E, Kermani RM, Mohhamadi AR, Nateghi MR, Fazeli AS, Kashi KM. Ocular Manifestations in Infants Resulted from Assisted Reproductive Technology (ART). Journal of family & reproductive health. 2013; 7(4):181. PMid:24971123 PMCid:PMC4064753

14. Tornqvist K, Finnström O, Källén B, Lindam A, Nilsson E, Nygren KG, Olausson PO. Ocular malformations or poor visual acuity in children born after in vitro fertilisation in Sweden. American journal of ophthalmology. 2010; 150(1):23-6. https://doi.org/10.1016/j.ajo.2010.01.035 PMid:20447615

15. Barker L, Bunce C, Husain S, Adams GG.,Is artificial reproductive technology a risk factor for retinopathy of prematurity

independent of the generation of multiple births? Eur J Ophthalmol. 2017; 27(2):174-178. https://doi.org/10.5301/ejo.5000832 PMid:27445066

16. McKibbin M, Dabbs TR. Assisted conception and retinopathy of prematurity. Eye. 1996; 10(4):476. https://doi.org/10.1038/eye.1996.105 PMid:8944102

17. Funnell CL, Dabbs TR. Assisted conception and retinopathy of prematurity: 8-year follow-up study. Eye. 2007; 21(3):383. https://doi.org/10.1038/sj.eye.6702215 PMid:16410811

18. McFaul PB, Patel N, Mills J, An audit of the obstetric outcome of 148 consecutive pregnancies from assisted conception: implications for neonatal services, Br J Obstet Gynaecol. 1993; 100(9):820-5. https://doi.org/10.1111/j.1471-0528.1993.tb14306.x PMid:8218001

19. Perri T, Chen R, Yoeli R, Merlob P, Orvieto R, Shalev Y, Ben-Rafael Z, Bar-Hava I. Clinical Assisted Reproduction: Are Singleton Assisted Reproductive Technology Pregnancies at Risk of Prematurity?. Journal of assisted reproduction and genetics. 2001; 18(5):245-9. https://doi.org/10.1023/A:1016614217411 PMid:11464574 PMCid:PMC3455328

20. Azad R, Chandra P, Patwardhan SD, Gupta A. Profile of asymmetrical retinopathy of prematurity in twins. Indian journal of ophthalmology. 2010; 58(3):209. https://doi.org/10.4103/0301-4738.62645 PMid:20413923 PMCid:PMC2886251

21. Friling R, Axer-Siegel R, Hersocovici Z, Weinberger D, Sirota L, Snir M. Retinopathy of prematurity in assisted versus natural conception and singleton versus multiple births. Ophthalmology. 2007; 114(2):321-4. https://doi.org/10.1016/j.ophtha.2006.11.010 PMid:17270680

22. Blumenfeld LC, Siatkowski RM, Johnson RA, Feuer WJ, Flynn JT. Retinopathy of prematurity in multiple-gestation pregnancies. American journal of ophthalmology. 1998; 125(2):197-203. https://doi.org/10.1016/S0002-9394(99)80092-0

23. Chernodrinska V, Oscar A, Cherninkova S. RetCam screening of prematurely born children and evaluation of the risk factors for the development of retinopathy. Pediatriya. 2011; 51(4):21-23.

24. Şekeroğlu MA, Hekimoğlu E, Çelik Ü, Kale Y, Baş AY. Retinopathy of prematurity in triplets. Turkish journal of ophthalmology. 2016; 46(3):114. https://doi.org/10.4274/tjo.94815 PMid:27800273 PMCid:PMC5076293

25. Watts P, Adams GG. In vitro fertilisation and stage 3 retinopathy of prematurity. Eye. 2000; 14:330-3. https://doi.org/10.1038/eye.2000.82 PMid:11026994

26. Wong R, Yonekawa Y, Sun G, DeAngelis MM, Morrison M, et al. Update: Assisted Conception and Progression of Retinopathy of Prematurity, Investigative Ophthalmology & Visual Science. 2009; 50:3143.

27. Chan P, Yonekawa Y, Morrison M, Sun G, Wong R, et al. Association between assisted reproductive technology and advanced retinopathy of prematurity. Clin Ophthalmol. 2010; 4:1385–1390. PMid:21179223 PMCid:PMC2999553

28. Yau GS, Lee JW, Tam VT, Yip S, Cheng E, Liu CC, Chu BC, Wong IY. Incidence and risk factors for retinopathy of prematurity in multiple gestations: a Chinese population study. Medicine. 2015; 94(18). https://doi.org/10.1097/MD.0000000000000867

29. Yau GS, Lee JW, Tam VT, Liu CC, Yip S, Cheng E, Chu BC, Yuen CY. Incidence and risk factors of retinopathy of prematurity from 2 neonatal intensive care units in a Hong Kong Chinese population. The Asia-Pacific Journal of Ophthalmology. 2016; 5(3):185-91. https://doi.org/10.1097/APO.0000000000000167 PMid:27183289

30. Minasian M, Fielder A, IVF babies with ROP at higher gestational age and birth weight: implications of changing screening criteria, Br J Ophthalmol. 2005; 89(8):1066. https://doi.org/10.1136/bjo.2004.062935 PMid:16024870 PMCid:PMC1772773

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Review Article

The Relationship between Resiliency and Burnout in Iranian Nurses: A Systematic Review and Meta-Analysis

Kolsoum Deldar[1], Razieh Froutan[2*], Sahar Dalvand[3], Reza Ghanei Gheshlagh[4], Seyed Reza Mazloum[5]

[1]Faculty of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran; [2] Department of Medical-Surgical Nursing, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran; [3]Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; [4]Clinical Care Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran; [5]Department of Medical-Surgical Nursing, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran

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Keywords: Resiliency; Burnout; Nursing; Meta-analysis; Iran

***Correspondence:** Razieh Froutan. Department of Medical-Surgical Nursing, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran. E-mail: froutanr@mums.ac.ir

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Abstract

BACKGROUND: The impact of resiliency on professional burnout in nurses has been evaluated in several studies.

AIM: This meta-analysis was conducted to examine the effect of resiliency on different aspects of nurses' professional burnout.

MATERIAL AND METHODS: Publications were identified through targeted literature review in national and international databases between 1980-2017, in Persian and English. Two independent coders assessed and extracted articles. Data analysis was done by a random effects model. Study heterogeneity was measured by the I2 test. The data were analysed by STATA software v. 14.

RESULTS: Initially, 227 articles were extracted. After titles and abstract screening, 108 articles were selected for full-text review. Only five of them had the necessary inclusion criteria for analysis. The meta-analysis performed on these observational studies showed that the correlation between resiliency and burnout was -0.57 with a 95% confidence interval of -0.354 to -0.726.

CONCLUSION: Regarding the inverse relationship between resiliency and burnout, it is recommended to plan for the interventions that can improve the resilience of nurses against burnout. Conducting interventional and resilient training courses for nurses in nursing education can be considered.

Introduction

Occupation is one of the most important sources of stress. If stress persists more than usual, it can endanger the person's health by causing physical, psychological, and behavioural harms [1] [2]. Among all types of jobs, health care providers, especially nurses, are commonly known as high-risk groups for stress and burnout [3], and they experience various forms of stress and physical and mental harms [4] [5].

Depression, anxiety, and fatigue are common problems in this occupational group. In various studies, the level of stress among nurses have been reported to be 90% [6]. The Institute for Occupational Health and Safety, which studies the relationship between mental illnesses and occupational stress, reports that among the 136 studied professions, nurses ranked 22 regarding acceptance of occupational, psychological problems. The US National Occupational Safety Administration has also introduced nursing among the top 40 high-stress professions [7].

Nurses are one of the important pillars of health care organisations, and any shortcomings in this group will have irreparable consequences due to their important role in patient treatment. Therefore, paying attention to the factors affecting the performance of nurses in this area is of paramount significance. Nurses are exposed to the highest levels of occupational harms, including burnout, due to

exposure to physical, psychological, and emotional stressors [8] [9] [10]. In some studies, the rate of burnout in nurses has been reported four times higher than other service occupations [11].

Burnout in nurses jeopardises patient recovery more than any other factor [12]. This problem is one of the main factors in reducing efficiency, loss of human resources, and physical and mental health problems [13] [14]. Experts believe that instead of focusing solely on the sources of stress, it is better to equip nurses with personal, psychological, and personal capacities.

One of the most important human abilities that facilitates effective adaptation to risk factors and is a good strategy for promoting mental health in individuals is the resiliency attribute [15] [16]. Personality characteristics such as resiliency act as a barrier against stressful events and work-related mental health problems, such as burnout [13]. Resiliency helps individuals to face and adapt to difficult living conditions and protect them from mental disorders and life problems. Resilient individuals have a high degree of adaptation to environmental stressors in their lives [17]. Nurses will also be able to withstand many psychological pressures using the resiliency element [18].

One of the most important goals of meta-analyses is to provide a precise and valid result due to an increase in the sample size through the combination of various studies, thus, reducing confidence interval and solving problems arising from the controversial results of former studies. According to numerous studies with different findings, in this study, we systematically reviewed the findings of previous studies and combined their data to obtain an accurate estimate of the relationship between resiliency and burnout in nurses. For any kind of planning and policy-making to prevent conditions such as burnout and promote resilience in nurses, a precise estimation of the target group is necessary. Given the fact that so far there have been a plethora of studies on the relationship between resiliency and burnout, it seems that accumulating their results in the form of a meta-analysis can help generalise their findings. For this reason, the present study aimed to investigate the relationship between resiliency and burnout in nurses in the form of meta-analysis.

Material and Methods

This meta-analysis was undertaken to investigate the association between resiliency and burnout in nurses in 2017.

We systematically searched SID, Magiran, Irandoc, Medlib, and IranMedex databases, as well as Science Direct, ProQuest, Embase, MEDLINE, SCOPUS, CINAHL, and The Web of Science for studies on nurses' resiliency and burnout that were completed by October 2017. The following keywords were used to select the studies on the relationship between resiliency and burnout in nurses: (Resiliency OR resilience) AND (burnout OR “occupational burnout” OR “occupational burnout syndrome” OR OBS) AND (nurse) AND (Iran). For example PubMed database was searched using the following script:

((((((Resiliency[All Fields] OR resilience[All Fields]) AND burnout[All Fields]) OR ("burnout, professional "[MeSH Terms] OR ("burnout" [All Fields] AND "professional" [All Fields]) OR "professional burnout" [All Fields] OR ("occupational" [All Fields] AND "burnout" [All Fields]) OR "occupational burnout" [All Fields])) OR (("burnout, professional" [MeSH Terms] OR ("burnout" [All Fields] AND "professional" [All Fields]) OR "professional burnout" [All Fields] OR ("occupational" [All Fields] AND "burnout" [All Fields]) OR "occupational burnout" [All Fields]) AND ("syndrome" [MeSH Terms] OR "syndrome"[All Fields]))) OR OBS [All Fields]) AND ("nurses" [MeSH Terms] OR "nurses" [All Fields] OR "nurse" [All Fields])) AND ("iran" [MeSH Terms] OR "iran" [All Fields]).

The Persian equivalents of these keywords were also used in Persian databases. At this stage of the study, all the studies in which the mentioned keywords were used in the title or abstract were included in the list for review. Also, the references list of the included articles was also examined for further articles.

Quality of the selected articles was also evaluated using the STROBE checklist. This checklist contains 22 items that examine all parts of a study. To reduce bias and error in data collection, two researchers independently screened the articles. After the initial review and deleting the duplicate and unrelated articles, finally, five studies were entered in the final analysis.

In cases where the researchers disagreed in choosing an article, the reasons for the disagreement were discussed and, if the issue was not resolved, the article was referred to a third researcher expert in the field of meta-analysis. Finally, we investigated the studies that were on the relationship between resiliency and burnout in nurses.

The inclusion criteria consisted of Persian or English-language studies, observational studies, available full-text articles, and studies on nurses working in medical centres. Considering that the subject of this study was to investigate the relationship between resiliency and burnout among nurses, studies on nursing students due to lack of employment, studies that examined burnout among other health care providers, interventional studies, and studies that did not use Conner-Davidson or Mason's tools for measuring resiliency were excluded.

We considered articles that addressed the correlation coefficient between the two variables of resiliency and burnout or Z-test between the two. Based on the inclusion and exclusion criteria, articles collected by the two authors (independent of each other) were examined once based on the title and abstract, and the second round the full text of the articles was considered for eligibility. To record the information, a form was used including the variables of the first author of the article, the year of publication, study setting, type of study, sample size, data collection tool, the correlation between resiliency and burnout, and the correlation between resiliency and dimensions of burnout.

Concerning the Connor-Davidson questionnaire, it can be said that this resiliency scale has 25 items rated using a 5-point Likert scale (i.e., never, rarely, theoretically, sometimes, and always) with the minimum and maximum possible scores of 25 and 125, respectively. This scale was customised for use in Iran by Mohammadi. He distributed the questionnaire among 248 individuals, and its reliability was confirmed by measuring the internal consistency of Cronbach's alpha (89%) (19). The other questionnaire was the Maslach Burnout Questionnaire, which is used to measure occupational burnout. This questionnaire consists of 22 separate items and includes three dimensions that include emotional exhaustion, depersonalization, and feeling of personal incompetence [20].

In this meta-analysis, data analysis was performed using the random effects model (Mantel-Haenszel). The standard error of the mean for each study was estimated using the normal distribution. The effect size in each study was estimated using the formula $Z = 0.5\ \mathrm{In}\frac{1+r}{1-r}$, where r is the correlation coefficient in each study. To convert Z to r, the formula $r = \frac{\exp(2z)-1}{\exp(2z)+1}$ was employed. After conversion of Z scores, the size of the joint effect was estimated using a random effects model. In order to investigate any heterogeneity in selected studies, Q-Cochran test and I^2 index (I^2 index in three classes 0.025 [low heterogeneity], 0.025-0.075 [medium heterogeneity], and more than 0.075 [high heterogeneity]) were used. $P < 0.01$ and $I^2 > 75\%$ were considered as significant heterogeneity of studies. Sensitivity analysis was used to investigate the effect of each study on the final result. All the statistical analyses were performed using STATA software, version 14.

In this study, all investigations in the area of the relationship between burnout and resiliency were based on the four steps of PRISMA. In the first stage of the search, 227 articles were retrieved. After screening the titles and abstracts of the articles, 119 articles were excluded from the study due to irrelevance to the research topic and being reviews. Finally, the full text of 108 articles was studied and, if the article met the inclusion criteria, the necessary information was extracted. In this phase, 103 studies were excluded due to irrelevance to the research question, being replications of other studies, lack of homogeneity of resiliency tools, and unreliable data analysis. In the end, only five articles met the required criteria to enter the analysis (Figure 1).

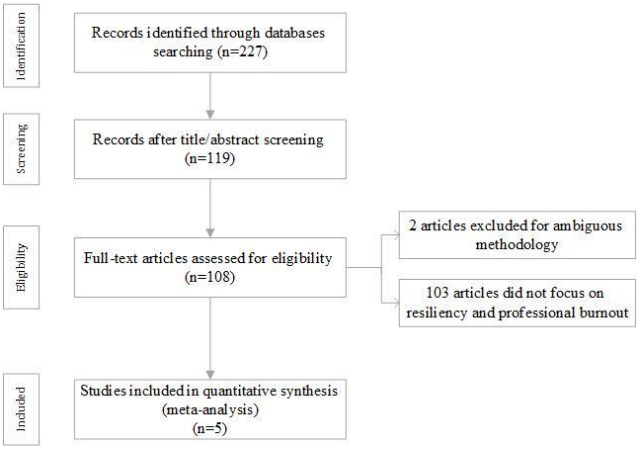

Figure 1: PRISMA flowchart for article identification and exclusion

In this meta-analysis, five studies published between 2009 and 2016 with a total sample size of 1169 people, investigated the relationship between resiliency and burnout in nurses. Specifications of the above studies are listed in Table 1.

Table 1: Summary of studies characteristics

No	First author	Year	Sample size	Location	Correlation	Z score	P-value
1	Khodabakhshi	2016	140	Semnan	-0/685	-0/838	<0/0001
2	Momeni	2010	426	Kermanshah	-0/602	-0/708	<0/0001
3	Amini	2013	304	Tehran	-0/355	-0/371	<0/001
4	Arbabshastan	2014	175	Shiraz	-0/208	-0/211	<0/0001
5	Shakerinia	2010	124	Rasht	-0/806	-1/112	<0/0001

In this study, considering that the heterogeneity index of studies was I^2=95.4%, the random effects model was used to estimate the joint correlation coefficient between resiliency and burnout.

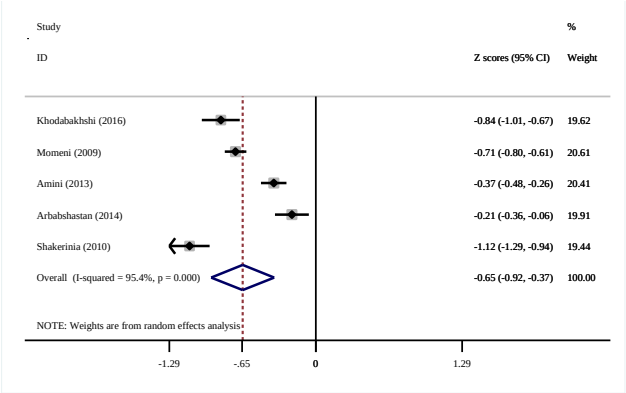

Figure 2: Association between resiliency and professional burnout

The highest and lowest sample sizes were related to the study by Momeni (2010) with 426 subjects [21] and Shakerinia (2010) with 124 cases

[22], respectively. The general correlation between resiliency and burnout was calculated using the random effects model and after converting the z-scores (-0.57) with confidence interval (95% CI: -0.726 to -0.354; Figure 2).

According to the results of correlation analysis between resiliency and burnout dimensions, the general correlation between resiliency and emotional exhaustion was -0.55 (95% CI: -0797 to -0.149; Figure 3), correlation with the depersonalization dimension was -0.388 (95% CI: -0.537 to -0.216; Figure 4), and correlation with the incompetence dimension was -0.254 (95% CI: -0.422 to -0.039; Figure 5).

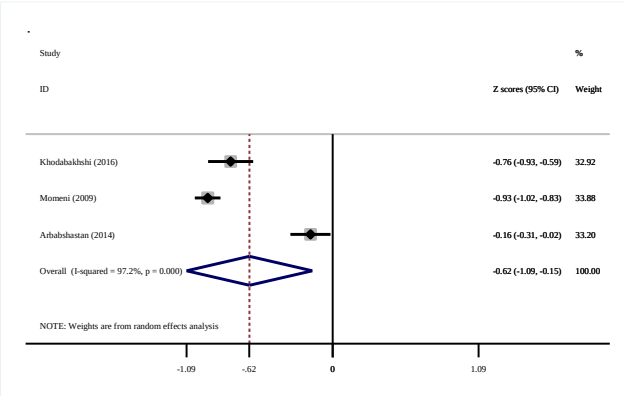

Figure 3: Association between resiliency and emotional exhaustion

In this study, the result of the sensitivity analysis showed that none of the studies alone had a significant impact on the outcome.

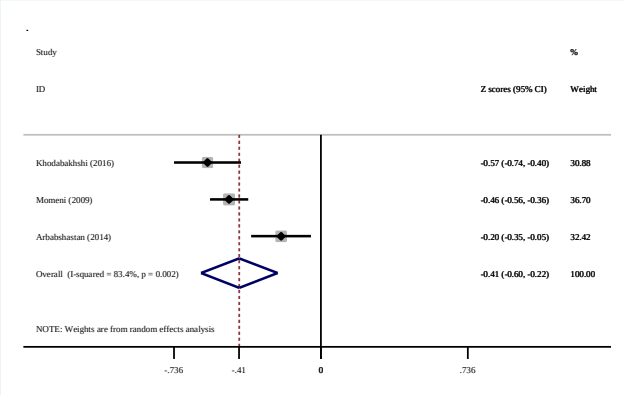

Figure 4: Association between resiliency and depersonalization

Discussion

The findings of this study that aimed to investigate the relationship between resiliency and burnout in the nursing community in Iran showed that the correlation between resiliency and burnout in Iranian nurses was -0.57. The results of the study by Rushton et al., (2015), which aimed to investigate the relationship between and burnout in nurses, showed that resiliency has a high correlation with the dimensions of emotional exhaustion, depersonalization, and personal incompetence, which is consistent with the findings of this research [23].

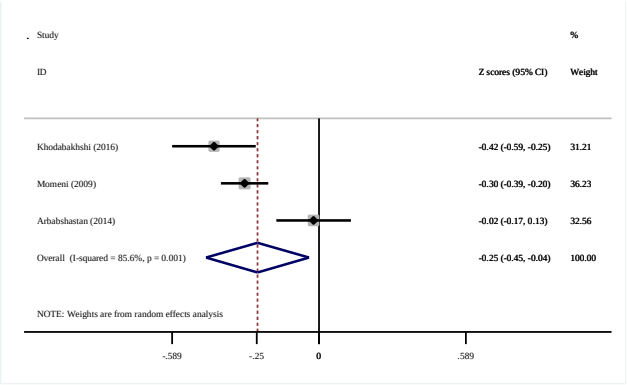

Figure 5: Association between resiliency and personal incompetence

Nurses use resilient behaviours in dealing with work problems to maintain their mental health and to go through negative experiences more easily and turn them into positive ones to feel burnout less [24]. The results of a study by Guo et al., in China indicated a weak correlation between resiliency and burnout in nurses [25]. The findings of various studies have also supported the relationship between resiliency and occupational burnout among nurses [26] [27]. Warelow et al., (2007) proposed that nurses who use resilient behaviours are less likely to develop burnout [24]. Jackson et al., (2008) studied resilience as a strategy for survival and growth in threatening work environments. They concluded that resilience is a kind of ability to adapt positively to problems and hardships, and it is positively associated with creating positive professional relationships, maintaining positivity, developing emotional insight, achieving balance in life, and promoting spirituality in nursing [18].

The results of Olson et al., (2015) showed that resiliency only has a significant negative correlation with emotional exhaustion and does not correlate with the other two dimensions [28]. In a cross-sectional study, Guo et al. investigated the correlation between resiliency and burnout in nurses in China. They noted a correlation between resiliency with all the three aspects of burnout in nurses [25)]. The results of the study by McCain et al., on physicians revealed a negative correlation between resiliency and burnout [29]. Accordingly, it can be stated that resiliency (successful coping capacity) in each occupational group can lead to a reduction in burnout.

Regarding the relationship between resiliency variable and burnout dimensions, the findings showed a significant negative correlation between the dimensions of burnout (emotional exhaustion,

depersonalization and personal incompetence) and resiliency variable. Considering the significant relationship between resilience and emotional exhaustion (-0.55), it can be noted that resiliency can help nurses to cope with stressful conditions and prevent emotional exhaustion, which can reduce psychological, motivational and emotional symptoms at work. Emotional exhaustion with symptoms such as chronic fatigue, sleep disturbance, and various physical symptoms and feeling of being pressured is associated with loss of emotional resources in an individual.

In the study of Talaee et al., about a quarter of nurses reported moderate emotional exhaustion [9]. The results of the study of Silvia et al. showed that burnout among nurses in emergency departments was high in the dimensions of emotional exhaustion and depersonalization, and it was low in the aspect of personal incompetence [30]. The findings of Spooner (2004) indicated that younger nurses experience higher levels of emotional exhaustion and depersonalization [31]. One of the important factors associated with emotional exhaustion is work experience. Emotional exhaustion is reported more often in less experienced nurses [32]. In the study by Momeni et al., (2010), which was aimed at comparing burnout among nurses working in treatment and education sectors, a significant direct relationship was noted between emotional exhaustion and employees' experience in the treatment sector, and in subjects with higher work experience, burnout rates in this dimension were greater [21].

Increased occupational burnout reduces the individual's ability to adapt to stressors and, as a result, causes behavioural and physical symptoms. Also, stresses induced from occupational conditions have a significant effect on the physical and mental health of individuals. Burnout due to persistent stresses reduces the ability of individuals to adapt to stressors [33]. The negative correlation between resiliency and burnout is indicative of the strength of prediction of resilience as a strong predictor of occupational burnout, that is, with higher resiliency, the degree of burnout is reduced. From the relationship between occupational burnout and resiliency, it can be noted that resiliency increases the individual's adaptability to stressors, which reduces the psychological, motivational and emotional symptoms at work, thus, reducing resiliency with increasing emotional exhaustion.

On the other hand, reduced resiliency in the subscale of depersonalization leads to the development of a negative attitude and the emergence of negative emotional responses about individuals and the environment. The low level of resilience in the subscale of personal incompetence reduces self-esteem and leads to the negative evaluation of the person's job and their ineffective relationship with colleagues and clients [21]. The negative correlation between resiliency and occupational burnout dimensions indicates that resiliency is a strong predictor of occupational burnout, such that with increasing resiliency, occupational burnout can be predicted in nurses. Nowadays, nurses' physical and mental health is emphasised to provide desirable health care and treatment services, and occupational burnout is one of the most important debilitating factors for nurses' health [34].

Measures and interventions that have been taken to reduce burnout and stress in nurses have often focused on organisational and managerial factors. If necessary, nurses should focus on improving individual factors to face workplace problems and difficulties. In this regard, Scholes (2008) has proposed 10 steps to promote resilient behaviours in nurses, which can cope with the professional identity crisis [35]. The literature on burnout and resilience shows that having a positive social network, good relationships with friends and colleagues, and having a supportive environment greatly affects these two variables [36]. Recently, the concept of resilience has been widely associated with the nursing profession [37]. Nurses must skillfully elaborate their resilience to cope with professional problems and maintain their mental health [24]. Resiliency has an effective effect on adaptation to risk factors and can play an effective role in reducing burnout [38].

Overall, the findings of this study showed a significant negative correlation between resiliency and burnout, thus, by improving the level of resiliency in nurses using strategies such as educating the importance of resiliency, monitoring and practising in the area of resiliency, and controlling occupational stresses the risk of burnout can be reduced. Also, promoting resiliency has other benefits, including maintaining peace of mind and increasing mental capacity and professional performance. In this regard, nursing and hospital authorities should, while paying attention to resiliency in their staff, create an effective management system to promote this characteristic. Also, since resiliency is learned over time, nursing education managers should pay attention to this issue in nursing students' curriculum.

There were several limitations to this meta-analysis. Few studies were found that had all of our inclusion criteria, for example, some articles did not address the correlation coefficient between the two variables of resiliency and their data sources did not report complete data for the meta-analysis.

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References

1. Rahmani F, Behshid M, Zamanzadeh V, Rahmani F. Relationship between general health, occupational stress and burnout in critical care nurses of Tabriz teaching hospitals. Iran Journal of Nursing. 2010; 23(66):54-63.

2. Saberi HR, Moravveji SAR, Ghoraishi F, Heidari Z. Post-traumatic stress disorder in Kashan and Arak emergency medicine departments' staffs during 2009. KAUMS Journal (FEYZ). 2009; 12(5):1-6.

3. Tummers GE, Janssen PP, Landeweerd A, Houkes I. A comparative study of work characteristics and reactions between general and mental health nurses: A multi-sample analysis. Journal of advanced nursing. 2001; 36(1):151-62. https://doi.org/10.1046/j.1365-2648.2001.01952.x PMid:11555059

4. Cho SH, Lee JY, Mark BA, Yun SC. Turnover of new graduate nurses in their first job using survival analysis. Journal of Nursing Scholarship. 2012; 44(1):63-70. https://doi.org/10.1111/j.1547-5069.2011.01428.x PMid:22233430

5. Niday P, Smithgall L, Hilton S, Grindstaff S, McInturff D. Redesigning nurse staffing plans for acute care hospitals: at Johnson City Medical Center in Tennessee, a team approach to better managing nurse staffing not only saved $7 million annually in contract labor costs, but also led to increased nursing satisfaction. Healthcare Financial Management. 2012; 66(6):112-7. PMid:22734326

6. Rahimi A, Ahmadi F, Akhond M. An investigation of amount and factors affecting nurses' job stress in some hospitals in Tehran. Hayat. 2004; 10(3):13-22.

7. Zandi A, Sayari R, Ebadi A, Sanainasab H. Abundance of depression, anxiety and stress in militant Nurses. Journal Mil Med. 2011; 13(2):103-8.

8. Abdi H, Shahbazi L. Correlation between occupation stress in nurses at intensive care unit with job burnout. Journal of Shahid Sadoughi University of Medical Sciences and Health Services. 2001; 9(3):58-63.

9. Talaee A, Mokhber N, Mohammad nejad M, Samari A. Burnout and its associated factors in Mashhad hospital employees. Journal of Semnan Medical Sciences University. 2008; 9(3):237-47.

10. Toubaei S, Sahraeian A. Burnout and job satisfaction of nurses working in internal, surgery, psychiatry burn and burn wards. The Horizon of Medical Sciences. 2007; 12(4):40-5.

11. Bakker AB, Van Der Zee KI, Lewig KA, Dollard MF. The relationship between the big five personality factors and burnout: A study among volunteer counselors. The Journal of social psychology. 2006; 146(1):31-50. https://doi.org/10.3200/SOCP.146.1.31-50 PMid:16480120

12. Vahey DC, Aiken LH, Sloane DM, Clarke SP, Vargas D. Nurse burnout and patient satisfaction. Medical care. 2004; 42(2 Suppl):II57. https://doi.org/10.1097/01.mlr.0000109126.50398.5a

13. Amini F. The Relationship between Resiliency and Burnout in Nurses. JGBFNM. 2013; 10(2):94-102.

14. McAllister M, McKinnon J. The importance of teaching and learning resilience in the health disciplines: a critical review of the literature. Nurse education today. 2009; 29(4):371-9. https://doi.org/10.1016/j.nedt.2008.10.011 PMid:19056153

15. Gobaribanab B. Spiritual intelligence. Andishe Novine Dini. 2008; 3(10):100-10.

16. Hosseini Ghomi T, Salimi Bajestani H. Effectiveness of resilience training on stress of mothers whose children, suffer from cancer in Imam Khomeini hospital of Tehran. Health Psychology. 2013; 1(4):97-109.

17. Hamid N, Keikhosravani M, Babamiri M, Dehghani M. The relationship between mental health, spiritual intelligence with resiliency in student of Kermanshah University of Medical Sciences. Jentashapir Journal of Health Research (Jentashapir) 2012; 3(2):331-8.

18. Jackson D, Firtko A, Edenborough M. Personal resilience as a strategy for surviving and thriving in the face of workplace adversity: a literature review. Journal of advanced nursing. 2007; 60(1):1-9. https://doi.org/10.1111/j.1365-2648.2007.04412.x PMid:17824934

19. Mohammadi M. The study of key factors influences on resiliency of substance abuse at risk. Tehran: University of Social Welfare and Rehabilitation Sciences; 2005.

20. Maslach C, Jackson SE. The measurement of experienced burnout. Journal of organizational behavior. 1981; 2(2):99-113. https://doi.org/10.1002/job.4030020205

21. Momeni H, Salehi A, Seraji A. The comparison of burnout in nurses working in clinical and educational sections of Arak University of Medical Sciences in 2008. AMUJ. 2010; 12(4):113-23.

22. Shakerinia I, Mohammadpour M. Relationship between job stress and resiliency with occupational burnout among nurses. J Kermanshah Univ Med Sci. 2010; 14(2):e79518. Epub 2010-09-01. en.

23. Rushton CH, Batcheller J, Schroeder K, Donohue P. Burnout and resilience among nurses practicing in high-intensity settings. American Journal of Critical Care. 2015; 24(5):412-20. https://doi.org/10.4037/ajcc2015291 PMid:26330434

24. Warelow P, Edward Kl. Caring as a resilient practice in mental health nursing. International Journal of Mental Health Nursing. 2007; 16(2):132-5. https://doi.org/10.1111/j.1447-0349.2007.00456.x PMid:17348964

25. Guo Yf, Luo Yh, Lam L, Cross W, Plummer V, Zhang Jp. Burnout and its association with resilience in nurses: a cross-sectional study Nurse burnout and resilience. Journal of clinical nursing. 2018; 27(1-2):441-9. https://doi.org/10.1111/jocn.13952 PMid:28677270

26. Aiken LH, Clarke SP, Sloane DM, Sochalski J, Silber JH. Hospital nurse staffing and patient mortality, nurse burnout, and job dissatisfaction. JAMA. 2002; 288(16):1987-93. https://doi.org/10.1001/jama.288.16.1987 PMid:12387650

27. Iglesias MEL, de Bengoa Vallejo RB. Prevalence and relationship between burnout, job satisfaction, stress, and clinical manifestations in Spanish critical care nurses. Dimensions of Critical Care Nursing. 2013; 32(3):130-7. https://doi.org/10.1097/DCC.0b013e31828647fc PMid:23571196

28. Olson K, Kemper KJ, Mahan JD. What factors promote resilience and protect against burnout in first-year pediatric and medicine-pediatric residents? Journal of evidence-based complementary & alternative medicine. 2015; 20(3):192-8. https://doi.org/10.1177/2156587214568894 PMid:25694128

29. McCain RS, McKinley N, Dempster M, Campbell WJ, Kirk SJ. A study of the relationship between resilience, burnout and coping strategies in doctors. Postgraduate medical journal. 2017:postgradmedj-2016-134683.

30. Silvia L, Gutiérrez C, Rojas PL, Tovar SS, Guadalupe J, Tirado O, et al. Burnout syndrome among Mexican hospital nursery staff. Rev Med IMSS. 2005; 43(1):11-5.

31. Spooner-Lane R. The influence of work stress and work support on burnout in public hospital nurses: Queensland University of Technology, 2004.

32. Kutluturkan S, Sozeri E, Uysal N, Bay F. Resilience and burnout status among nurses working in oncology. Annals of general psychiatry. 2016; 15(1):33. https://doi.org/10.1186/s12991-016-0121-3 PMid:27895699 PMCid:PMC5109709

33. Khodabakhshi M, Abdollahi M, Gholamrezaei S, Habibi E. Work place burnout prediction based on resiliency in nurses in

relation to gender. Koomesh. 2016; 17(4):845-55.

34. Rafiee F, Oskouie F, Nikravesh M. Key factors in nurses' reaction to Burnout: A qualitative study. Razi Journal of Medical Sciences. 2007; 13(53):83-94.

35. Scholes J. Coping with the professional identity crisis: is building resilience the answer? International Journal of Nursing Studies. 2008; 45(7):975-8. https://doi.org/10.1016/j.ijnurstu.2007.12.002 PMid:18255070

36. Treglown L, Palaiou K, Zarola A, Furnham A. The dark side of resilience and burnout: a moderation-mediation model. PloS one. 2016; 11(6):e0156279. https://doi.org/10.1371/journal.pone.0156279 PMid:27336304 PMCid:PMC4918953

37. Tusaie K, Dyer J. Resilience: A historical review of the construct. Holistic nursing practice. 2004; 18(1):3-10. https://doi.org/10.1097/00004650-200401000-00002 PMid:14765686

38. Garmezy N, Masten AS. The protective role of competence indicators in children at risk, 1991.

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Review Article

Hydrofluoric Acid: Burns and Systemic Toxicity, Protective Measures, Immediate and Hospital Medical Treatment

Emilija Bajraktarova-Valjakova[1*], Vesna Korunoska-Stevkovska[1], Silvana Georgieva[2], Kiro Ivanovski[2], Cvetanka Bajraktarova-Misevska[3], Aneta Mijoska[1], Anita Grozdanov[4]

[1]Department of Prosthodontics, Faculty of Dentistry, „Ss Cyril and Methodius", University of Skopje, University Dental Clinical Centre "St Pantelejmon" "Mother Theresa" 43, 1000 Skopje, Republic of Macedonia; [2]Department of Oral Pathology and Periodontology, Faculty of Dentistry, „Ss Cyril and Methodius", University of Skopje, University Dental Clinical Centre "St Pantelejmon" "Mother Theresa" 43, 1000 Skopje, Republic of Macedonia; [3]Department of Orthodontics, Faculty of Dentistry, „Ss Cyril and Methodius", University of Skopje, University Dental Clinical Centre "St Pantelejmon" "Mother Theresa" 43, 1000 Skopje, Republic of Macedonia; [4]Faculty of Technology and Metallurgy, „Ss Cyril and Methodius", University of Skopje, Ruger Boskovic 16, 1000 Skopje, Republic of Macedonia

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Keywords: Hydrofluoric acid; Skin burns; Eye injury; Ingestion; Inhalation; Systemic toxicity; Decontamination; Medical treatment

***Correspondence:** Emilija Bajraktarova-Valjakova. Faculty for Dental Medicine, Ss "Cyril and Methodius University" of Skopje, Skopje, Republic of Macedonia. E-mail: emibaj@yahoo.com

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Abbreviations: HFA - Hydrofluoric acid; OSHA - Occupational Safety and Health Administration (USA); AIHA - The American Industrial Hygiene Association; NIOSH - The National Institute for Occupational Safety and Health; ppm - parts per million; BSA - Body Surface Area; CaG - Calcium Gluconate; D5W - 5% Dextrose in water

Abstract

BACKGROUND: Hydrofluoric acid is a commonly used chemical in many industrial branches, but it can also be found as an ingredient in household products such as cleaning agents. Possessing high corrosive potential, HF acid causes burns and tissue necrosis, while when absorbed and distributed through the bloodstream, its extremely high toxic potential is expressed. Acute symptoms are often followed by pain, particularly in the case of skin burns, which intensiveness does not often correlate with the expressiveness of the clinical findings. Even exposure to low-concentrated solutions or gasses, or low-doses of high-concentrated acid, may provoke delayed systemic disorder which may eventually have a lethal outcome.

AIM: Therefore, having information regarding the possible hazardous effects of hydrofluoric acid usage, a variety of symptoms, as well as a treatment approach, is of great importance in the case of HF exposure.

METHODS: Available scientific articles published in literature databases, scientific reports and governmental recommendations from the internet websites, written in English, using the following search terms "Hydrofluoric acid, skin burns, eye injury, ingestion, inhalation, systemic toxicity, decontamination, antidote, medical treatment" have been reviewed.

RESULTS: This review is useful not only for physicians but for everyone who may come in contact with a person exposed to HF acid.

CONCLUSION: It highlights the mechanism of action, presents the acute and chronic symptoms, personal and general protective measures and devices that should be used, as well as decontamination procedures, immediate, antidote and hospital medical treatment.

Introduction

Hydrofluoric acid as a chemical compound with a high reactivity is used in many industrial branches: fluorine industry, glass industry, stevedoring and transportation industries, semiconductor industry, stainless steel and aluminum manufacturing, electronic components manufacturing, metal finishing and metal rust removal industry, inorganic and organic chemical manufacturing, mineral processing, petroleum refining, fire extinguishers manufacturing, as well as waste and disposable service sectors [1], [2], [3], [4], [5]. In the dental industry and every-day clinical practice, HF acid is used as an etchant agent for ceramic materials [6], [7], [8]. Hydrofluoric acid can be found in consumer products for marble, brick and stone cleaning, as a rust removal, in automobile wheel cleaners, toilet bowl cleaners, air conditioners cleaners, and as insecticides [9], [10], [11], [12], [13], [14].

Unintentional exposure to hydrofluoric acid or gases, with health consequences, may occur in case of inappropriate operation, inadequate protection, when having machine problems, explosion of HF containers and tanks, during the traffic accidents [1], and inattentive usage of household agents [10], while intentionally, in case of suicide [11], [12], [14] and murder. There are cases of intoxicated children when domestic cleaners, in appropriately stored, were accidentally ingested [13].

Dermal burns, eye injury and gastrointestinal or respiratory acute symptoms may be provoked when in direct skin/eye contact with HF acid, ingestion of the solutions or inhalation of fumes or HF vapours. Systemic intoxication including electrolytic misbalance, enzyme inhibition, cardiovascular, pulmonary, renal and neuromuscular symptoms happens when fluoride ions are absorbed into the bloodstream after dermal exposure and other mucous membranes, and distributed in all tissues and organs. Chronic symptoms may even occur several months after HF ingestion [12] or may persist with months after the long-time respiratory exposure [3]. Sometimes, systemic intoxication has a lethal outcome [15], [16], [17], [18], [19], [20].

The tissue damage and the degree of toxicity are determined by the acid concentration, the exposure time, the contaminated body surface area, the time elapsed between exposure and decontamination i.e. hospital care [21], [22], [23], and person's age as well [13], [24].

Decontamination, antidote therapy and hospital treatment, implemented promptly, are of great importance to prevent penetration of the hydrofluoric acid deep into the tissue, to disable dissemination of the fluoride ions through the bloodstream, or to minimise the progression of organs' damage.

The purpose of this review is to present the properties of a hydrofluoric acid, its corrosiveness, toxicity and mechanism of action, acute and chronic symptoms after the HF exposure, describes preventive and protective measures, as well as procedures for decontamination and HF neutralisation, antidote and hospital medical treatment.

HF Acid Properties

Hydrogen fluoride (HF) has several synonyms: Hydrofluoric acid, Fluoric acid, Hydrofluoride, Fluorine monohydride, Fluorane.

Physical properties: As a gas, HF is a diatomic compound of hydrogen and fluorine atoms, while as a liquid, it is a polymeric compound with strong hydrogen bonds between the chains [8]. Both, anhydrous hydrofluoric acid and aqueous solutions are colourless, fuming gas or liquid with a strong, irritating odour. Its disagreeable, pungent odour, even at the concentration of 0.04 ppm (which is considerably less than the OSHA PEL - Permissible Exposure Limit of 3 ppm), is a warning sign of the presence of the potentially dangerous substance. It readily dissolves in water forming a colourless hydrofluoric acid solution that when diluted (exothermal reaction) is visibly indistinguishable from water [4], [24], [25], [26].

Some of the physical properties of the Hydrofluoric acid are presented in Table 1.

Table 1: Physical properties of the hydrofluoric acid

Molecular Formula:	HF
Molecular Weight:	20.006 g/mol
Boiling point:	20 °C (68°F) at 760 mm Hg
Freezing Point:	-83 °C (-117.4 °F)
Specific gravity:	0.99 at -7 °C (19,4 °F); 1 for liquid at 20 °C (68 °F) (water = 1)
Density:	1.002 at 0 °C / 4 °C
Vapour pressure:	783 mm Hg at 20 °C (68 °F); 400 mmHg at 2.5 °C (36.5 °F)
Vapour density:	1.27 at 34 °C (air=1)
Surface Tension:	10.2 mN/m at 0 °C
pKa:*	3.15
The heat of Vaporization:	7.493 KJ/mol at 101.3 KPa
Solubility:	Miscible with water, very soluble in alcohol, soluble in many organic solvents
Flammability:	Nonflammable, explosive or oxidising

*acid dissociation constant

Chemical properties: Hydrofluoric acid is characterised by high reactivity - it reacts with metals, glass, concrete, enamels, pottery, rubber, leather and many organic compounds [24], [25], [26]. Consequently, it is commonly used in many industrial sectors and as a domestic cleaning agent. HF has a unique ability to react with many silicon compounds, including glass [9], thus commonly used as an etchant agent in the glass industry and before adhesive luting of ceramic restorations in dentistry [27].

Preventive Measures when Working with Hydrofluoric Acid

Because of its high corrosiveness and toxicity, the extraordinary caution when using is recommended. Hydrofluoric acid should be exclusively used inappropriately equipped industrial sectors and laboratories, must not be applied on the restorations in the oral cavity (when used in dentistry), while attending, when using household and cleaning agents, is recommended. The last one should be stored out of reach of children. Personal and general protective equipment and measures should be implemented.

The person who uses HF acid should be aware of the toxicity of this agent and be familiar with all information and procedures regarding the safety when using, way of transporting and storing the acid, managing with HF containing waste, decontamination

procedures, antidote and medicaments that should be used in case of contact and intoxication [4], [5], [24], [25], [26], [27]. Protective equipment and measures, as well as managing with HF containing waste and spills are presented in Table2.

Table 2: Personal and general protective equipment and measures, medicaments used in the first aid kit, and managing HF containing waste and acid spills

Personal Protective Equipment when working with/using HFA	***General Protective Equipment*** at the working/laboratory place
Laboratory coat	Handled inside of a fume hood
Long pants	Ventilation/exhaust system
Acid resistant apron	Sign "Danger, Hydrofluoric Acid Used in this Area."
Close-toed shoes	Easy access to a good supply of running water
Tightly sealed goggles	Safety shower and eyewash
Full-face shield in conjunction with goggles	The Standard Operating Procedure (SOP) document
Rubber gloves: nitrile, butyl or neoprene	
Respiratory filter device	
Protective Measures HFA containers	***First aid kit*** medicaments
Polyethylene or Teflon	2.5% calcium gluconate gel
Clearly labelled	1% calcium gluconate eyewash
Securely supported and not likely to tip over	a solution of 0.13% benzalkonium chloride
Tightly closed and kept in a safe place	
Away from heat and direct sunlight	
Managing HF Containing Waste	***Managing HF Acid Spills***
Neutralisation of the solution using powder: Na_2CO_3, Ca_2CO_3	HF-specific absorbents Aqueous $Ca(OH)_2$ or $Mg(OH)_2$
Neutralised diluted solution should be disposed under running water	The neutralisation should be performed slowly to avoid an exothermic reaction that will speed up the evaporation of the HF and increase the risk of exposure and intoxication
Chemically resistant container, clearly labelled with a "Hazardous Waste" tag	

Corrosiveness and Toxicity

Hydrofluoric acid is characterised by its corrosiveness and high local and systemic toxicity. At room temperature (20°C), it has a strong acidic pH-value of 2.0 [27]. However, the devastating effects are not based on the low pH value, but on the toxicity of this acid [9], [21], [22].

Routes of exposure: There are three different pathways through which HF acid could be absorbed into the human body - skin/eye contact, inhalation and ingestion. The most frequent exposure is by cutaneous contact with the aqueous solution, no matter if the skin is intact or damaged [28], [29], [30], [31], [32]; it could also be absorbed through eyes [33], [34]. Inhalation intoxication occurs not only from exposure to hydrogen fluoride gas [17], [35], but also from vapors arising from concentrated hydrogen fluoride liquid [20], [36], while ingestion [14], [37], [11] of even a small amount of this acid is likely to produce systemic effects and may be fatal [13], [16], [18], [38], [39].

Mechanism of action: Easy penetration through the skin, soft tissues and lipid membranes are enabled by low charging of undissociated hydro fluoride molecules. Once in the tissue, the HF molecules dissociate in hydrogen cations and fluoride anions [9].

There are two primary mechanisms through which HF acid causes tissue destruction. The first occurs due to the activity of corrosive hydrogen ion when using a high concentration of this acid (>50%) and is associated with cutaneous and ocular lesions, as well as digestive and respiratory mucous membrane damage. Corrosive burns are similar to those provoked by other acids: they occur immediately, with visible tissue destruction, grey areas, ulceration or necrosis, followed by intense pain [9].

The latter is caused by cytotoxic fluoride anion responsible for local and systemic toxicity when HF acid products with high, as well as with low concentrations have been used [23], [40]. The fluoride ion is very small and diffuses readily in the aqueous media [9]. Absorbed into the bloodstream, it is carried to all body organs in proportion to their vascularity and fluoride concentration in the blood [16], [41]. When reacting with cellular calcium and magnesium, forms insoluble chelates, CaF_2 and MgF_2, thus provoking local calcium depletion and inhibition of Na^+K^+ ATP-ase pump. Subsequently, the cell membrane's permeability to potassium is increased resulting in local hyperkalemia. High lipid affinity induces liquefaction necrosis and cellular death, thus destructing the nerve and blood vessels, tendons, bone structures and all other tissues [23],[42]. These effects are due to the presence of fluoride ion and differ from other acids, in which the feature of the free hydrogen cations to provoke coagulative necrosis, which retards the further penetration into the tissues, is expressed [9], [11].

Fluoride Distribution: Deposition of the fluoride in the different tissues is characteristic: as the fluoride is excreted through the urine, after an acute exposure to fluoride-containing solutions, the most of the fluoride is deposited in the kidney, then in the liver and the spleen; insignificant amount has been detected in the bones [16]. Rapid excretion and removal of the fluoride from the kidney occur within 24 hours [43]. However, after chronic exposes, a large amount (about half of the dose) is deposited in the bones, while the kidney serves as a temporary depositing organ.

Referential exposure limits: Systemic effects are potentially lethal, depending on the acid concentration and available amount of free fluoride ions [44]. Referential exposure limits and HF burns with a high risk to develop lethal electrolyte imbalances are presented in Table 3 [4], [24].

Special attention should be paid if intoxicated persons are children [13]. Because of their relatively larger surface area: body weight ratio, children are more vulnerable to the hydrofluoric acid absorbed through the skin. When exposed to its evaporations, larger doses are inhaled because of greater lung surface area: body weight ratio and increased minute ventilation per kg weight compared to adults. Additionally, vulnerability to corrosive agents is greater because of the relatively smaller diameter of

their airways [5], [24], [25].

Table 3: Exposure limits and HF burns that occupy various BSA depending on the acid concentrations that could have a lethal outcome

Exposure limits	**3 ppm**[*] - maximum concentration of a chemical substance that an employee may be exposed to over an 8-hour work shift; eyes and throat irritation, if not protected, have been noted. **20 ppm**[**] - maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to 1 hour without experiencing or developing irreversible or other serious health effects or symptoms, which could impair an individual's ability to leave the contaminated area, and take protective action. **30 ppm**[***] - immediately dangerous to health or life concentration.
HF burns with a high risk to develop lethal electrolyte imbalances	1% BSA burn with anhydrous HF 5% BSA burn with >70% concentrated HF 7% BSA burn with 50–70% concentrated HF 10% BSA burn with 20–50% concentrated HF 20% BSA burn with <20% concentrated HF Prolonged exposure or long delay for treatment in minor HF burns Ingestion of HF at concentrations >5% Inhalation of HF at concentrations >5%

allowed by OSHA[*]; according to AIHA[**]; established by NIOSH[***]*1%, BSA is an area equal to a hand palm.

Symptoms

Dermal exposure: Skin burns

A 10-year [1], a 11-year [20], a 15-year [30], a 20-year [2] and a 22-year [29] retrospective epidemiological studies revealed that chemical burns encountered in a Burn centres caused by hydrofluoric acid are one with the most frequent occurrence, especially in the regions with highly developed industry. The most common sites of injury are the head and neck, hands, legs and arms [1], [45]. HF burns might be accompanied by ocular injuries, respiratory and digestive tract disorders [1].

Transdermal penetration of the fluoride ions depends on the exposure time and HF acid concentration, while intradermal accumulation is dose-dependently. Fluoride penetration increases four-times by extending the exposure time from 1 to 3 min (with no further increase when the exposure is prolonged to 10 min) and is exponentially increased with increasing the HF concentration from 5% up to 50%. Also, intradermal pH decreases with increasing the HF concentration and exposure time [46]. Accordingly, epidermal alterations can be detected after 3 min exposure to only 5% HF. Severe damage including coagulation necrosis of deeper dermal layers is provoked by HF concentration of ≥ 30%, with a considerable intradermal accumulation of 13-67% of total absorbed fluoride [47]. When exposed to 70% HF for only 20 sec, cellular alteration in four to five epidermal layers is noted by 1 min after the exposure. Within 5 min, HF acid completely penetrates to the human dermis [44], [48]. Coagulation necrosis including acidophilic cellular cytoplasm and pyknotic nuclei in all skin layers have been detected 1 h after exposure, while complete necrosis of the epidermis and disintegration of the structures have been observed by 24 hours [49].

Further penetration of the fluoride ion through all epidermal and dermal layers into the subcutaneous tissue, causes ulceration and severe destruction such as complete loss of the soft tissues on the place of contact, including myolysis, tenosynovitis, decalcification and osteolysis [4], [9], [32], [50].

Skin damage symptoms are directly related to the acid concentration. Strong concentrations of more than 50% HF acid cause immediate, severe, throbbing pain and a whitish discolouration of the skin [51], vesicle surrounded by an erythematous flare [52], which eventually transforms into the blisters containing necrotic tissue. Reduced motor activity, decreased sensitivity and ischemia provoked by arterial vasospasm might be additional symptoms in fingers' burns [53]. Necrotic dermal tissue detached from the subcutaneous tissue has been observed in third-degree skin burns provoked by 50% HF acid [19].

An autopsy finding of a man who died from a cardiopulmonary arrest 30 min after the exposure to 60% HF acid, with the skin burns that occupy 30% of the TBSA, revealed greenish-gray or black coloring skin with thin circumferential erythema and severe liquefaction necrosis, with completely lost elastic fibers within the dermis, and severely affected wall of large vessels within the subcutaneous layers. Immediate penetration of the fluoride ions into the deep layer of the skin and their rapid dissemination through the bloodstream resulted in the development of acute systemic toxicity with the lethal outcome [15].

Dilute solutions from 20% to 50% may provoke pain and swelling, with erythema and vesicles formation on the contact area, which may be delayed up to 8 hours [21].

Acid solutions with concentration lower than 20% may cause serious injury (deep burns and tissue necrosis) 12 to 24 hours or several days after the exposure, without immediate pain [9], [22], [23], [32], [42], [54].

The severe pain, that is the first symptom of an intensive HF burns, results from the neuronal depolarization, disruption in neuronal conductivity and nerve ending irritation provoked by hyperkalemia in extracellular spaces, a compensatory mechanism for reduced levels of calcium ions which have been bound by the fluoride [23], [28], [40]. In case of burns provoked by low-concentrated HF acid, the onset of pain is a result of the long lag time in pH drop [46].

Ocular exposure: Eye burns

Irritation and immediate severe pain, followed by lacrimation are first signs after eye exposure. Conjunctivitis followed by oedema and congestion [45], oedema of other structures of the eye, mydriasis, nystagmus, and corneal erosion and ulceration, with corneal opacification and non-visible iris as

complications, may result from even minor hydrofluoric acid splashes [33], [55]. Prolonged HF exposure can lead to loss of vision and total eye destruction. Ocular burn with a complete diffusion into the cornea has been detected within 4 min exposure to 2.5% HF acid [48].

Gastrointestinal exposure

Ingestion of a solution of hydrofluoric acid [13], [14], [37], [38], causes strong caustic effect on the mouth and throat, erythema, ulcerations and bleeding of the oral mucosa, with the risk of perforation of the esophagus and/or stomach, erosive gastritis [16] followed by severe abdominal pain, dysphagia, nausea, vomiting and diarrhea, that eventually may progress to hemorrhagic gastritis, hematemesis and melena [11], [12], [39], [41]. Liver congestion, hepatocellular swelling and pancreatitis may also be present [16].

An autopsy finding of a person who accidentally ingested a mixture of hydrochloric and hydrofluoric acid revealed haemorrhages in the gastrointestinal tract, brownish discolouration of the oesophagal, gastric, duodenal and small intestinal mucous membranes with necrosis foci reaching the deeper layers of the walls, numerous ulcerations in the oesophagus and the stomach, and congested mucous membrane of the large intestine [18].

Respiratory exposure

Symptoms from respiratory system may occur when exposed to HF gas, fumes or vapours which arise when liquid acid evaporates on increased ambient temperature; in the case of a pulmonary aspiration while ingesting [1]; as a systemic toxicity symptom after HF acid ingestion [16], [18]; as a respiratory complication after HF splashes on the skin [24], or after severe dermal burns [45].

The toxicological effect of HF gases or vapours on the airway epithelia depends on the inhaled doses: when exposed up to 1.5 mM HF, no toxic effect has been observed; repairable damage to the epithelial cells has been detected when inhaled 7.5 mM HF, while severe, irreversible damage has been caused by 75 mM inhaled HF gas [48].

Inhalation of toxic gases or vapours provokes nasal irritation and inflammation, dryness and mucosal bleeding with subsequent ulceration and/or perforation of nasal septum, erythema and oedema of the oral, nasal end laryngeal mucous membrane. Continued exposure can results in coughing, dyspnea, laryngitis, laryngospasm and retrosternal pain, followed by chills, fever, and cyanosis [5], [56]; it has a devastating effect on the trachea and bronchi causing tracheobronchitis, bronchiolar obstruction [10] and bleeding accompanied by stridor and wheezing. Gaseous HF when reaches the pulmonary tissue provokes pulmonary oedema and congestion, pleural effusion, pneumonia [16], [57], and even partial or complete lung collapse [9], [40]. Chest radiograph revealed pulmonary oedema [57] or diffuse infiltrative shadows over the lungs' parenchyma [35], [56]. Acute respiratory failure may even lead to a lethal outcome [20] with little to no additional signs of trauma [17]. Chronic, repeated exposure to hydrofluoric acid-containing fumes may provoke pulmonary alveolar proteinosis followed by long-lasting dyspnea [3].

The presence of pale bronchial mucus on the bronchial membrane and congested, edematous mucosa several days after skin exposure have been confirmed by fiberoptic bronchoscopy [45]. Extravasations and hemorrhagic infarcts have been detected in the lungs after fatal accidental ingestion of a mixture of hydrochloric and hydrofluoric acid [18].

Systemic poisoning

Absorption of the fluoride ions into the bloodstream after dermal HF exposure, ingestion, or inhalation, and their distribution in all cells [16] may result in systemic toxicity [28]. The degree of toxicity and the outcome depends on the HF concentration and duration of exposure, burn surface area and burn depth, and the time elapsed between exposure and decontamination/medical treatment [15], [45].

When exposed to diluted solutions or low-saturated gas, the symptoms are often delayed. Even a severe dermal burns provoked by concentrated HF acid with symptoms of systemic poisoning, when treated in timely manner with appropriate therapy, may have favorable outcome, as in the following cases: face and neck exposure to 100% HFA [56], [57], skin burns provoked by 71% HFA occupying 7%TBSA [58], skin burns provoked by 70% HFA occupying 10%TBSA [59], face and neck exposure to 53% HFA [60].

Cases with expressed systemic disorder including electrolyte imbalance, enzyme inhibition, symptoms of hypovolemic shock, multiorgan failure, acute respiratory failure and numerous ventricular fibrillation or asystole, in which resuscitation procedures have been ineffective, have a lethal outcome [15], [18], [19].

Laboratory findings

Due to the high affinity of fluoride ion towards calcium and magnesium, and formation of insoluble salts, massive systemic electrolyte imbalance, which is difficult to counterbalance, may occur [53], [60]. This includes hypocalcemia, hypomagnesemia [45], hyperkalemia [14], [15], [19] or hypokalemia [2], [61], [62], fluorosis and metabolic acidosis [1], [19]. Enzyme inhibition and coagulation disorders [15], [22] are common findings as well.

Cardiovascular system disorder

The electrolytic imbalance affects the cardiovascular system, provoking hypotension and vasospasm [53], [63]. Hypocalcemia causes decreased myocardial contractility and intermittent prolongation of the Q-T interval, which is associated with degenerative rhythms such as torsades de pointes, a specific type of ventricular tachycardia that may cause sudden death. Hypomagnesemia is also associated with prolonged QTc and lethal dysrhythmias [9], [14], [38]. Moderate hyperkalemia can cause ECG changes, while severe hyperkalemia, due to the suppression of the electrical activity of the myocardium, may cause cardiac arrest. Additionally, free fluoride ions activate myocardial adenylyl cyclase thus increasing cyclic adenosine monophosphate (cAMP), which stimulates the myocardium inducing refractory ventricular fibrillation. Severe fluoride intoxication may provoke sudden cardiac arrest with a lethal outcome [1], [2], [13], [16], [18], [19], [23].

Recurrent ventricular dysrhythmias [14] or asystolia [62] can be developed several hours after HF acid ingestion or dermal exposure, while congestive heart failure provoked by toxic myocarditis has been detected as a long-term complication, four months after the hydrofluoric acid ingestion [12].

Renal symptoms

Fluorosis causes renal dysfunction, insufficiency and renal cortical necrosis followed by hematuria, proteinuria and azotemia [9], [15], [19], [22].

Osteosceletal symptoms

As the significant amount of fluoride deposits in the bones, osteolysis caused by HF intoxication has been observed.

Neuromuscular symptoms

Intoxication caused by acute exposure to HF acid may influence the function of the neuromuscular system. Hypocalcemia and hyperkalemia cause depolarisation of nerves and muscle fibres and interfere with the normal transmission of electrical signals throughout the neurons. The intoxicated person may become anxious, confused with headaches, may have seizures, paresthesia, paresis and paralysis [9], and may develop carpopedal and generalised tetany. When exposed to high doses, brain oedema followed by deep coma may occur [15], [16], [19].

Immediate, Decontamination/ Neutralization Procedures and Hospital Medical Treatment of the HF-Exposed Person

Correct diagnosis and timely treatment are of great importance when one is exposed to HF acid. Before starting any procedure, the exposed person should be checked for regular respiration and pulse, and in the case of suspected trauma, cervical immobilisation with a cervical collar and a backboard should be conducted. Decontamination implemented within the first minute and topical treatment including the use of the antidote is a critical procedure to prevent or minimise on-going HF absorption and progressive tissue destruction caused by fluoride ions [44].

Sometimes, systemic support by qualified medical staff should be obtained [54]. Immediate transport to the hospital is mandatory for the decontaminated person who has been in contact with the concentrated HF acid solutions/ vapours, if exposure to the low HF concentration lasted longer, still having acute symptoms or systemic complications are expected (no matter of the route of exposure). During the transport, skin and eye irrigation should continue, assistant ventilation if breathing has stopped and cardiopulmonary resuscitation (CPR) in the case of cardiac arrest should be given. The person who has been exposed to HF fumes only, and does not have any symptoms of skin/eyes burns, should be transported to the hospital as well and held for observation for at least 24 hours, the period when swelling of the respiratory tract is expected [24],[25],[26].

Treatment of the HF skin burns

Decontamination is mandatory for the victims with the dermal or ocular contact; contaminated clothing, as well as jewellery that could trap HF, should be immediately removed and double-bagged [31]. There are several methods that can be used for decontamination and neutralisation of the exposed skin and hair: rinsing with water, saline or solution of soap and water, and neutralisation performed with calcium gluconate, benzalkonium chloride, polyethylene glycol, magnesium oxide or Hexafluorine.

Rinsing with cold or warm (16°C) running water [29], [31], [33], [45], [51], [56], [59] or saline, for at least 30 minutes is the most commonly used decontaminating procedure. A solution of soap and water which should have a pH value of at least 8 (should not exceed a pH value of 10.5), is also recommended to neutralise low pH of the HF acid [52], using a soft brush and moving in a downward motion (from head to toe). Five per cent solution of

sodium bicarbonate can be successfully used as well [45]. Rinsing should be thoroughly performed until the contaminant is removed [4]. Ice pack on the affected area may reduce symptoms by vasoconstriction it provokes and retarding diffusion of the ion into the bloodstream [4]. Special attention should be paid when decontaminating children or the elderly because of the risk of hypothermia (blankets or warmers may be used) [4], [24].

Calcium gluconate (CaG) in its various formulations (solution, gel or ointment), administered immediately, is used as the most appropriate antidote when one is exposed to hydrofluoric acid. Sterile gauze moistened with 10% solution of calcium gluconate may be used to cover burn areas [45]. When used as a gel, 2.5 % CaG should be applied and rubbed into the affected area for 15-30 minutes [25], [40], wearing a new pair of chemical resistant gloves in order to prevent possible secondary HF burns [26]; upon reaction with the acid and forming CaF_2 precipitate, CaG gel turns white. The gel should be re-applied, every 10-15 minutes until the ambulance arrives or a physician gives medical treatment [26]. If used as a definitive treatment, 2.5% calcium gluconate should be applied 4 to 6 times daily, for 3 to 4 days [24]. Used as an ointment, it should be applied every two hours [51]. Application of surgical jelly consisting of 50% calcium gluconate and 50% dimethyl sulphoxide is recommended as well [29].

An iced solution of 0.13 % benzalkonium chloride can be used for immersion or as soaked compresses for at least 2 hours (a total of 4 to 6 hours); compresses should be changed or soaked with additional solution approximately every 2 to 4 minutes [5], [49]. This solution should not be used for burns of the face, ears or other sensitive areas.

Other useful agents are polyethylene glycol, magnesium oxide [59], 5% sodium bicarbonate [45], and Hexafluorine solution [48], [64].

Different agents have different ability in skin decontamination, neutralisation of the fluoride ion and minimising on-going HF absorption. According to Dennerlein et al. [65], polyethylene glycol reduces the cumulative penetrated amount of fluoride in the skin by 28%, flushing with water by 49%, while rinsing with CaG or Hexafluorine© is the most effective method with a reduction rate of 64% [65].

Rinsing the 20-sec exposed skin to 70% HF, with running tap water for 15 min followed by one topical application of 2.5% CaG gel, has limited preventive effect over the integrity of the cellular structure in the epidermis and the papillary and reticular dermis. Recovery, after the initial cellular deteriorations, observed 1h after the exposure, turned into edematous changes in the epidermal basal layer 3 hours later. No deterioration of the structures of either the epidermis or dermis have been detected after washing the exposed skin with Hexafluorine applied as a spray of 400 ml over 10 min, whatever the time of observation was [49].

Hultén et al. [66] have not observed any differences in the electrolyte misbalance after dermal exposure to HF acid, between the Hexafluorine-treated animals and those treated with water only. Further on, Brent [67], reviewing the data from 69 relevant papers, concluded that water-based solutions are the best, widely available and inexpensive decontaminating fluids for dermal corrosive exposures [67]. However, Yoshimura et al. [59], concluded that even after a 3 hours delay, skin washing with Hexafuorine (followed by intravenous, intradermal, perilesional, and topical injunction of calcium gluconate) is beneficial in the treatment of first- to third-degree skin burns occupying approximately 10% of the TBSA, provoked by 70% HF. Decontamination with Hexafuorine has prevented the development of significant systemic toxicity which otherwise occurs when exposed to concentrated HF acid and often results with a fatal outcome [59].

Different neutralising abilities of agents are due to the different mechanism of action. Water has a mechanical rinsing and a diluting effect only; it has no active binding or chelating properties for any chemical substance, including hydrofluoric acid. As a hypotonic liquid, it cannot stop penetration of HF throughout skin layers and may enhance such penetration. Besides mechanical rinsing of the surface, a residue of fluoride ion might be still present on the skin in a sufficient quantity to provoke secondary necrosis.

Unlike water, calcium gluconate is capable of chelating the free fluoride ions, forming insoluble salts, thus neutralising their toxical effect over the cells' metabolism. A single application of CaG gel helps in delaying the onset of the skin injury and mitigating the severity of the damage, but the tissue lesions continue to evolve; this is the reason for recommendation, CaG gel to be applied multiple times [49].

Hexafluorine® has a triple effect over the HF acid-exposed skin, eye or mucous membranes. As a sterile water-solution, it has the same rinsing and diluting effect as the pure water has. As an amphoteric compound, Hexafluorine actively acts against both primary mechanisms (corrosive and cytotoxic) through which HF acid causes tissue destruction: it has a property to neutralise the H^+ ion and chelates the F^- ion. Consequently, decontamination with Hexafluorine® completely prevents cutaneous and eye tissue injuries [49], [64].

Vapour burns of the skin are treated the same as liquid HF burns [36].

Hydrogen fluoride burns are followed by intense pain that should not be suppressed with local infiltration of anaesthetic because the degree of pain is an indicator of treatment efficacy.

Topically applied, calcium gluconate has limited ability in term of chelating the F− ions that have

been already penetrated deeply into the skin tissues. In the case of large and/or deeply penetrating burns, when exposed to hydrogen fluoride concentrations greater than 50%, when the treatment is delayed, or if pain-relief is not achieved by previously applied CaG, subcutaneous injection of sterile aqueous calcium gluconate solution underneath the burned area and into the immediately adjacent skin should improve the neutralizing efficacy [29], [30], [52], [59], [68]; the recommended dose is limited to 0.5 mL/cm^2 affected skin surface area of a 5% or 10% calcium gluconate solution (with a maximum of 0.5 mL per digit for finger burns), using a small gauge needle (#30) [29]. However, multiple injections into the fingers may lead to increased tissue pressure thus worsening an existing swelling, impairing circulation and causing ischemic necrosis [69]. It is recommended subcutaneous injection to be applied, only if there is a central grey burn with surrounding erythema, or when having severe throbbing pain [70].

According to De Capitani et al. [51], "intra-arterial calcium gluconate might be considered for finger burns caused by highly concentrated HF, when topical treatment is considered useless, or when intradermal and subcutaneous calcium injections cannot be performed" [51].

Calcium gluconate gel is not effective in treating burns of the nails as well. It may be necessary to drill, split or even remove nails to allow the topical methods of treatment to be effective [29], [30], [68]. Sometimes, removing the nails may be avoided by immediate immersion in benzalkonium chloride (Zephrian®) solution [24], [25].

The skin blisters, if already formed, should be opened and necrotic tissue should be debrided as soon as possible, as early debridement may facilitate healing [59]. There are several chirurgical methods which can be used for the treatment of the severe HF dermal burns: escharotomy or fish-mouth fasciotomy followed by intravenous administration of prostaglandin in order to maintain maximal distal circulation [71], succeed by skin grafting (a split-thickness or full-thickness skin grafting) or flap transfer for wound closure and reconstruction [1]. In case of deep layer finger injuries of weight bearing portions such as finger pulp, a partial toe pulp-free flap should be performed to reconstruct the digits [71].

Treatment of the eye burns

After ocular exposure or irritation, the eyes should be immediately irrigated with a large amount of gently flowing cool plain water or sterile 0.9 % saline solution for 15-30 minutes [45], while holding eyelids apart, and moving the eyeball in every direction, thus ensuring the irrigator reaches all the surfaces. If the exposed person is wearing contact lenses, the lenses should be carefully removed [5], [24], [26]. If sterile 1% calcium gluconate solution is available, water washing may be limited to 5 minutes.

Usage of 1-10% sterile aqueous solution of calcium-gluconate eye-drops [23], [33], [34], [45], although widely recommended as a preferred flushing agent, should be done with great care, because sometimes it may worsen the clinical outcome [4], [32].

Subconjunctival injection of a 1% CaG has been successfully used as well [25].

An in-vitro study revealed that 20% solution of mannitol used for 15 min immediately after the corneal HF exposure is an effective decontaminating agent [72].

According to Spöler et al. [55], Hexafluorine is the only decontaminating solution that preserves the transparency of the corneal surface, with no reported injuries or long-term consequences [55]. A five-year follow-up study revealed that chemical burns have not developed, nor further medical or surgical treatments have been needed in workers sustained an eye or skin HF splashes when treated with Hexafluorine [64]. According to Soderberg et al. [73], medical treatment other than initial decontamination with Hexafluorine is not required in workplaces where water decontamination followed by calcium gluconate injunction failed to prevent HF dermal and ocular burns and systemic toxicity [73].

One or two drops of proparacaine or tetracaine should provide rapid-onset ocular anaesthesia for 20 minutes to an hour, as severe pain is felt when erosion or corneal ulcer have already occurred. Corneal damage should be treated by an ophthalmologist.

Treatment after HF acid ingestion

When HF solution is ingested, it is very dangerous to induce vomiting. Instead, if the exposed person is conscious, his/her mouth should be thoroughly rinsed with water or with a 5-10% solution of calcium gluconate [9], [45].

Gastric suction and lavage using a small flexible nasogastric tube are recommended within the first hour of ingestion [11] if a large dose of HF solution has been ingested or the patient has oral lesions or persistent oesophagal discomfort. Large amounts of room temperature water, milk (120-240 ml), 10% CaG, magnesium-containing beverages (60-120 ml) as well as 60 gr sodium polystyrene sulfonate should be taken orally or by nasogastric tube in order to dilute the acid and bind the remaining fluoride ions that have not been absorbed yet [9], [11], [24], [25], [26]. Anyway, good assessment should be done if using a nasogastric tube excludes the risk of oesophagal or gastric perforation [9].

According to Heard and Delgado [39], oral administration of calcium- or magnesium-containing

solutions does not alter the toxic effect following hydrofluoric acid ingestion. It is forbidden to administrate sodium bicarbonate to neutralise the acid, as the carbon dioxide byproduct could cause severe burns [39].

Endoscopy may be performed to define the extent of the injury, to assess the state of mucous membrane and to prognosticate the course of recovery [11]. However, this procedure should be performed after the neutralisation of the HF acid content into the stomach, as the HF destroys optic fibres while attempting endoscopic examination [25].

Treatment after HF inhalation

After inhalation of HF gases, fumes or vapours, the affected person should be immediately moved to fresh air. One hundred per cent oxygen (10 to 12 L/min flow rate) should be administered as soon as possible [45], and a bag-valve-mask for assistant ventilation can be used if breathing has stopped [24].

Calcium gluconate solution, 2.5% - 5%, given by intermittent positive-pressure ventilation using a nebuliser is the therapy of choice when starting the hospital treatment [17], [56]. Aerosolised bronchodilator should be administered in the patients with bronchospasm considering the myocardial condition; the risk of cardiac arrhythmias (especially in the elderly) should be estimated. Racemic epinephrine aerosol (0.25–0.75 mL of 2.25% racemic epinephrine solution in 2.5 cc water), repeated every 20 minutes if needed, might be useful for children who developed stridor [5], [24]. In the case of pulmonary oedema, calcium gluconate and N-Acetyl cysteine given intravenously on the first day and with a nebuliser for 48 h after the exposure have been proved to be efficient in reducing the pulmonary secretion [57]. Aspiration and lavage of the affected bronchi [45] or lungs [3] may also be performed.

If the respiration is compromised (in case of oedema, laryngospasm and hypoxemia), an airway may be secured via endotracheal intubation [11], [19], cricothyroidotomy or tracheotomy [35], [45], while the respiratory function should be established by mechanical ventilation.

Fiberoptic bronchoscopy is a prognostic procedure that is performed to assess the extent of damage to the respiratory tract and to evaluate the efficacy of the conducted therapy [3].

Systemic toxicity treatment

As mentioned before, the fluoride is a low-molecular-weight anion that is easily absorbed through the skin, mucous membranes of the gastrointestinal and respiratory tracts, diffusing readily into the bloodstream causing fluorosis and acidosis, hypocalcemia, hypomagnesemia and hyperkalemia or hypokalemia [2], [61], [62]. The half-life of fluoride is 12 to 24 hours and is eliminated primarily through renal excretion. Because of its highly toxic potential, increasing the renal elimination by administrating diuretic therapy and alkalization of the urine with sodium bicarbonate are of great importance [45].

Even if the serum electrolytes and blood saturation with oxygen are already normalised, recurrent ventricular fibrillation may still occur. It is assumed that the reason is fluoride-induced cardiotoxicity due to the high fluoride levels in the serum and urine. In such cases, hemodialysis enables full recovery of the intoxicated person [58]. Continuous renal replacement therapy, hemofiltration or hemodialysis, should be conducted as an effective and potentially lifesaving treatment in patients with severe systemic toxicity [23], [45], [60]. Antar-Shultz et al. [37], have confirmed 70% reduction of the fluoride level in the blood after three hours of hemodialysis, with the recommendation, the initial hemodialysis to be prolonged beyond the standard four-hour treatment session [37]. Continuous venovenous hemodialysis up to 72 hours followed by continuous venovenous hemofiltration up to 10 days has been performed to remove delayed release of fluoride ions to avoid fluoride-related cardiac toxicity [45].

According to Pu et al., “cardiac arrhythmia is the leading cause of death during the early stage, mainly due to polymorphic ventricular tachycardia and ventricular dysrhythmias” [45]. Hypocalcemia is considered as the main factor that provokes disturbances in the cardiac rhythm. Therefore calcium-containing substances are mainstays of therapy for fluoride toxicity [11]. Normal calcium level can be achieved by ordering intravenous (IV) infusions of 10% calcium gluconate [74], [75], [76], with doses of 0.1 to 0.2 mL/kg. Infusions can be repeated until serum calcium, ECG, or symptoms improve. If hand or forearm is affected, Hatzifotis et al. [29] recommended regional IV infusion of 40 ml 10% CaG with 5000 U heparin. Pu et al. [45] in a person with severe cutaneous injuries involving approximately 60% of the TBSA (with third-degree burns present on approximately 13% of the burn area), provoked by 10% HF and 50% nitric acid, after an initial 20mL IV bolus of 10% CaG, continued the infusion with 6 g/h until ultimate normalization of calcemia and stabilization of the cardiac rhythm (a total of 55g of CaG during the first 24 hours) [45]. A total of 8.4 gr of elemental calcium administered as 10% calcium gluconate at 20 mL/h, is a dosage that has normalised Ca level when ingested 120 ml of 20-25% HFA [11].

Intra-arterial infusion of calcium gluconate, firstly reported by Köhnlein and Achinger [50], is indicated when exposed to high HF concentrations with severe burns [51], rapid destruction of tissues and acute systemic toxicity especially in patients with upper and lower extremities- and facial burns [29], [30], [63], [77], [78]. According to ASTDR, the initial dosage is 10 mL of 10% calcium gluconate diluted

with 40 mL D_5W given intra-arterially over 4 hours. If the pain is unrelieved, 20% concentrations should be used. The ultimate goal is achieving a pain-free condition for up to 4 hours [24].

Nguyen et al. [79] reported a case of calcium gluconate infusion via the external carotid artery in a person with severe face burn. When severe burns of the digits, the brachial or the radial artery [51], [77], [80] are catheterised, depending on the fingers involved [53]. Intra-arterial infusions of 2% CaG solution in 5% dextrose have been given through radial artery at wrist level, every four hours for 36 hours when burned middle and fourth fingers with 70% HF acid [51]. Pain symptoms have been improved and sensory, and motor functions have been restored in the fingers D II to D V injured with 60% HFA, after intra-arterial infusion of 10 ml 20% CaG in 40 ml 0.9 % NaCl administered over 4 hours. Thomas et al. [63] concluded that intra-arterial calcium gluconate injection is a successful and well-tolerated therapy for HF burn of the hand associated with Raynaud's syndrome [63]. Vasospasm of the common palmar digital artery has been eliminated by vasoactive therapy with alprostadil while platelet aggregation has been inhibited with acetylsalicylic acid and clopidogrel [53].

However, a great precaution and Intensive Care Unit (ICU) monitoring is required when ordering intra-arterial infusion (in terms of infusion solution, concentration, and time interval) as some serious complications may occur including artery spasm and bleeding, hematomas followed by median nerve palsies, carpal tunnel syndrome, hypercalcemia and even a high morbidity when brachial artery-cannulation [23].

Hypomagnesemia, associated with a prolonged QTc and possible lethal dysrhythmias, can be solved by 2 to 4 mL of 50% of magnesium sulfate intravenously, over 40 minutes [4], [24], [61], [62], [74], or continuous IV infusion of 25% magnesium sulfate at 1.5 g/h according to the concentration of serum magnesium [11], [45].

Hyperkalemia, provoking ECG disturbances or cardiac arrest, should be treated with calcium gluconate (10-20 ml of 10% solution IV) to protect myocardium, in conjunction with 10 U of regular insulin intravenously administered along with 50 mL of 50% dextrose (or glucose) [11] to enhance shifting potassium from the vascular space into the cells; 10-20 mg (5 mg/ml) Albuterol (Ventolin) administered by nebulizer, has additive effect to that of insulin, while 20-40 mg Furosemide (Lasix) IV, increases renal excretion of potassium, thus, both of them, decreasing the free potassium level. Sodium bicarbonate administered intravenously as 8.4 % solution and 60 g sodium polystyrene sulfonate given via the nasogastric tube or in 30 ml of sorbitol solution administered orally (in the case of HFA ingestion), removes potassium from the blood or gastrointestinal tract in exchange for sodium [11].

Patients with a life-threatening condition (substantial skin burns, persistent hypotension, respiratory distress, cardiac arrhythmias, seizures and coma) should be admitted at an intensive care unit [30], and treated according to Advanced Life Support (ALS) protocol.

In the case of ventricular fibrillation, defibrillation should be conducted, as many times as needed [11], [14], [45] and administrate dobutamine to improve left ventricular contraction. If acute respiratory distress syndrome (ARDS) appears, and routine mechanical ventilation or higher level of applied positive end-expiratory pressure (PEEP) cannot improve oxygenation, extracorporeal membrane oxygenation (ECMO) is a method for relief from hypoxemia and/or carbon dioxide retention [45], [57].

Additionally, glucocorticoid (methylprednisolone 40mg/8h) and antibiotic to prevent bacterial infection should be administered [35], [45].

Unfortunately, when exposed to high concentration of HFA solution (50% and higher), with extensive and deep skin burns (third-degree), even a vigorous medical treatment consisted of a continuous administration of calcium gluconate (50 ml/h, 8,5% solution) and magnesium sulfate, a massive transfusion of saline with catecholamine (vasopressor) for the treatment of shock, Midazolam (2 mg), vecuronium (8 mg), and buprenorphine (0.2 mg), extensive skin debridement and even leg amputation in order to prevent HFA flowing into systemic circulation, defibrillation and cardiopulmonary resuscitation could not stop the progression of disseminated intravascular coagulation that eventually lead to cardiopulmonary arrest, progressive organ damage and lethal outcome [19].

Conclusion

Due to its high reactivity, hydrofluoric acid is a commonly used the chemical compound in many industrial branches and as a domestic cleaning agent. It possesses corrosive potential causing burns and tissue necrosis on the site of contact, while when absorbed into the bloodstream and distributed to all organs and tissues, it provokes potential life-threatening systemic toxicity and organs failure. All this imposes an extraordinary caution and great awareness of health consequences when using, and implementation of all personal and general protective measures.

The kinetics of the fluoride ion penetration into the skin and mucous membrane and organ distribution imposes appropriate urgent first aid and

secondary medical management. Initial decontamination procedures started within the first minute after the exposure, neutralisation and antidote agents implemented promptly are of great importance to avoid or minimise the extent and depth of local tissue damage or necrosis, as well as to prevent absorption and systemic distribution of the fluoride ions and massive systemic electrolyte imbalance. Electrolyte replacement therapy including calcium gluconate and magnesium sulfate, fluid resuscitation, bronchodilators, glucocorticoids and antibiotics, vasopressors, in conjunction with insulin, furosemide and anticoagulants, are medicines of choice. Extracorporeal membrane oxygenation used to improve oxygenation and to support hemodynamic profile in case of acute respiratory distress syndrome or cardiac arrest, as well as renal replacement therapy, to remove serum fluorides and excess potassium, are sometimes necessary procedures to sustain life in severe fluoride intoxication.

References

1. Zhang Y, Zhang J, Jiang X, Ni L, Ye C, Han C, Sharma K, Wang X. Hydrofluoric acid burns in the western Zhejiang Province of China: a 10-year epidemiological study. J Occup Med Toxicol. 2016 11:55. https://doi.org/10.1186/s12995-016-0144-3 PMid:27980604 PMCid:PMC5142164

2. Wu ML, Yang CC, Ger J, Tsai WJ, Deng JF. Acute hydrofluoric acid exposure reported to Taiwan Poison Control Center, 1991-2010. Hum Exp Toxicol. 2014; 33(5):449-54. https://doi.org/10.1177/0960327113499165 PMid:23892993

3. Kim YJ, Shin JY, Kang SM, Kyung SY, Park J-W, Lee SP, Lee SM, Jeong SH. Pulmonary alveolar proteinosis induced by hydrofluoric acid exposure during fire extinguisher testing. J Occup Med Toxicol. 2015; 10(6):1-3. https://doi.org/10.1186/s12995-015-0048-7

4. Hydrogen Fluoride/Hydrofluoric Acid: Systemic Agent. Center for Disease, Control and Prevention. The National Institute for Occupational Safety and Health (NIOSH). https://www.cdc.gov/niosh/ershdb/emergencyresponsecard_29750030.html Accessed August 20, 2018

5. Recommended Medical Treatment for Hydrofluoric Acid Exposure. Honeywell International Inc. 2018:1-23.

6. Ho GW, Matinlinna JP. Insights on ceramics as dental materials. Part II: chemical surface treatments. Silicon. 2011; 3:117-23. https://doi.org/10.1007/s12633-011-9079-6

7. Blatz MB, Sadan A, Kern M. Resin-ceramic bonding: a review of the literature. J Prosthet Dent. 2003; 89(3):268-74. https://doi.org/10.1067/mpr.2003.50 PMid:12644802

8. Bajraktarova-Valjakova E, Grozdanov A, Guguvcevski Lj, Korunoska-Stevkovska V, Kapusevska B, Gigovski N, Mijoska A, Bajraktarova-Misevska C. Acid etching as surface treatment method for luting of glass-ceramic restorations, part 1: Acids, application protocol and etching effectiveness. Open Access Maced J Med Sci. 2018; 6(3):568-73. https://doi.org/10.3889/oamjms.2018.147 PMid:29610622 PMCid:PMC5874387

9. Makarovsky I, Markel G, Dushnitsky T, Eisenkraft A. Hydrogen fluoride - the protoplasmic poison. Isr Med Assoc J. 2008; 10(5):381-5. PMid:18605366

10. Franzblau A, Sahakian N. Asthma following household exposure to hydrofluoric acid. Am J Ind Med. 2003; 44(3):321-4. https://doi.org/10.1002/ajim.10274 PMid:12929153

11. Whiteley PM, Aks SE. Case files of the Toxikon Consortium in Chicago: survival after intentional ingestion of hydrofluoric acid. J Med Toxicol. 2010; 6(3):349-54. https://doi.org/10.1007/s13181-010-0088-4 PMid:20661686 PMCid:PMC3550485

12. Gradinger R, Jung C, Reinhardt D, Mall G, Figulla HR. Toxic myocarditis due to oral ingestion of hydrofluoric acid. Heart Lung Circ. 2008; 17(3):248-50. https://doi.org/10.1016/j.hlc.2007.04.011 PMid:17822953

13. Ozsoy G, Kendirli T, Ates U, Perk O, Azapagasi E, Ozcan S, Baran C, Goktug A, Dindar H. Fatal refractory ventricular fibrillation due to ingestion of hydrofluoric acid. Pediatr Emerg Care. 2018. https://doi.org/10.1097/PEC.0000000000001548 PMid:30020244

14. Vohra R, Velez LI, Rivera W, Benitez FL, Delaney KA. Recurrent life-threatening ventricular dysrhythmias associated with acute hydrofluoric acid ingestion: observations in one case and implications for mechanism of toxicity. Clin Toxicol (Phila). 2008; 46(1):79-84. https://doi.org/10.1080/15563650701639097 PMid:17906993

15. Ohtani M, Nishida N, Chiba T, Muto H, Yoshioka N. Pathological demonstration of rapid involvement into the subcutaneous tissue in a case of fatal hydrofluoric acid burns. Forensic Sci Int. 2007; 167(1):49-52. https://doi.org/10.1016/j.forsciint.2005.12.008 PMid:16426786

16. Martínez MA, Ballesteros S, Piga FJ, Sánchez de la Torre C, Cubero CA. The tissue distribution of fluoride in a fatal case of self-poisoning. J Anal Toxicol. 2007; 31(8):526-33. https://doi.org/10.1093/jat/31.8.526 PMid:17988468

17. Zierold D, Chauviere M. Hydrogen fluoride inhalation injury because of a fire suppression system. Mil Med. 2012; 177(1):108-12. https://doi.org/10.7205/MILMED-D-11-00165 PMid:22338991

18. Smędra-Kaźmirska A, Kędzierski M, Barzdo M, Jurczyk A, Szram S, Berent J. Accidental intoxication with hydrochloric acid and hydrofluoric acid mixture. Arch Med Sadowej Kryminol. 2014; 64(1):50-8. https://doi.org/10.5114/amsik.2014.44590 PMid:25184427

19. Onohara T, Komine M, Yoshidomi Y, Amari K, Fujita R, Matsumoto Y, Koyama T, Koga M, Mitsumizo S, Satake Y, Masumoto K, Matsunaga T, Mae T, Fujita N. Chemical burn caused by high-concentration hydrofluoric acid: a case that followed a lethal course. Glob Dermatol. 2015; 2(6):215-7.

20. Blodgett DW, Suruda AJ, Crouch BI. Fatal unintentional occupational poisonings by hydrofluoric acid in the U.S. Am J Ind Med. 2001; 40(2):215-20. https://doi.org/10.1002/ajim.1090 PMid:11494350

21. Bertolini JC. Hydrofluoric acid: a review of toxicity. J Emerg Med. 1992; 10(2):163-8. https://doi.org/10.1016/0736-4679(92)90211-B

22. Özcan M, Allahbeickaraghi A, Dündar M. Possible hazardous effects of hydrofluoric acid and recommendations for treatment approach: a review. Clin Oral Investig. 2012; 16(1):15-23. https://doi.org/10.1007/s00784-011-0636-6 PMid:22065247

23. McKee D, Thoma A, Bailey K, Fish J. A review of hydrofluoric acid burn management. Plast Surg (Oakv). 2014; 22(2):95-8. https://doi.org/10.1177/229255031402200202

24. Hydrogen Fluoride. Agency for Toxic Substances and Disease Registry ATSDR, Federal public health agency of the U.S. Department of Health and Human Services: 1-23. https://www.atsdr.cdc.gov/mhmi/mmg11.pdf Accessed August 10, 2018.

25. Emergency response guidelines for anhydrous hydrogen fluoride (HF). American Chemistry Council. August 2007. 1-58. https://www.americanchemistry.com/ProductsTechnology/Hydrogen-Fluoride-2/Emergency-Response-Guidelines-for-AHF.pdf Accessed August 15, 2018.

26. Guidelines for the safe use of hydrofluoric acid. Harvard University, Department of Chemistry and Chemical Biology. Cambridge, MA, USA. February 2013:1-8.

https://chemistry.harvard.edu/files/chemistry/files/safe_use_of_hf_0.pdf Accessed August 10, 2018.

27. http://www.ivoclarvivadent.us/zoolu-website/media/document/4176/IPS+Ceramic +Etching+ gel+ (document last reviewed on 04/14/2016). Accessed May 1, 2016.

28. Bartlett D. Dermal exposure to hydrofluoric acid causing significant systemic toxicity. J Emerg Nurs. 2004; 30(4):371-3. https://doi.org/10.1016/j.jen.2004.06.014 PMid:15282524

29. Hatzifotis M, Williams A, Muller M, Pegg S. Hydrofluoric acid burns. Burns. 2004; 30(2):156-9. https://doi.org/10.1016/j.burns.2003.09.031 PMid:15019125

30. Stuke LE, Arnoldo BD, Hunt JL, Purdue GF. Hydrofluoric acid burns: a 15-year experience. J Burn Care Res. 2008; 29(6):893-6. https://doi.org/10.1097/BCR.0b013e31818b9de6 PMid:18849854

31. Barker L. Hydrofluoric acid skin exposure. Nursing. 2012; 42(6):72. https://doi.org/10.1097/01.NURSE.0000414644.43323.66 PMid:22627830

32. Wang X, Zhang Y, Ni L, You C, Ye C, Jiang R, Liu L, Liu J, Han C. A review of treatment strategies for hydrofluoric acid burns: current status and future prospects. Burns. 2014; 40(8):1447-57. https://doi.org/10.1016/j.burns.2014.04.009 PMid:24946967

33. Atley K, Ridyard E. Treatment of hydrofluoric acid exposure to the eye. Int J Ophthalmol. 2015; 8(1):157-61. PMid:25709926 PMCid:PMC4325260

34. Bentur Y, Tannenbaum S, Yaffe Y, Halpert M. The role of calcium gluconate in the treatment of hydrofluoric acid eye burn. Ann Emerg Med. 1993; 22(9):1488-90. https://doi.org/10.1016/S0196-0644(05)82003-7

35. Kawaura F, Fukuoka M, Aragane N, Hayashi S. Acute respiratory distress syndrome induced by hydrogen fluoride gas inhalation. Nihon Kokyuki Gakkai Zasshi. 2009; 47(11):991-5. PMid:19994593

36. Siéwé C-L, Barbe J-M, Mathieu L, Blomet J, Hall AH. Hexafluorine decontamination of 70% hydrofluoric acid (HF) vapor facial exposure: Case report. J Chem Health Saf. 2012; 19(1):7-11. https://doi.org/10.1016/j.jchas.2011.05.011

37. Antar-Shultz M, Rifkin SI, McFarren C. Use of hemodialysis after ingestion of a mixture of acids containing hydrofluoric acid. Int J Clin Pharmacol Ther. 2011; 49(11):695-9. https://doi.org/10.5414/CP201532 PMid:22011695

38. Kavakli AS, Ozturk NK. Recurrent ventricular fibrillation associated with acute ingestion of hydrofluoric acid. J Clin Anesth. 2018; 46:8-9. https://doi.org/10.1016/j.jclinane.2018.01.009 PMid:29320713

39. Heard K, Delgado J. Oral decontamination with calcium or magnesium salts does not improve survival following hydrofluoric acid ingestion. J Toxicol Clin Toxicol. 2003; 41(6):789-92. https://doi.org/10.1081/CLT-120025343 PMid:14677788

40. Upfal M, Doyle C. Medical management of hydrofluoric acid exposure. J Occup Med. 1990; 32(8):726-31. PMid:2401930

41. Hydrogene Fluoride. In: Acute Exposure Guideline Levels for Selected Airborne Chemicals: Volume 4. Committee on Toxicology, National Research Council (US). National Academies Press (US) Washington, D.C.; 2004:123-98. https://www.ncbi.nlm.nih.gov/books/NBK207733/

42. Bosse GM, Matyunas NJ. Delayed toxidromes. Emerg J Med. 1999; 17(4):679-90. https://doi.org/10.1016/S0736-4679(99)00064-5

43. https://inis.iaea.org/collection/NCLCollectionStore/_Public/16/077/16077051.pdf

44. Burgher F, Mathieu L, Lati E, Gasser P, Peno-Mazzarino L, Blomet J, Hall AH, Maibach HI. Experimental 70% hydrofluoric acid burns: histological observations in an established human skin explants ex vivo model. Cutan Ocul Toxicol. 2011; 30(2):100-7. https://doi.org/10.3109/15569527.2010.533316 PMid:21077748 PMCid:PMC3116716

45. Pu Q, Qian J, Tao W, Yang A, Wu J, Wang Y. Extracorporeal membrane oxygenation combined with continuous renal replacement therapy in cutaneous burn and inhalation injury caused by hydrofluoric acid and nitric acid. Medicine. 2017; 96(48):e8972. https://doi.org/10.1097/MD.0000000000008972 PMid:29310404 PMCid:PMC5728805

46. Kilo S, Dennerlein K, Korinth G, Göen T, Drexler H. Dermal absorption of fluoride and hydrogen ions following topical exposure to hydrofluoric acid. Occup Environ Med. 2018; 75(Suppl 2):A409.

47. Dennerlein K, Kiesewetter F, Kilo S, Jäger T, Göen T, Korinth G, Drexler H. Dermal absorption and skin damage following hydrofluoric acid exposure in an ex vivo human skin model. Toxicol Lett. 2016; 248:25-33. https://doi.org/10.1016/j.toxlet.2016.02.015 PMid:26930472

48. Mathieu L, Burgher F, Constant S, Huang S, Hall AH, Blomet J, Fortin J-L, Schrage NF. Hydrofluoric acid: update about knowledge on eye/skin and respiratory exposure damages and decontamination. Occup Environ Med. 2018; 75(Suppl 2):A19.

49. Burgher F, Mathieu L, Lati E, Gasser P, Peno-Mazzarino L, Blomet J, Hall AH, Maibach HI. Part 2. Comparison of emergency washing solutions in 70% hydrofluoric acid-burned human skin in an established ex vivo explants model. Cutan Ocul Toxicol. 2011; 30(2):108-15. https://doi.org/10.3109/15569527.2010.534748 PMid:21083510 PMCid:PMC3116720

50. Köhnlein H, Achinger R. A new method of treatment of hydrofluoric acid burns of the extremities. Chir Plast. 1982; 6(4):297-305. https://doi.org/10.1007/BF00288763

51. De Capitani EM, Hirano ES, Zuim Ide S, Bertanha L, Vieira RJ, Madureira PR, Bucaretchi F. Finger burns caused by concentrated hydrofluoric acid, treated with intra-arterial calcium gluconate infusion: case report. Sao Paulo Med J. 2009; 127(6):379-81. https://doi.org/10.1590/S1516-31802009000600011

52. Park SE, Lee JY, Kim CW, Kim SS. Hydrofluoric acid burn on a fingertip treated successfully with single session of subcutaneous injection of 6.7% Calcium Gluconate. Ann Dermatol. 2016; 28(5):639-40. https://doi.org/10.5021/ad.2016.28.5.639 PMid:27746647 PMCid:PMC5064197

53. Peter J, Maksan S-M, Eichler K, Schmandra TC, Schmitz-Rixen T. Hydrofluoric acid burn of the hand – a rare emergency. Eur J Vasc Endovasc Surg. 2012; 24(4):e19-e20.

54. Wedler V, Guggenheim M, Moron M, Künzj W, Meyer VE. Extensive hydrofluoric acid injuries: a serious problem. J Trauma. 2005; 58(4):852-7. https://doi.org/10.1097/01.TA.0000114528.15627.65 PMid:15824669

55. Spöler F, Frentz M, Först M, Kurz H, Schrage NF. Analysis of hydrofluoric acid penetration and decontamination of the eye by means of time-resolved optical coherence tomography. Burns. 2008; 34(4):549-55. https://doi.org/10.1016/j.burns.2007.05.004 PMid:17869429

56. Kono K, Watanabe T, Dote T, Usuda K, Nishiura H, Tagawa T, Tominaga M, Higuchi Y, Onnda M. Successful treatments of lung injury and skin burn due to hydrofluoric acid exposure. Int Arch Occup Environ Health. 2000; 73 Suppl:S93-7. https://doi.org/10.1007/PL00014634 PMid:10968568

57. Shin JS, Lee S-W, Kim N-H, Park J-S, Kim KJ, Choi S-H, HongY-S. Successful extracorporeal life support after potentially fatal pulmonary oedema caused by inhalation of nitric and hydrofluoric acid fumes. Resuscitation. 2007; 75(1):184–8. https://doi.org/10.1016/j.resuscitation.2007.04.004 PMid:17507140

58. Björnhagen V, Höjer J, Karlson-Stiber C, Seldén AI, Sundbom M. Hydrofluoric acid-induced burns and life-threatening systemic poisoning - favorable outcome after hemodialysis. J Toxicol Clin Toxicol. 2003; 41(6):855-60. https://doi.org/10.1081/CLT-120025351 PMid:14677796

59. Yoshimura CA, Mathieu L, Hall AH, Monteiro MG, de Almeida DM. Seventy per cent hydrofluoric acid burns: delayed decontamination with hexafluorine® and treatment with calcium gluconate. J Burn Care Res. 2011; 32(4):e149-54. https://doi.org/10.1097/BCR.0b013e31822240f7 PMid:21747332

60. Zhang Y, Wang X, Liu Y, Jiang X, Ye C, Ni L, Zhang L, Zhang J, Xu B, Han C. Management of a rare case with severe hydrofluoric acid burns: important roles of neutralizers and continuous renal replacement therapy. Int J Low Extrem Wounds. 2017; 16(4):289-295. https://doi.org/10.1177/1534734617736198 PMid:29132247

61. Dalamaga M, Karmaniolas K, Nikolaidou A, Papadavid E. Hypocalcemia, hypomagnesemia, and hypokalemia following hydrofluoric acid chemical injury. J Burn Care Res. 2008; 29(3):541-3. https://doi.org/10.1097/BCR.0b013e3181711152 PMid:18388571

62. Wu ML, Deng JF, Fan JS. Survival after hypocalcemia, hypomagnesemia, hypokalemia and cardiac arrest following mild hydrofluoric acid burn. Clin Toxicol (Phila). 2010; 48(9):953-5. https://doi.org/10.3109/15563650.2010.533676 PMid:21171855

63. Thomas D, Jaeger U, Sagoschen I, Lamberti C, Wilhelm K. Intra-arterial calcium gluconate treatment after hydrofluoric acid burn of the hand. Cardiovasc Intervent Radiol. 2009; 32(1):155-8. https://doi.org/10.1007/s00270-008-9361-1 PMid:18506520

64. Mathieu L, Nehles J, Blomet J, Hall AH. Efficacy of hexafluorine for emergent decontamination of hydrofluoric acid eye and skin splashes. Vet Hum Toxicol. 2001; 43(5):263-5. PMid:11577928

65. Dennerlein K, Hahn T, Göen T, Drexler H, Kilo S. Hydrofluoric acid – effects of skin decontamination on the bioavailability of fluoride. Occup Environ Med. 2018; 75 (Suppl 2):A408-A409.

66. Hultén P, Höjer J, Ludwigs U, Janson A. Hexafluorine vs. standard decontamination to reduce systemic toxicity after dermal exposure to hydrofluoric acid. J Toxicol Clin Toxicol. 2004; 42(4):355-61. https://doi.org/10.1081/CLT-120039541 PMid:15461243

67. Brent J. Water-based solutions are the best decontaminating fluids for dermal corrosive exposures: a mini review. Clin Toxicol (Phila). 2013; 51(8):731-6. https://doi.org/10.3109/15563650.2013.838628 PMid:24003912

68. Ohata U, Hara H, Suzuki H. 7 cases of hydrofluoric acid burn in which calcium gluconate was effective for relief of severe pain. Contact Dermatitis. 2005; 52(3):133-7. https://doi.org/10.1111/j.0105-1873.2005.00521.x PMid:15811026

69. Anderson WJ, Anderson JR. Hydrofluoric acid burns of the hand: mechanism of injury and treatment. J Hand Surg Am. 1988; 13(1):52-7. https://doi.org/10.1016/0363-5023(88)90200-6

70. Strausburg M, Travers J, Mousdicas N. Hydrofluoric acid exposure: a case report and review on the clinical presentation and management. Dermatitis. 2012; 23(5):231-6. https://doi.org/10.1097/DER.0b013e31826e457a PMid:23010832

71. Han HH, Kwon BY, Jung SN, Moon SH. Importance of initial management and surgical treatment after hydrofluoric acid burn of the finger. Burns. 2017; 43(1):e1-e6. https://doi.org/10.1016/j.burns.2016.07.031 PMid:27650188

72. Nosé RM, Daga FB, Nosé W, Kasahara N. Optical coherence tomography analysis of hydrofluoric acid decontamination of human cornea by mannitol solution. Burns. 2017; 43(2):424-8. https://doi.org/10.1016/j.burns.2016.08.012 PMid:27608526

73. Soderberg K, Kuusinen P, Mathieu L, Hall AH. An improved method for emergent decontamination of ocular and dermal hydrofluoric acid splashes. Vet Hum Toxicol. 2004; 46(4):216-8. PMid:15303400

74. Henry JA, Hla KK. Intravenous regional calcium gluconate perfusion for hydrofluoric acid burns. J Toxicol Clin Toxicol. 1992; 30(2):203-7. https://doi.org/10.3109/15563659209038631

75. Gupta R. Intravenous calcium gluconate in the treatment of hydrofluoric acid burns. Ann Emerg Med. 2001; 37(6):734-5. https://doi.org/10.1067/mem.2001.115842 PMid:11385351

76. Zhang Y, Wang X, Ye C, Liu L, Jiang R, Ni L, Xia W, Han C. The clinical effectiveness of the intravenous infusion of calcium gluconate for treatment of hydrofluoric acid burn of distal limbs. Burns. 2014; 40(4):e26-30. https://doi.org/10.1016/j.burns.2013.12.003 PMid:24418646

77. Lin TM, Tsai CC, Lin SD, Lai CS. Continuous intra-arterial infusion therapy in hydrofluoric acid burns. J Occup Environ Med. 2000; 42(9):892-7. https://doi.org/10.1097/00043764-200009000-00008 PMid:10998764

78. Yuanhai Z, Liangfang N, Xingang W, Ruiming J, Liping L, Chunjiang Y, Wenhao X, Chunmao H. Clinical arterial infusion of calcium gluconate: the preferred method for treating hydrofluoric acid burns of distal human limbs. Int J Occup Med Environ Health. 2014; 27(1):104-13. PMid:24464441

79. Nguyen LT, Mohr WJ 3rd, Ahrenholz DH, Solem LD. Treatment of hydrofluoric acid burn to the face by carotid artery infusion of calcium gluconate. J Burn Care Rehabil. 2004; 25(5):421-4. https://doi.org/10.1097/01.BCR.0000138288.15403.BA PMid:15353934

80. Miyamoto K, Shimizu M, Tanaka K, Minemura A, Tamatsukuri T, Miyake Y, Aruga T. Case of continuous trans-arterial calcium gluconate infusion using a direct arterial sphygmomanometry line that exhibited dramatic improvement of chemical burns on the fingers caused by hydrofluoric acid. Chudoku Kenkyu. 2014; 27(4):343-7. PMid:25771670

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Review Article

Seborrheic Keratoses – The Most Common Benign Skin Tumor of Humans. Clinical presentation and an update on pathogenesis and treatment options

Uwe Wollina*

Städtisches Klinikum Dresden, Department of Dermatology and Allergology, Dresden, Germany

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***Correspondence:** Uwe Wollina. Städtisches Klinikum Dresden, Department of Dermatology and Allergology, Dresden, Germany. E-mail: uwollina@gmail.com

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Abstract

Seborrheic keratoses (SK) are the most common skin tumour of humanity. The incidence of this purely benign epithelial proliferation is increasing with age and exposure to ultraviolet light. It has a remarkable variability in its clinical presentation raising some differential diagnoses. Recently, oncogenic mutations have been detected involved in the development of SK, which, however, do not bear the risk of malignant transformation. SK may also develop with the use of modern targeted drugs for the treatment of malignancies. The classical treatment options for SK are cryotherapy and curettage. Recently, topical treatment with 40% hydrogen peroxide and the nitric-zinc complex has been investigated. Ablative laser therapy is an effective treatment as well.

Introduction

Seborrheic keratoses (SKs) are the most common benign epithelial tumours of humanity with an increasing incidence with age. The predilection zones of SKs are trunk and forehead. An important clinical sign is the formation of multiple horn pearls [1]. In dermoscopy, comedo-like openings, milia-like cysts, and fissures and ridges are characteristic [2]. Although most SKs have a maximum diameter of less than 4 cm, sometimes giant lesions develop that raises some possible differential diagnoses including Buschke-Löwenstein tumours [3].

On the histologic level six subtypes can be differentiated: - hyperkeratotic type; - acanthotic type; - reticular/adenoid type; - clonal type; - irritated type; - melanoakanthoma [1]. Multiple eruptive SKs was known as Leser-Trélat-syndrome gain importance as a paraneoplasia [4].

Pathogenesis

The etiopathology of SKs is not completely understood. They are considered a sign of ageing skin and UV-exposure may be associated with. The viral hypothesis is suggesting the involvement of human papillomavirus has not been substantiated by recent studies [5], [6].

Expression of the amyloid precursor protein (APP) has been evaluated in SKs versus normal skin by immunohistochemistry, Western blotting and quantitative real-time polymerase chain reaction (PCR). APP and its downstream products (i.e. amyloid-β42) were stronger expressed in SK than in paired adjacent normal skin tissues. In contrast, the expression of its key secretase (i.e. β-secretase1) was low.

Furthermore, APP expression was higher in UV-exposed than non-exposed skin sites and the

older age group. APP expression correlated positively with age in the epidermis ($p < 0.05$), but not in the dermis. These findings suggest that overexpression of APP may promote the onset of SK and is a marker of skin ageing and UV damage [7].

Exome sequencing of SKs indicated three mutations per megabase pairs of the targeted sequence. The mutational pattern depicted typical UV signature with the majority of alterations being C > T and CC > TT base changes at dipyrimidinic sites. The *FGFR3* mutations were the most frequent, detected in 48% of lesions, followed by the *PIK3CA* (32%), *TERT* promoter (24%) and *DPH3* promoter mutations (24%) [8].

Neel et al., (2016) investigated SKs, which frequently have acquired oncogenic mutations in the receptor tyrosine kinase/phosphatidylinositol 3-kinase/Akt signalling cascade. They demonstrated that SKs have a hypersensitivity to Akt inhibition. FoxN1 is a novel biomarker of the oncogenically activated but benign phenotype in SKs.

They also established that Akt inhibition caused an increase in p53 protein expression, but not RNA expression and that Akt-mediated apoptosis was dependent on p53 and FoxO3, a target of Akt [9].

Hyperkeratotic type

The histologic feature of this common subtype is orthohyperkeratosis with papillomatosis. Acanthosis is mild or absent. Horn pearls are missing (Figure 1) [1].

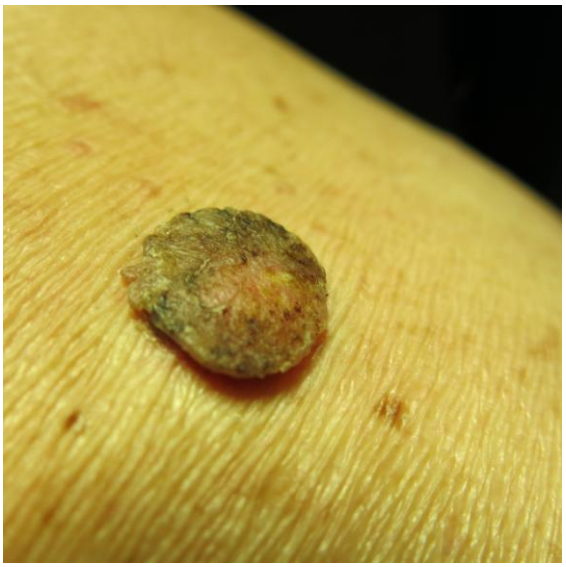

Figure 1: Hyperkeratotic seborrheic keratosis

This is the most common subtype (Figure 2). The tumours are characterised by huge acanthosis but mild or missing hyperkeratosis and papillomatosis. Horny invaginations that on cross-sections appear as “pseudo-horn cysts” are numerous. True horn cysts are also seen, which show sudden and complete keratinisation with a very thin granular layer surrounding it [1].

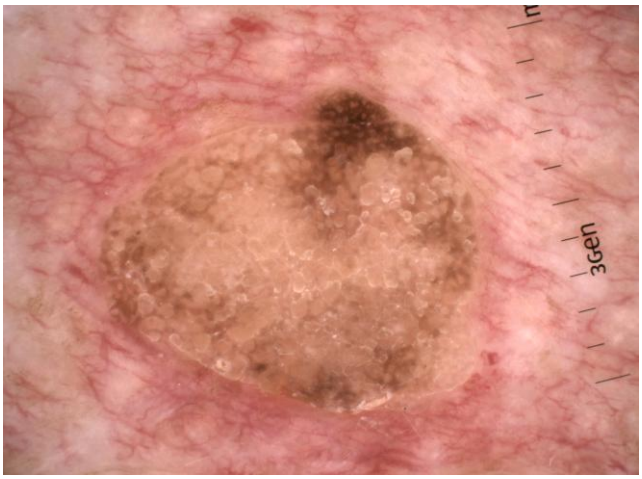

Figure 2: Dermoscopic presentation of acanthotic seborrheic keratosis with multiple pseudo-horn cysts

Reticular or adenoid type

The SKs of this subtype is characterised by reticular acanthosis of thin, double row with mild to moderate hyperkeratosis and papillomatosis. These SKs are often hyperpigmented. Horn pearls are uncommon. This subtype is more common in sun-exposed areas (Figures 3 and 4) [10].

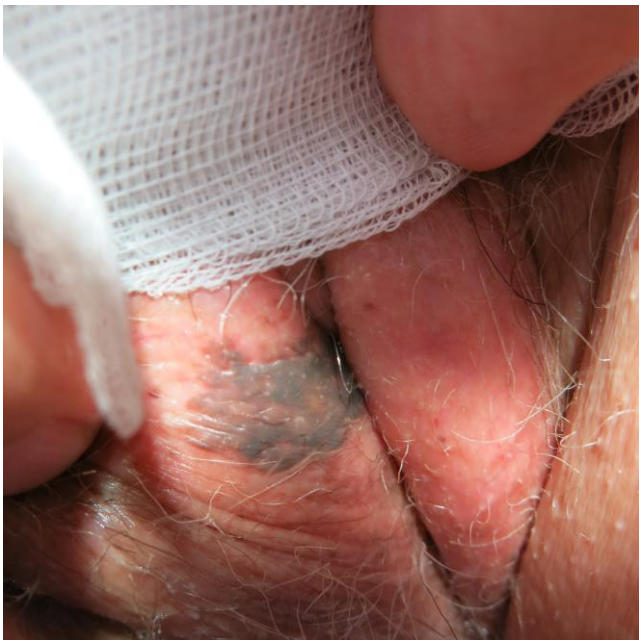

Figure 3: Hyperpigmented adenoid seborrheic keratosis of the vulva

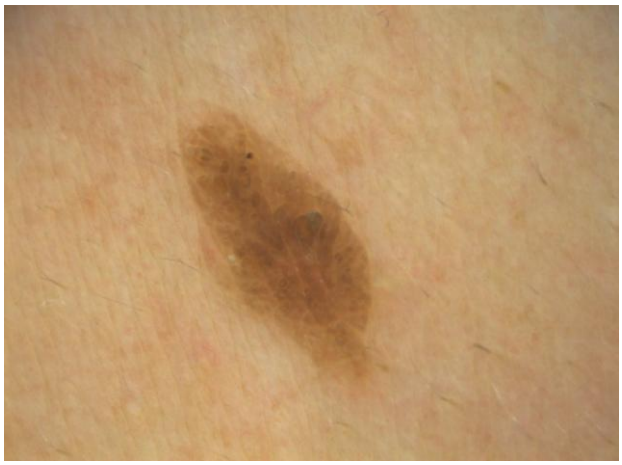

Figure 4: Reticular seborrheic keratosis with an oak-leave appearance

Clonal type

In this type marked acanthosis and papillomatosis are associated with orthohyperkeratosis. Tumour cells are spindle-shaped. With a tumour demarcated tumour, islands of basaloid cell nests are found. Major differential diagnosis to clinal SK is pagetoid Bowen's disease (Figure 5). Immunohistochemistry may be helpful in selected cases to distinguish both entities. Cytokeratin-10 is more expressed in clonal SK, while increased Ki-67-positive cells and the presence of > 75% positive p16 cells is in favour of pagetoid Bowen's disease [11].

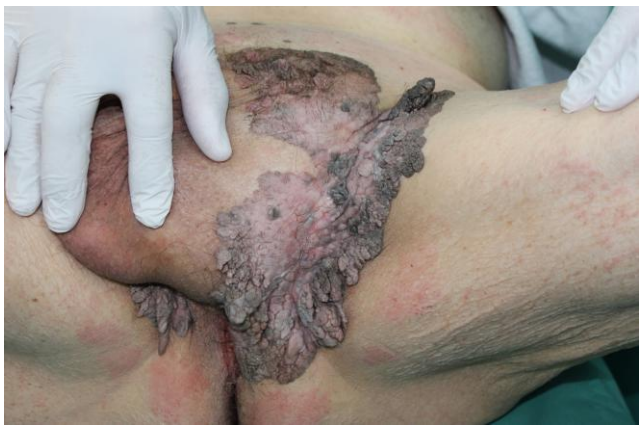

Figure 5: Giant hyperpigmented clonal seborrheic keratosis resembling Buschke-Löwenstein a tumour

Irritated type

Here, a proliferation of spindle-shaped eosinophilic tumours cells is found, sometimes in whorl-like formations. Dyskeratotic cells may be present. In clinical practice, it is important to separate irritated SK from cutaneous squamous cell carcinoma (SCC) (Figure 6). Immunohistochemistry may be helpful to distinguish both entities. In a recent study, the combination of U3 small nucleolar ribonucleoprotein protein IMP3 and B-cell lymphoma 2 regulatory protein (BCL-2) may has been shown diagnostic utility in distinguishing between irritated SK and SCC in daily clinical practice while epidermal growth factor receptor (EGFR) immunohistochemistry did not appear to be useful in this setting [12].

Figure 6: Irritated seborrheic keratosis resembling cutaneous squamous cell carcinoma

In the illustrated case of an SK prepatellar, koilocytes were present as a histological sign of a secondary viral infection. A higher prevalence of human papillomavirus p16 expression has been found preferably in genital SKs with 65% to 69.6% [13], [14]. The prevalence is lower in vulvar SKs in women older than 50 years with 14.3% [15]. Herpes virus 6A is another virus that can be detected in SKs [16]. In a recent study, Merkel cell polyomavirus could be detected in 6 of 23 SKs by polymerase chain reaction (PCR) and fluorescence in situ hybridisation (FISH) [14]. Expression of p16 and p21 had been detected in SKs with Bowenoid transformation [17].

With the development of checkpoint inhibitors, new adverse events have been seen in treated patients. Rhambia et al., (2018) reported on inflammatory reactions in SKs induced by programmed cell death-1 (PD1) inhibitor nivolumab. Chronic viral infections are known to result in T-cell tolerance because of persistent antigen stimulation. A viral infection is a secondary event in SKs. PD-1 inhibition can reinvigorate exhausted T cells, which are accordingly able to decrease viral load. Thus, the inflammatory reaction of SKs may be the result of PD-1 inhibition reactivating virally driven T lymphocytes [18].

LL-37 is a naturally occurring 37-amino-acid peptide that is part of the innate immune system in human skin. Studies have shown that intra-tumoral

injections of LL-37 stimulate the innate immune system by activation of plasmacytoid dendritic cells, which mediate tumour destruction. It has been reported that LL-37 induces an inflammatory response to SKs characterised by a lichenoid cellular infiltrate [19].

Melanoakanthoma

This is the hyperpigmented acanthotic type with a proliferation of basaloid cells with mild or even absent hyperkeratosis. Numerous melanocytes are intermingled. Melanophages are located within the dermis underneath a tumour (Fig. 7) [1].

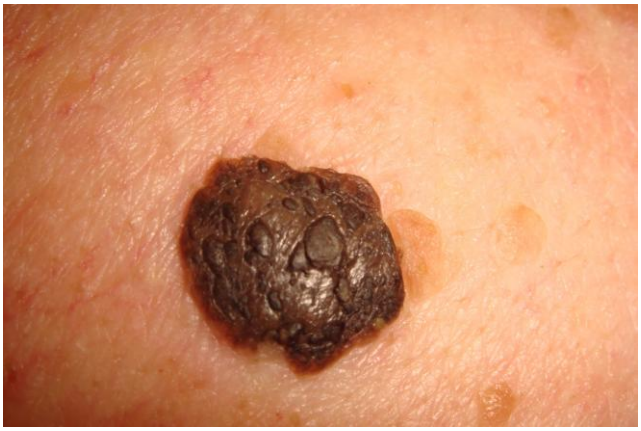

Figure 7: Melanoacanthoma with prominent horn cysts

Treatment

Patients have wide ranges of motivations for treating or removing SKs, including embarrassment from the stigmatising appearance of the lesion, physical irritation or pruritus, and a desire to look younger [20].

Lesions that are inflamed, bleeding, ulcerated, or sufficiently irritated should be further characterised by a biopsy or excision to rule out malignancies [21].

Two recent randomised trials (ClinicalTrials.gov identifiers: NCT02667236 and NCT02667275) compared the safety and efficacy of 40% hydrogen peroxide topical solution (HP40; ESKATA^{TM}, Aclaris Therapeutics, Inc., Wayne, PA) versus vehicle (VEH) for the treatment of SKs. This trial included 937 patients with 4 SKs who were randomised 1:1 to HP40 or VEH.

The clinical response was graded using the Physician's Lesion Assessment (PLA) scale (0: Clear; 1: Near-Clear; 2: ≤ 1 mm thick, and 3: > 1 mm thick). After one treatment, SKs with PLA > 0 were re-treated 3 weeks later. At day 106, significantly more HP40 patients versus VEH achieved PLA = 0 on all 4 SKs (4% or 8% vs 0%) and 3 of 4 SKs (13% or 23 % vs 0%). A higher mean per-patient percentage of SKs were Clear (25% vs 2 % or 34% vs 1%) and Clear/Near-Clear (47% vs 10% or 54% vs 5%) with HP40 versus VEH. Most local skin reactions were mild and resolved by day 106 [22]. HP40 may act not only through its direct oxidation of organic tissues, generation of reactive oxygen species, and local lipid peroxidation but also by the generation of local concentrations of oxygen that are toxic to SK cells [23] [24].

A nitric-zinc complex has been used to treat 50 SK lesions. The topical formulation is an aqueous solution containing nitric acid, zinc and copper salts, and organic acids (acetic, lactic, and oxalic acid). Treatment consisted of the topical application to obtain a whitening or yellowish reaction on top of SKs. Application of the nitric-zinc solution was performed every other week until clinical and dermoscopic clearance or crust formation, for a maximum of 4 applications. All subjects, who reported no or minimal discomfort during and after the application of the solution, completed the study. After eight weeks, complete clearance was observed in 37 lesions after an average of 3 applications/lesion. A partial response, with minimal persistent residual spots, was detected in the remaining 13 lesions. All patients with complete clearance showed no relapses at a 6-month follow-up [25].

Case reports have been published with the successful use of either diclofenac gel, imiquimod, dobesilate or calcitriol [26], [27], [28], [29].

Patients preferred cryotherapy over curettage in a small trial (n = 25). But physicians rating observed more redness at 6 weeks and the tendency for hypopigmented scar formation at greater than 12 months with curettage. Leftover SK lesion occurred more frequently with cryotherapy in the short and long term [30].

The use of ablative lasers and cryotherapy for SKs has a long tradition. But which method is better? A comparative trial was carried out on 42 patients with SK of 0.5-3 cm, located on the back, chest, face and neck. Lesions with a similar size and location on the same patient were matched. In the same session, half of the lesions were treated with cryotherapy, while the other half were treated with Er: YAG laser [31].

Following the first treatment, complete healing was detected in all of the lesions (100%) treated with Er: YAG lasers, while the healing rate was 68% in the cryotherapy group ($p < 0.01$). In the Er: YAG laser-treated group, hyperpigmentation was significantly lower and more erythema developed than in the cryotherapy group [31]. The results are in one line with our own experiences with Er: YAG laser [32].

The CO_2 laser is another opportunity but with

a higher risk of scarring and pigmentary changes [33]. Small case series have used intense pulsed light [34].

In conclusion, SKs are very common. Their clinical variability can confuse with other potentially malignant skin diseases. Treatment options include topical and surgical options.

References

1. Jackson JM, Alexis A, Berman B, Berson DS, Taylor S, Weiss JS. Current Understanding of Seborrheic Keratosis: Prevalence, Etiology, Clinical Presentation, Diagnosis, and Management. J Drugs Dermatol. 2015; 14(10):1119-25. PMid:26461823

2. Takenouchi T. Key points in dermoscopic diagnosis of basal cell carcinoma and seborrheic keratosis in Japanese. J Dermatol. 2011; 38(1):59-65. https://doi.org/10.1111/j.1346-8138.2010.01093.x PMid:21175757

3. Wollina U, Chokoeva A, Tchernev G, Heinig B, Schönlebe J. Anogenital giant seborrheic keratosis. G Ital Dermatol Venereol. 2017; 152(4):383-6. PMid:25604463

4. Thiers BH, Sahn RE, Callen JP. Cutaneous manifestations of internal malignancy. CA Cancer J Clin. 2009; 59(2):73-98. https://doi.org/10.3322/caac.20005 PMid:19258446

5. Tsambaos D, Monastirli A, Kapranos N, Georgiou S, Pasmatzi E, Stratigos A, Koutselini H, Berger H. Detection of human papillomavirus DNA in nongenital seborrhoeic keratoses. Arch Dermatol Res. 1995; 287(6):612-5. https://doi.org/10.1007/BF00374085 PMid:7487151

6. Kambiz KH, Kaveh D, Maede D, Hossein A, Nessa A, Ziba R, Alireza G. Human Papillomavirus Deoxyribonucleic Acid may not be Detected in Non-genital Benign Papillomatous Skin Lesions by Polymerase Chain Reaction. Indian J Dermatol. 2014; 59(4):334-8. https://doi.org/10.4103/0019-5154.135475 PMid:25071248 PMCid:PMC4103265

7. Li Y, Wang Y, Zhang W, Jiang L, Zhou W, Liu Z, Li S, Lu H. Overexpression of Amyloid Precursor Protein Promotes the Onset of Seborrhoeic Keratosis and is Related to Skin Ageing. Acta Derm Venereol. 2018; 98(6):594-600. https://doi.org/10.2340/00015555-2911 PMid:29487944

8. Heidenreich B, Denisova E, Rachakonda S, Sanmartin O, Dereani T, Hosen I, Nagore E, Kumar R. Genetic alterations in seborrheic keratoses. Oncotarget. 2017; 8(22):36639-49. https://doi.org/10.18632/oncotarget.16698 PMid:28410231 PMCid:PMC5482683

9. Neel VA, Todorova K, Wang J, Kwon E, Kang M, Liu Q, Gray N, Lee SW, Mandinova A. Sustained Akt Activity Is Required to Maintain Cell Viability in Seborrheic Keratosis, a Benign Epithelial Tumor. J Invest Dermatol. 2016; 136(3):696-705. https://doi.org/10.1016/j.jid.2015.12.023 PMid:26739095

10. Roh NK, Hahn HJ, Lee YW, Choe YB, Ahn KJ. Clinical and Histopathological Investigation of Seborrheic Keratosis. Ann Dermatol. 2016; 28(2):152-8. https://doi.org/10.5021/ad.2016.28.2.152 PMid:27081260 PMCid:PMC4828376

11. Kalegowda IY, Böer-Auer A. Clonal Seborrheic Keratosis Versus Pagetoid Bowen Disease: Histopathology and Role of Adjunctive Markers. Am J Dermatopathol. 2017; 39(6):433-9. https://doi.org/10.1097/DAD.0000000000000669 PMid:28475506

12. Richey JD, Deng AC, Dresser K, O'Donnell P, Cornejo KM. Distinguishing between irritated seborrheic keratosis and squamous cell carcinoma in situ using BCL-2 and IMP3 immunohistochemistry. J Cutan Pathol. 2018; 45(8):603-9. https://doi.org/10.1111/cup.13269 PMid:29726030

13. Harvey NT, Leecy T, Wood BA. Immunohistochemical staining for p16 is a useful adjunctive test in the diagnosis of Bowen's disease. Pathology. 2013; 45(4):402-7. https://doi.org/10.1097/PAT.0b013e328360c064 PMid:23635817

14. Hillen LM, Rennspiess D, Speel EJ, Haugg AM, Winnepenninckx V, Zur Hausen A. Detection of Merkel Cell Polyomavirus in Seborrheic Keratosis. Front Microbiol. 2018; 8:2648. https://doi.org/10.3389/fmicb.2017.02648 PMid:29375515 PMCid:PMC5767171

15. Reutter JC, Geisinger KR, Laudadio J. Vulvar seborrheic keratosis: is there a relationship to human papillomavirus? J Low Genit Tract Dis. 2014; 18(2):190-4. https://doi.org/10.1097/LGT.0b013e3182952357 PMid:24556611

16. Ding L, Mo X, Zhang L, Zhou F, Zhu C, Wang Y, Cai C, Liu Y, Wei F, Cai Q. High prevalence and correlates of human herpesvirus-6A in nevocytic nevus and seborrheic diseases: Implication from a pilot study of skin patient tissues in Shanghai. J Med Virol. 2018; 90(9):1532-40. https://doi.org/10.1002/jmv.25217 PMid:29727474

17. Wu YH, Hsiao PF, Chen CK. Seborrheic keratosis with bowenoid transformation: the immunohistochemical features and its association with human papillomavirus infection. Am J Dermatopathol. 2015; 37(6):462-8. https://doi.org/10.1097/DAD.0000000000000285 PMid:25747812

18. Rambhia PH, Honda K, Arbesman J. Nivolumab induced inflammation of seborrheic keratoses: a novel cutaneous manifestation in a metastatic melanoma patient. Melanoma Res. 2018; 28(5):475-77. https://doi.org/10.1097/CMR.0000000000000477

19. Dolkar T, Trinidad CM, Nelson KC, Amaria RN, Nagarajan P, Torres-Cabala CA, Ivan D, Prieto VG, Tetzlaff MT, Curry JL, Aung PP. Dermatologic toxicity from novel therapy using antimicrobial peptide LL-37 in melanoma: A detailed examination of the clinicopathologic features. J Cutan Pathol. 2018; 45(7):539-44. https://doi.org/10.1111/cup.13262 PMid:29665030

20. Del Rosso JQ. A closer look at seborrheic keratoses: patient perspectives, clinical relevance, medical necessity, and implications for management. J Clin Aesthet Dermatol. 2017; 10(3):16-25. PMid:28360965 PMCid:PMC5367878

21. Karadag AS, Parish LC. The status of the seborrheic keratosis. Clin Dermatol. 2018; 36(2):275-7. https://doi.org/10.1016/j.clindermatol.2017.09.011 PMid:29566932

22. Baumann LS, Blauvelt A, Draelos ZD, Kempers SE, Lupo MP, Schlessinger J, Smith SR, Wilson DC, Bradshaw M, Estes E, Shanler SD. Safety and efficacy of hydrogen peroxide topical solution, 40%(w/w) in patients with seborrheic keratoses: results from two identical, randomized, double-blind, placebo-controlled, phase 3 studies (A-101-SEBK-301/302). Journal of the American Academy of Dermatology. 2018. https://doi.org/10.1016/j.jaad.2018.05.044

23. Bekeschus S, Kolata J, Winterbourn C, et al. Hydrogen peroxide: a central player in physical plasma-induced oxidative stress in human blood cells. Free Radic Res. 2014; 48(5):542-9. https://doi.org/10.3109/10715762.2014.892937 PMid:24528134

24. Oyewole AO, Wilmot MC, Fowler M, et al. Comparing the effects of mitochondrial targeted and localized antioxidants with cellular antioxidants in human skin cells exposed to UVA and hydrogen peroxide. FASEB J. 2014; 28(1):485-94. https://doi.org/10.1096/fj.13-237008 PMid:24115050

25. Lacarrubba F, Nasca MR, Verzì AE, Micali G. A novel topical agent in the treatment of seborrheic keratoses: A proof of concept study by clinical and dermoscopic evaluation. Dermatol Ther. 2017; 30(5).

26. Aktaş H, Ergin C, Keseroğlu HÖ. Diclofenac gel may be a new treatment option for seborrheic keratosis. Indian Dermatol Online J. 2016; 7(3):211-2. https://doi.org/10.4103/2229-5178.182363 PMid:27294065 PMCid:PMC4886602

27. Karadag AS, Ozlu E, Uzuncakmak TK, Akdeniz N, Cobanoglu B, Oman B. Inverted follicular keratosis successfully treated with imiquimod. Indian Dermatol Online J. 2016; 7(3):177-9.

https://doi.org/10.4103/2229-5178.182354 PMid:27294052 PMCid:PMC4886589

28. Cuevas P, Angulo J, Salgüero I, Giménez-Gallego G. Clearance of seborrhoeic keratoses with topical dobesilate. BMJ Case Rep. 2012; 2012.

29. Lu'o'ng Kv, Nguyễn LT. The roles of vitamin D in seborrhoeic keratosis: possible genetic and cellular signalling mechanisms. Int J Cosmet Sci. 2013; 35(6):525-31. https://doi.org/10.1111/ics.12080 PMid:23859137

30. Wood LD, Stucki JK, Hollenbeak CS, Miller JJ. Effectiveness of cryosurgery vs curettage in the treatment of seborrheic keratoses. JAMA Dermatol. 2013; 149(1):108-9. https://doi.org/10.1001/2013.jamadermatol.275 PMid:23324775

31. Gurel MS, Aral BB. Effectiveness of erbium:YAG laser and cryosurgery in seborrheic keratoses: Randomized, prospective intraindividual comparison study. J Dermatolog Treat. 2015; 26(5):477-80. https://doi.org/10.3109/09546634.2015.1024597 PMid:25798694

32. Wollina U. Erbium-YAG laser therapy – analysis of more than 1,200 treatments. Glob Dermatol. 2016; 3(2):268-72. https://doi.org/10.15761/GOD.1000171

33. Bruscino N, Conti R, Campolmi P, Bonan P, Cannarozzo G, Lazzeri L, Moretti S. Dermatosis Papulosa Nigra and 10,600-nm CO2 laser, a good choice. J Cosmet Laser Ther. 2014; 16(3):114-6. https://doi.org/10.3109/14764172.2013.854640 PMid:24131098

34. Piccolo D, Di Marcantonio D, Crisman G, Cannarozzo G, Sannino M, Chiricozzi A, Chimenti S. Unconventional use of intense pulsed light. Biomed Res Int. 2014; 2014:618206. https://doi.org/10.1155/2014/618206 PMid:25276803 PMCid:PMC4167959