ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):813-817.
https://doi.org/10.3889/oamjms.2017.207
eISSN: 1857-9655
Basic Science

Histological Characteristics of Bruises with Different Age

Irena Kostadinova-Petrova[1], Elida Mitevska[1]*, Biljana Janeska[2]

[1]Institute for Histology and Embryology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia, [2]Institute for Forensic Medicine and Criminalistics, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Kostadinova-Petrova I, Mitevska E, Janeska B. Histological Characteristics of Bruises with Different Age. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):813-817. https://doi.org/10.3889/oamjms.2017.207

Keywords: bruises; histological analysis.

***Correspondence:** Elida Mitevska, Institute of Histology and Embryology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje. E-mail: elida.mitevska@gmail.com

Received: 20-Sep-2017; **Revised:** 15-Oct-2017; **Accepted:** 17-Oct-2017; **Online first:** 16-Oct-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: In forensics bruises as injuries take an important part in the interpretation of the causes of death. Since activating the inflammatory response of the body in their formation, histological analysis of the bruised tissue can provide data on the determination the time when the injury occurred.

AIM: The aim of this study is to compare the histological features of 1-day and 5-days old bruises.

MATERIAL AND METHODS: Bruised human skin samples, 1-day old in group A and 5-day-old in group B, obtained at autopsy from individuals who died from a violent death, were analyzed in this study. The qualitative microscopic analysis was performed on serial paraffin sections of tissues stained with Hematoxylin-eosin and Pearls Prussian Blue method, using a light microscope connected to a digital camera.

RESULTS: Qualitative histological analysis of the studied group A presented with fresh bruises, less than 24 hours old, showed ruptured smaller vessels and extravasated red blood cells in the connective tissue of the skin, with subsequent expansion and infiltration of fibrous septa of the skin. In the area of bleeding an initial infiltration by macrophages was observed. In the studied group B, presented with bruises 3-7 days old, histological analysis showed a marked presence of hemosiderin-laden macrophages and presence of hematoidin granules in the area of bleeding, as well as ruptured small blood vessels and red blood cells extravasation in the dilated fibrous septa.

CONCLUSION: A detailed analysis of tissue changes in bruises every day from the initiation until their recovery, a detailed description of the histological finding can be given, which will be supported in the precise determination of the age of the injuries themselves.

Introduction

Bruises are skin injuries which present with extravasation of blood in the connective tissue of the skin or deeper in the underlying tissues [1]. At the time of the formation of bruises, due to the impact of the force, small blood vessels break in the skin and blood extravasates into the surrounding connective tissue, resulting in activation of the cellular response, followed by infiltration of the field of bleeding firstly with neutrophil granulocytes and then with macrophages. In addition to the initial signs of an injury, such as oedema near the haemorrhage resulting in enlargement of the fibrous septa, the erythrocytes outside blood vessels activate the inflammatory response of the body, hence inflammation of the field of bleeding rapidly occurs [2]. Most commonly blood is extravasated between the dermis and hypodermissince there is a larger amount of loose connective tissue.

Three criteria must be met to create a bruise. The first criterion is the force acting on the skin; it should cause blood vessels to rupture in it, but not to disrupt the integrity of the skin. The pressure of force is usually transmitted by a blunt object. Otherwise the skin would be damaged. The second criterion is the blood pressure, which should be high enough to allow extravasation of the blood in the connective tissue of the skin. The third criterion is the location of the field of bleeding. The blood should be extravasated sufficiently close to the surface of the skin to be detected as a bruise [3]. Bruises formed during life

may not be visible due to opalescence of the skin [3, 4], but will be seen postmortem after the appearance of the skin reflection [5]. The early manifestation of blood drainage is dependent on two factors: the extravasation of blood from the blood vessels in the surrounding tissue and the depth of the skin where the blood is drained [6-8].

Bruises are not minor injuries because they can lead to death if they are extensive [9]. They are quite frequent injuries, and the determination of their age is one of the most sought data for forensic examinations, especially when it comes to cases of violent deaths and abuse in children [10] when the causes of death and the timing of the injuries are important to be known. Once the bruise is formed, the body reacts by activating the inflammatory response, and at the site of the bleeding, there is a migration of inflammatory cells, first neutrophilic granulocytes and later mononuclear macrophages. Their infiltration occurs at a certain time interval, which allows through the specific histological finding to interpret the age of the bruise [11].

In the routine examination procedures of injuries, when reviewing and interpreting bruises, the most commonly used method is observation as a method for rapid orientation for the age of the bruises. This approach as a standalone is commonly used in clinical assessment of these injuries, while in modern forensic medicine it is a baseline analysis complemented by microscopic and molecular analyses, to accurately determine the age of bruises.

Histological analysis through a detailed analysis of the cellular structure of affected tissue, as one of the additional methods for a detailed analysis of the age of bruises, provides an interpretation of the time when they occurred, as well as their age, data that can be further used in the investigation of cases of abuse in women and children, with possibility to be used as evidence in medico-legal cases.

The aim of this study was to make a comparison in the features of the bruised tissue by histological analysis with determination of the main histological findings in bruises 1-day and 5-day old, findings that further may be used as data for determining the age of bruises in addition to other methods.

Material and Methods

The research was performed on human skin samples, obtained at autopsy from individuals who died from a violent death, at the Institute of Forensic Medicine in Skopje. All tissue samples were taken in depth across the entire skin together with the hypodermis, with dimensions of 4 cm², and then fixed in a 40% formalin solution. Tissue samples contained the entire bruise and its peripheral edges where the bruise passed into the healthy tissue. The qualitative histological analysis was performed on serial paraffin sections of skin tissues of 4 μm thickness, stained according to the standard Hematoxylin-eosin method. Additionally staining with the Pearls Prussian Blue method was applied for detection of macrophages. A light microscope, model Olympus CX21FS1 and lenses 40x, 20x and 10x were used for microscopic assessment of the tissue. Figures used in this study were made using micro camera Bresser, tv lens c-0.45x connected to Nikon Eclipse E400 microscope.

Samples were divided into three analysed groups, using the findings of a court expert, who classified the bruises by age, by using an observational method during the initial analysis of the bruised tissue during the autopsy. For this kind of classification, there are more published patterns (Table 1).

• Control group contained 12 samples of human skin with resectional edges that penetrated the surrounding healthy tissue of each autopsy material.

• Group A contained six tissue samples from human skin with fresh bruises, not older than 24 hours.

• Group B contained six tissue samples from human skin with bruises aged 3-7 days. The material in this group of orientational observation was classified into bruises with an approximate age of 3 days (two samples), four days (one sample), five days (two samples) and 5-6 days (sample of the decomposed body). Except for the last sample, the remaining samples were obtained from corpses shortly after death.

Table 1: Classification of the age of bruises by colour, by five schemes published by different authors

	Adelson	Rentable	Camps	Poison	Spitz
Initial colour	Red/blue	Violet	Red	Red, black	Blue/red
1-3 days	Blue/brown	Dark blue	Blue/brown	Purple, black	Purple, black
1 week	Yellow/green	Green	Green	Green	Green/yellow
8-10 days		Yellow	Yellow		Brown
2 weeks		Normal	Normal	Yellow	Normal

Results

Control group

The control group served us as a prototype for pattern construction and was used for comparison of the histological changes that occurred in bruised skin samples.

Histological analysis of all samples in the control group showed a normal skin structure, with an intact epidermis, dermis and hypodermis, and intact blood vessels. Red blood cells were observed only in

blood vessels. The cellular population of the connective tissue of the dermis was predominantly represented by fibrocytes and was less commonly present. Collagen fibres were a dominant component, with the irregular layout. The superficial tissue of the hypodermis consisted of clustered adipocytes in the form of lobes, wrapped with intact fibrous lobe-like septum with uniform obesity. The skin annexes had normal morphology (Figure 1).

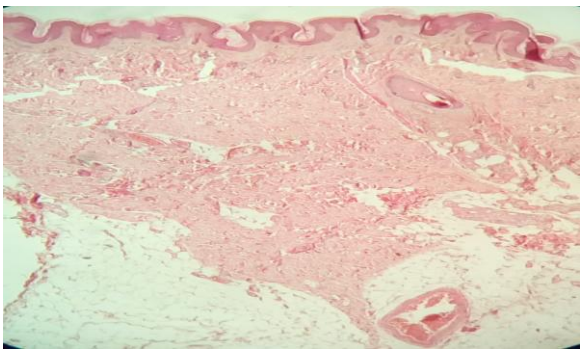


Figure 1: Normal human skin. The microphotography shows normal morphology of human skin, epidermis, dermis and part of the hypodermis (Haematoxylin-eosin, X10)

Group A

Histological analysis of tissue sections with fresh bruises, not older than 24 hours, showed a change in the skin structure only in the hypodermis, while all other layers of the skin were unchanged in comparison to the control group. In the area of the fibrous septa of hypodermis, extravasated red blood cells were present from the ruptured vessels. The extravasated red blood cells penetrated the tissue of the fibrous septum, where only the peripheral contours of the septa and the collagen remained after the erythrocytes infiltration. The red blood cells retained their morphological form and appearance. Fibrous septa were damaged, corresponding to the initial oedema observed during the observation of injuries that resulted in a bruise. The bleeding area was located in the fibrous septa in the hypodermis, partly penetrating into the loose connective tissue between the adipocytes.

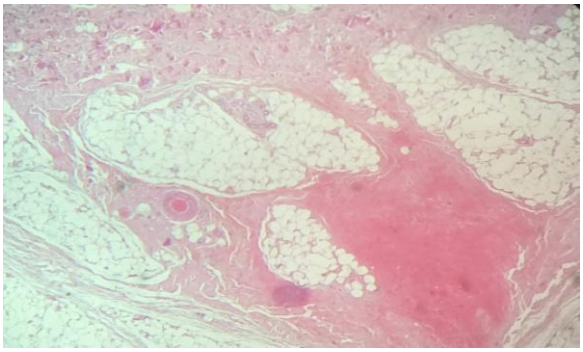


Figure 2: Fresh bruise, a few hours old. The microphotography shows the bleeding area filled with erythrocytes and the expansion of fibrous septae (Haematoxylin-eosin, X10)

Solid infiltration with polymorphonuclear cells and initial infiltration with rare mononuclear cells were noticed. Infiltration was present throughout the bleeding area, with greater intensity in the peripheral parts (Figures 2 and 3). In 4 tissue samples in this group, the bleeding area entered the adipose tissue lobes, thus separating the adipocytes one from another, presenting with the microscopic impression as if adipocytes were found in the bleeding area.

The microscopic analysis of the peripheral parts in the bleeding area with surrounding healthy tissue showed a gradual transition from the bleeding area located in the fibrosis septa of the hypodermis, filled with red blood cells and inflammatory cells, into the normal structure of the skin in all its structural parts. There was a gradual narrowing of the fibrous septa in which the morphologic change was located, passing through to the healthy tissue where their structural features were noticed. Compared to the control group, their histological structure showed normal structure, with irregularly thick connective tissue woven with blood vessels and a normal structure of the border between the dermis and the hypodermis.

Figure 3: Fresh bruise, a few hours old. The microphotography shows the bleeding area (fibrous septum in the hypodermis) with initial infiltration with granulocytes (Haematoxylin-eosin, X20)

Conclusion: In the first 24 hours after the appearance of the bruise, the morphologic change was located in the fibrous septa of the hypodermis. Their collagen bundles were damaged due to the abundant extravasation. There was infiltration in the area of bleeding with granulocytes and rare macrophages. Infiltration was most pronounced in the peripheral zone of bleeding, with a wide belt. The bleeding area limits remained in the contours of the fibrous septa.

Group B

Histological analysis of bruised tissue samples with 3 to 7 days old bruises showed extravasated erythrocytes from damaged blood vessels in the connective tissue of the hypodermis, located in the fibrous septa. Fibrous septa were dilated, their structural elements barely spun due to red blood cells infiltration. In the bleeding area, pronounced infiltration with mononuclear cells-

macrophages was noted. Residues from the erythrocytes phagocytosis in the form of hemosiderin were observed in them. Macrophage infiltration affected the entire bleeding area, both in its peripheral and in central parts. Among the erythrocytes, hematoidin particles were noticed, and they were diffusely spread through the bleeding area, seen brown-coloured. As in the analysed group A, in this group, there was also blood extravasation, located in the septa of connective tissue. The area of bleeding entered the delicate connective tissue between the adipocytes, isolating them; hence they were surrounded by densely hematoidin residues. In this examined group, bruises with different ages were analysed and there was a gradation of the presence of macrophages. The examined material consisted of 2 samples of bruises with an approximate age of 3 days, one sample of 4 days, two samples with an approximate age of 5 days and one sample of tissue originating from the decomposing body and its age corresponded approximately to 5-6 days.

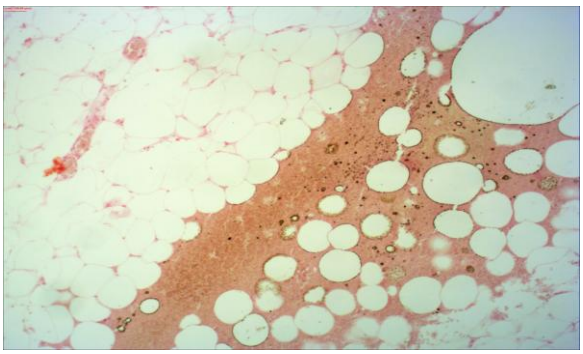


Figure 4: Five days old bruise. The microphotography shows the localisation of the bleeding area into the fibrous septa in the hypodermis (Pearls Prussian Blue, X 10)

The most pronounced presence of macrophages was observed in the bruises aged five days. The structure of the dermis was not altered; the analysed bruises were located in the adipose tissue of the hypodermis, within the contours of the fibrous septa.

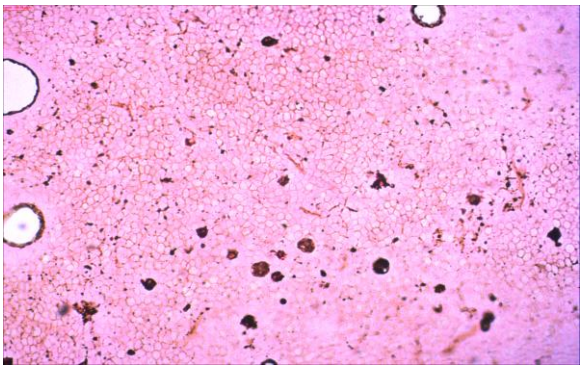


Figure 5: Five days old bruise. The microphotography shows macrophages filled with hemosiderin, as well as hematoidin granules between erythrocytes in the bleeding area (Pearls Prussian Blue, X 100)

The surrounding edges of the bruise showed a gradual narrowing of the fibrous septae containing the bleeding area and passage to healthy tissue.

Due to the presence of a bleeding area in the hypodermis, a slight change in its thickness did not show a subsequent change in the appearance and thickness of the dermis. Since this study included tissue samples with small bruises, the question remains of further analysis of the changes that would cause oedema in the surrounding tissue in massive bruises (Figures 4 and 5).

Conclusion: The intensity of infiltration in the bleeding area with macrophages in our examined samples gradually increased until the fifth day after the appearance of the bruise. Infiltration affected the entire bleeding area. Hematoidin, among erythrocytes, was also extracellularly observed. The change was located and limited in the hypodermis fibrotic septa. Their collagen bundles were damaged due to abundant cell effusion.

Discussion

In determining the age of bruises, the histological analysis in this study showed changes in the bruised tissue that occurred at a different time from the appearance of the bruise. In the first studied group, in bruises not more than 24 hours old, in addition to other tissue changes, the finding of infiltration of the bleeding area with polymorphonuclear leucocytes and rare mononuclear leukocytes indicating fresh bleeding. This finding is supported by literature data describing the course of the inflammatory response after the occurrence of the bleeding. In addition to the initial signs of injury, such as oedema in the area of bleeding, resulting in the enlargement of fibrous septa, the erythrocytes outside the blood vessels activate the inflammatory response of the body, which results in infusion of the connective tissue with inflammatory cells [2]. Approximately 4 hours after the initial bleeding, polymorphonuclear leukocytes-neutrophils migrated. These cells cannot phagocyte the red blood cells. Neutrophils are not present in normal skin, and therefore their presence is considered to be a significant finding [11]. Mononuclear leukocytes-macrophages are present in normal skin, and this makes their interpretation difficult when their infiltration begins in the bleeding area [11].

In the second examined group, with bruises aged 3-7 days, our analysis noted infiltration of the bruise with mononuclear cells-macrophages. These cells gradually infiltrated the bleeding area, which was also shown in the analysis of bruise samples with different ages within the second examined group. In the 5-day old bruises, the most intense infiltration with

macrophage in the bleeding area was observed. In addition to this finding, haematoidin granules extracellularly, as well as the presence of hemosiderin in macrophages, were noted in the bleeding area. The literature describes that approximately 9 hours after the initiation of the bruise, there is macrophages migration [12]. They can phagocyte extravasated erythrocytes. Phagocyted erythrocytes in macrophages are observed after 15-17 hours after the appearance of the bruise [12]. Erythrophagocytosis occurs no later than three days [13]. In human skin lesions, macrophages are found at the earliest after 3 or 7 hours [13], with a peak of presence at 1-2 days after injury [14]. Macrophages filled with hemosiderin, as residues from phagocyted red blood cells, detected by specific Pearls Prussian Blue method (Figure 3), can be seen at the earliest 24-48 hours after the appearance of the bruise, but are usually observed after 4-8 days. Hemosiderin is usually found in the tissue 90 hours after the inflammation, while the hematoidin is occasionally found 9 hours after the appearance of the bruise [12]. Hematoidin is a pigment that is chemically identical to bilirubin. It occurs in tissues as a result of the haemoglobin metabolism, especially in conditions of reduced oxygen concentration. Hematoidin does not contain iron; it is formed intracellularly, probably in reticuloendothelial cells, but is often located extracellularly, after 5-7 days, in the foci of previous bleeding. Our histological analysis showed refractory, yellow-brown or orange-red granules, but more typical were rhomboid plates arranged in a radial pattern, the so-called hematoidin burrs. The finding of hematoidin was a sign of bleeding which was in the direction of healing. The Pearls Prussian Blue staining method makes it easy to differentiate between hematoidin and hemosiderin. Hemosiderin produces blue coloured particles in the bleeding area, while hematoidin remains with light brown colouring [12].

In the further processing of this material, quantitative histological descriptions of cellular infiltration of bruises were made, as well as support for existing and new findings with additional molecular analyses. Quantification of macrophage infiltration in the bleeding area can be done with specific staining of macrophages with the Pearls Prussian Blue method that allows us to visualize hemosiderin in macrophages or this method can be supported by a molecular analysis of the gene expression for HO-1, an enzyme expressed in macrophages. In a more recent morphometric study, a significant amount of hemosiderin in the bleeding area has been described, about 20% and more of the field of vision, indicating the age of the bruise of about 1 week [15], which gives us the opportunity for a new field of research in interpretation of the histological finding in the bruises to determine their age.

The histological analysis gives a detailed insight into tissue structure. When using this method of analysis, the inflammatory response of the body to the injury sustained can be monitored, with the possibility to note and process the stages in the repair of bruises on time. This method is used in the post-mortem analysis of bruises such as injuries in forensic medicine. A detailed analysis of tissue changes in bruises every day from the appearance until their recovery and a detailed description of the histological finding can be given, which will support the precise determination of the age of the injuries themselves.

References

1. Langlois NEI. The science behind the quest to determine the age of bruises—a review of the English language literature. Forensic Science Medicine and Pathology. 2007;3:241-251. https://doi.org/10.1007/s12024-007-9019-3 PMid:25869263

2. Vanezis P. Interpreting bruises at necropsy. J Clin Pathol. 2001;54:348-355. https://doi.org/10.1136/jcp.54.5.348 PMid:11328832 PMCid:PMC1731416

3. Cluroe AD. Superficial soft-tissue injury. Am J Forensic Med Pathol. 1995:16:142-146. https://doi.org/10.1097/00000433-199506000-00013 PMid:7572870

4. Camps F. Interpretation of wounds. Br Med J. 1952;2:770-772. https://doi.org/10.1136/bmj.2.4787.770 PMid:12978325 PMCid:PMC2021602

5. Hiss J, Kahana T. Medicolegal investigation of death in custody: a postmortem procedure for detection of blunt force injuries. Am J Forensic Med Pathol. 1996;17:312-314. https://doi.org/10.1097/00000433-199612000-00007 PMid:8947356

6. Edwards EA, Duntley SQ. The pigments and colour of living human skin. Am J Anat. 1939;65:1-33. https://doi.org/10.1002/aja.1000650102

7. Hardy JD, Hammel HT, Murgatroyd D. Spectral transmittance and reflectance of excised human skin. J Appl Physiol. 1956;9:257-264. PMid:13376438

8. Bonhert M, Baumgartner R, Pollak S. Spectrophotometric evaluation of the colour of intro- and subcutaneous bruises. Int J Legal Med. 2000;113:343-348. https://doi.org/10.1007/s004149900107

9. Hiss J, Kahana T, Kugel C. Beaten to death: why do they die? J Trauma. 1996;40:27- 30. https://doi.org/10.1097/00005373-199601000-00006 PMid:8576994

10. Gall J, Payne-James J.Current practice in forensic medicine. John Wiley & Sons, Ltd. Publication, 2011.

11. Tarran S, Langlois NEI, Dziewulski P, Sztynda T. Using the inflammatory cell infiltrate to estimate the age of human burn wounds: a review and immunohistochemical study. Med Sci Low. 2006;46:115-126. https://doi.org/10.1258/rsmmsl.46.2.115 PMid:16683466

12. Microscopy of bruises. Available from http://www.forensicmed.co.uk/wounds/blunt-force-trauma/bruises/microscopy-of-bruises. Accessed April 24th, 2014

13. Betz P. Histological and enzyme histochemical parameters for the age estimation of human skin wounds. Int J Legal Med. 1994;107:60-68. https://doi.org/10.1007/BF01225491 PMid:7529545

14. Oenmichen M. Vitality and time course of wounds. Forensic Sci Int. 2004;144:221-231. https://doi.org/10.1016/j.forsciint.2004.04.057 PMid:15364394

15. Betz P, Eisenmenger W. Morphometrical analysis of hemosiderin deposits in relation to wound age. Int J Leg Med. 1996;108:262–264. https://doi.org/10.1007/BF01369823

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):818-824.
https://doi.org/10.3889/oamjms.2017.181
eISSN: 1857-9655
Basic Science

The Palm-Heart Diameter: A Prospective Simple Screening Tool for Identifying Heart Enlargement

Adegbenro Omotuyi John Fakoya*, David Adeiza Otohinoyi, Terrence Marcelle, Joshua Yusuf

Department of Anatomical Sciences, All Saints University School of Medicine Dominica, Dominica

Citation: Fakoya AOJ, Otohinoyi DA, Marcelle T, Yusuf J. The Palm-Heart Diameter: A Prospective Simple Screening Tool for Identifying Heart Enlargement. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):818-824. https://doi.org/10.3889/oamjms.2017.181

Keywords: Palm size; Embryology; Ultrasound; Cardiology; Hypertension; Heart enlargement.

***Correspondence:** Adegbenro Omotuyi John Fakoya. Department of Anatomical Sciences, All Saints University School Of Medicine Dominica, Dominica. E-mail: adegbenro.fakoya@allsaintsu.org

Received: 24-Jun-2017; Revised: 05-Sep-2017; Accepted: 2ep-2017; Online first: 28-Nov-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Several speculations have linked the size of the fist to be equal to the size of the heart. However, the substantial scientific report still lacks to support this theory.

AIM: This study aims to provide the validity of the fist-heart assumption by correlating the palm and heart diameters while benchmarking it as a reference tool for determining the normal heart size.

MATERIALS AND METHOD: Volunteers from the public were recruited during a health fair organised by the school. A self-administered questionnaire for necessary information was distributed after the volunteers signed the consent forms. The palm of both hands was measured in duplicates using a flexible ruler. Ultrasound examination was used in measuring the diameter of the heart with the landmark being from the anterior fibrous pericardium to the lowest part of the posterior fibrous pericardium. The level of significance was kept at $P < 0.05$.

RESULTS: A total of 275 people, consisting of 123 males and 152 females participated in the study. The age range was from 15 to 80 years with a mean age of 28.16 ± 16.18. The measurement showed that the size of both palms correlated with the heart diameter, $p < 0.05$. Other factors such as age and height showed a substantial level of correlation. However, this correlation ceased with older participants. Palm size did not correlate among participants with previously diagnosed prehypertension. However, participants with previously diagnosed hypertension with good medication compliance maintained the correlation.

CONCLUSION: This study establishes the correlation between the palm and heart diameters. Since the heart tissue and the upper limb share a similar embryonic origin, being the mesoderm, this study prospects the fact that heart enlargement could be preliminarily identified by measuring the size of the hand.

Introduction

The universal perception that the size of the fist and heart are equal has been well known among the general population. Various individuals have presented their views on how the size of the heart and the hand correlate. However, there has been no proper scientific report on the validity of this theory until now. The fact that the heart muscles and musculoskeletal system are both derivatives of the mesodermal layer [1, 2] gives a logical reason why the hand and the heart could be characteristically related, as some properties may be residual in these tissues.

The limbs and the cardiac muscles share a similar mesodermal origin which is the lateral plate of the mesoderm. Similarly, these mesodermal cells share some transcription factors such as Nkx2.5, GATA, Mef2, Tbx and Hand [3]. Other factors like fibroblast growth factor (FGF) [4], WNT [2, 3], and transforming growth factor beta (TGFβ) superfamily to include bone morphogenic proteins (BMP) [4, 5] have been demonstrated to influence both heart and limb development. Detailed and well laid out reviews on the development of the heart and upper limb have already been properly documented [3-8]. To further support this association, it has been recorded that the defect in some of these factors has resulted in both cardiac and limb abnormalities. For instance, defect in the TGFβ-1 has not only been associated with heart abnormalities such as congenital aortic stenosis, regurgitation [9], and arrhythmogenicity [10] but the differential expression of BMP (a member of the TGFβ-1 superfamily) as a result of Tfap2b loss has been shown to result in both heart and limb abnormalities [11].

It is, however, astonishing that an organ as important and critical as the heart still lacks reliable referencing data for the dimensions of the heart

cavities. Though it is a known fact that the body surface area (BSA) determines the dimensions of the heart chambers, this is however not resulted in any concrete and reliable referencing data. However, predictors such as age, sex, height, and weight have been linked to the heart dimensions [12].

Hence, this study was to find a customised value for easy and fast identification of a possible heart enlargement from any underlying cause by relating the heart to the palm, which is another structure of similar embryological origin. The findings from this study are expected to help redirect and solve the heart referencing issues for better diagnosis and prognostication.

Materials and Method

Participants

Volunteers from the public were recruited during a health fair organised by the school. A consent form detailing the procedure to be performed given to the volunteers, and the procedure was duly explained to them. The participants were asked about any history of palmar injuries, and the palms were equally observed for any form of injury. A total of 275 people, 123 males and 152 females with an age range of 15 to 80 years, agreed to participate by signing the consent after which a self-administered questionnaire was given.

Selection Criteria

The participants used for this study were only right-handed people. Left-handed individuals were left out of the study for the sake of consistency, and moreover, we could not gather a significant number of left-handed people. Also, we excluded subjects with known cardiac problems like valvular stenosis' or insufficiencies and other conditions and activities that can predispose to remodelling of the heart tissue like athletics, subjects with known hemodynamic states like Sickle cell disease, hyperthyroidism, and pregnancy. Also, participants with known and established abnormal blood pressure were used for a further study.

Measurements

A double-blinded technique was used for measurements of the heart and palm dimensions. Here, the ultrasound measurements were taken by an investigator in the ultrasound room and records kept away from the other investigator, taking the palm dimensions to avoid an observer bias.

Palm measurement

The right and the left palms were measured with a soft ruler from medial to lateral, specifically from the edge of the first palmar crease (heart line), where the fist is made, to the edge of a secondary palm line (headline) if visible or by extension [13] (Fig. 1). The measurement for each palm was done twice with the mean measurements calculated.

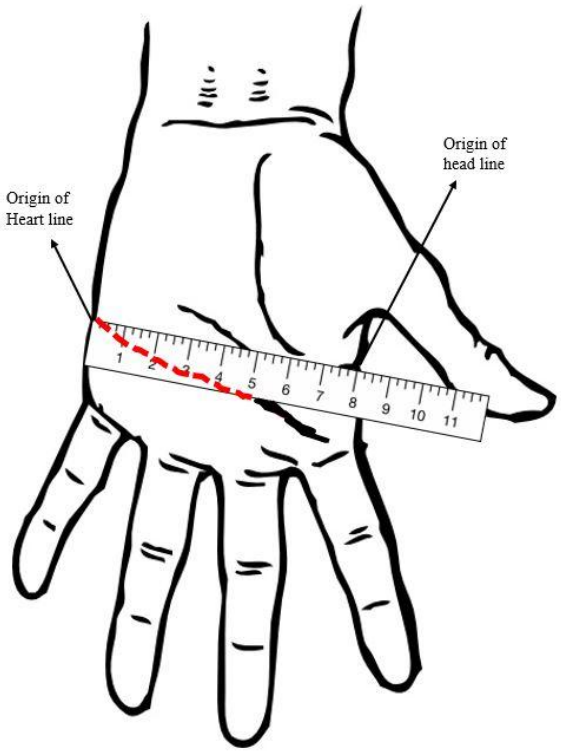


Figure 1: Schematics for palm measurement

Ultrasound measurement

The measurement of the heart diameter was carried out by a single experienced investigator using a DUS 5000 ultrasound machine (Miami, Florida, USA) using a low-frequency micro-convex transducer preset to "adult cardiac" with a default frequency of 4MHz.

The participants were requested to expose their chest to around the 5th intercostal and were given a clean drape to cover themselves to make them comfortable and were required to lie supine on the examination bed. During the scan, only the required area was exposed (the left thoracic region up to about the 5th intercostal space). The transducer was applied to the subject's chest wall to find a window on the 2nd to the 5th intercostal space left the parasternal area looking at the heart at its longitudinal axis (left parasternal view), PLAX [14, 15]. Using this view, we can visualise and measure the largest diameter of the heart in two-dimensions from the anterior fibrous pericardium to the lowest part of the posterior fibrous pericardium. The measurement is taken in late diastole when the mitral valve is almost

closing, and also this is when the left ventricular wall is expected to be thinnest. Although the 4-chambered view is the mainstay 2D echocardiographic technique for the imaging of the left ventricle [12] but requires more time, skill and exposure of the subject. Whereas, the PLAX is opposite regarding time, skill and exposure, especially if a quick view is desired. In this view, we can capture from superficial to deep the right ventricle, the interventricular septum, the left ventricle, the left atrium and two valves, aortic and mitral. Other studies have concentrated on the size of the left ventricular chamber [16-22]. However, reference ranges are still very limited for the other cavities despite the known prognostic significance of the left atrium and its volume [12]. Using this simple approach (PLAX), we can capture the essential cavity and volumetric changes from any underlying defects that might alter the normal size (diameter) of the heart.

Statistical analysis

Statistical analysis was performed by STATA/IC 13.0 (for windows). The Chi-square test was used for comparing categorised variables. Multiple regression analysis was also performed to predict the diameter of the heart from both the right and left hands measurements. Statistical hypothesis tests with $p < 0.05$ were considered as significant. ANCOVA was used so that measurements of the right palm, left palm, and heart diameter could be taken into account when comparing the groups regarding varying blood pressures. Values are presented as mean ± standard deviation or number (%).

Results

A total of 275 individuals volunteered in the study. Participants included 123 (44.73%) males and 152 (55.27%) females. Mean age was 28.16 ± 16.18 years. Age distribution of the participants (Table 1) showed that the most predominant age bracket was between 15-20 year.

Table 1: Age distribution of participants with their palmer and heart measurements

Age group	Frequency n (%)	Right palm Mean (cm) ±. S.D	Left palm Mean (cm) ±. S.D	Heart Diameter Mean (cm) ±. S.D
15-20	131 (47.64)	8.54 ± 0.67	8.40 ± 0.70	8.38 ± 0.84
21-26	68 (24.73)	8.78 ± 0.72	8.55 ± 0.76	8.78 ± 0.92
27-32	14 (5.09)	8.69 ± 0.77	8.55 ± 0.76	8.76 ± 0.74
33-38	9 (3.27)	8.57 ± 0.50	8.39 ± 0.57	9.12 ± 0.84
39-44	10 (3.64)	8.87 ± 0.51	8.76 ± 0.54	9.29 ± 0.62
45-50	9 (3.27)	8.51 ± 0.56	8.44 ± 0.55	9.50 ± 0.53
51-56	8 (2.91)	8.88 ± 0.72	8.36 ± 0.60	8.93 ± 1.27
57-62	5 (1.82)	8.50 ± 0.19	8.70 ± 0.74	9.58 ± 0.79
63-68	5 (1.82)	8.50 ± 0.19	8.24 ± 0.18	9.94 ± 0.83
69-74	10 (3.64)	8.82 ± 0.40	8.67 ± 0.43	9.7 ± 1.07
75-80	6 (2.18)	8.73 ± 0.77	8.61 ± 0.62	9.73 ± 0.89

In this study, relating the palm size of both hands using linear regression showed a good fit ($P = 0.000$), with 91.2% coefficient of determination (95% confidence interval from 0.89 to 0.96). Multiple regression to predict the diameter of the heart from the palm size of both the right and left palm statistically significantly predicted the diameter of the heart, $F(2, 273) = 96.18$, $p < 0.0005$, $R2 = 0.4133$, $p = 0.008$ (95% confidence interval from 0.15 to 1.0)

Also, among the participants, 56 individuals were identified with previously diagnosed cases of abnormal blood pressures, with an equal number of 28 persons in each category of prehypertensive and hypertensive blood pressure, respectively. The hypertensive volunteers also acknowledged being on medication with good compliance. A control group of 28 individuals with normal blood pressure and belongs to the same age group of those with abnormal blood pressure was also selected for further comparisons (Table 2). Efforts were also made to ensure no gender was predominating in any of the study groups.

Table 2: Role of high blood pressure on the relationship between the fist size and heart diameter

Group	Right palm Mean (cm) ±S.D	Left palm Mean (cm) ±S.D	Heart diameter Mean (cm) ± S.D	Chi-Squared evaluation in comparing right and left palm to the diameter of the heart, respectively
Normal blood pressure	8.42 ± 0.55	8.27 ± 0.59	8.83 ± 0.96	0.00 and 0.02
Prehypertensive blood pressure	8.79 ± 0.49	8.62 ± 0.5	9.74 ± 0.83	0.355 and 0.682
Hypertension	8.39 ± 0.79	8.25 ± 0.76	8.91 ± 1.0	0.001 and 0.042

There was no relationship when the right palm, left palm, and heart diameter of prehypertensive and hypertensive blood pressure groups was collectively analysed using ANCOVA, with P values of 0.39, 0.54, and 0.07, for right palm, left palm, and heart diameter, respectively.

The sonographic images demonstrate how the measurements were taken during the scan (Fig. 2).

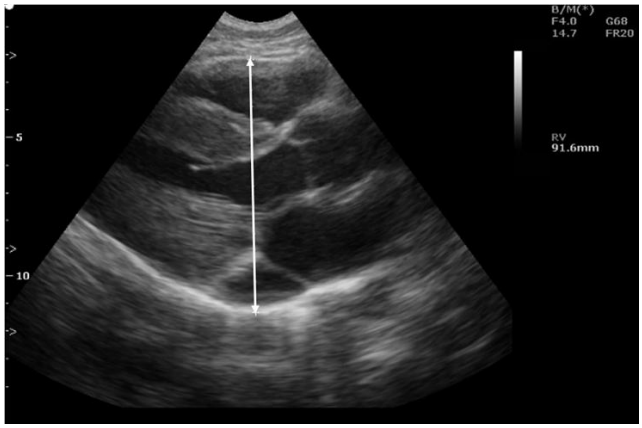


Figure 2: Ultrasound scan of a heart from the anterior fibrous pericardium to the lowest part of the posterior fibrous pericardium

Age distribution showed a link between age and heart diameter (chi-square with 210 degrees of freedom = 356.65, p = 0.000). However, there was no correlation when compared with the palm sizes of both hands (P > 0.05). A simple regression was run to predict the diameter of the heart from the age group of teenagers to middle-aged adults (15-55 years).

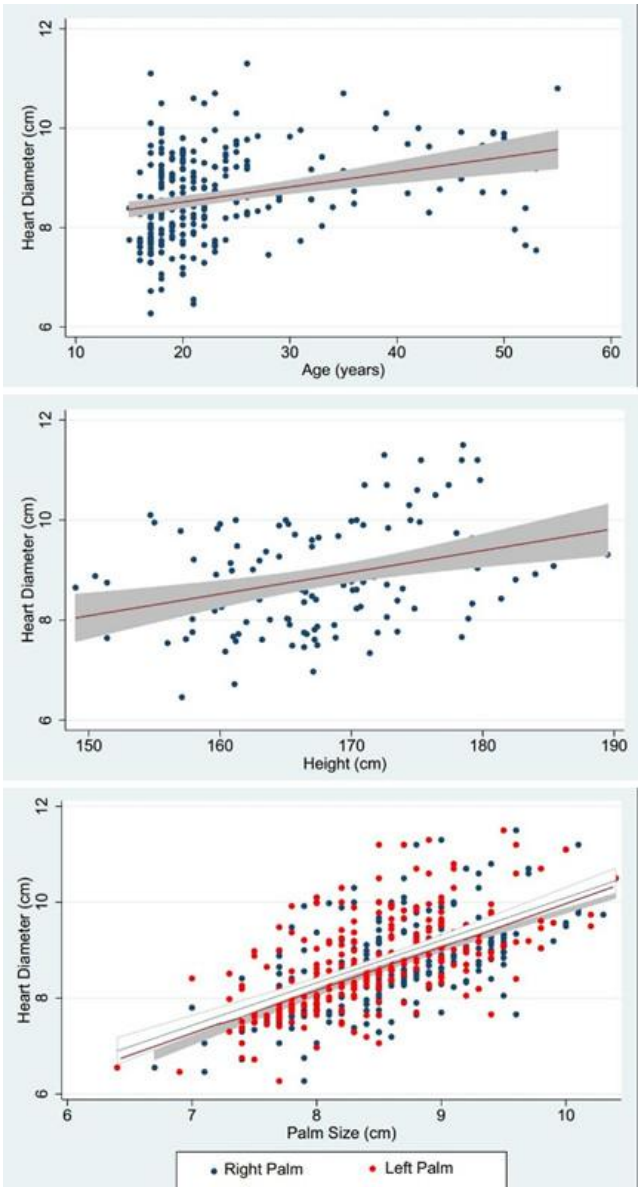


Figure 3: Predictive graph, showing the relationship of age (A), height (B), and the fist size (C) of both palms on the diameter of the heart. (Confidence interval of 95% of means, P<0.05 in each plot)

These variables statistically significantly predicted the diameter of the heart, F (1, 246) = 21.79, p < 0.0005, R2 = 0.0814. The variable added statistically significantly to the prediction, p < 0.05. The coefficient indicated that for each increase in age there is a 2% increase in heart diameter (95% confidence interval from 0.04 to 0.02). However, for participants above 55 years, the variable was not statistically significant. History retrieved from each participant revealed 26 (9.42%) with previous palmar injuries. However, the injuries had no impact on the correlation between the fist size and the diameter of the heart when analysed (chi-square with 10 degrees of freedom = 8.30, p = 0.60). Comparing the diameter of the heart based on gender showed that within a particular age group, males had a bigger heart diameter and palm size of both hands than females, P = 0.000, in all comparisons. Multiple regression analysis in comparing males to females further indicated that in any age group, males had their heart diameter, palm size of both the right and left hands bigger than females by 11.4%, 29.3%, and 18.5%, respectively, with 59.52% coefficient of determination. The linear regression of participants' heights below 40 years of age related to the diameter of the heart (P = 0.000, 95% confidence interval from 0.07 to 0.02). No correlation was observed among patients above the age of 40. The Linear prediction graph was also used in interpreting the link between age (15-55 years), height, and fist size on the diameter of the heart (Fig. 3).

Discussion

Heart disease remains one of the major illnesses leading to increased mortality rate [23]. An estimate of 17.5 million people dies each year from cardiovascular-related diseases, thus giving an estimate of 31% of deaths worldwide [24]. Cardiovascular problems can be diagnosed by electrocardiogram, echocardiogram, chest x-rays, cardiac computed tomography, cardiac magnetic resonance, positron emission tomography, coronary angiography, single-photon emission tomography and radionuclide ventriculography. Other additional diagnostic tests include haematological and biochemical analysis of Brain-type Natriuretic peptide (BNP), exercise testing, endomyocardial biopsies, and genetic testing. Amongst all, echocardiography has been proven to be the most useful and widely available test in diagnosing cardiac problems. It has also been shown to be accurate, safe and cost-effective [25]. Cardiovascular diseases are usually as a result of various illnesses. However, not all of these illnesses lead to heart muscle hypertrophy or enlargement [26]. The idea of linking the size of one's heart to their palm dimensions may appear irrational. However, few speculations have proposed the theory with several debates, but without any substantial evidence until now. The objective of this study was not just to confirm the speculations but also prospect how the information could prove beneficial in clinical medicine. As previously stated, the cardiac muscles and the appendages share the lateral plate mesoderm layer as a common derivative [1, 2]. This could also illustrate that all cells from the mesoderm, irrespective of differentiation may share a few linking properties [27].

Correlation with Previous studies

This study showed a considerable association between heart size and factors such as age, sex, and height (Table 1 and Figure 2), which was similar to previous reports [12, 28]. Previous studies have demonstrated that age has a direct proportionality with the heart diameter until the age of 50 years, where the heart begins to achieve a constant size, although the rate of growth was not elucidated upon [29, 30, 31]. However, this study demonstrates a 2% increase in heart diameter for each increase in age till 55 years. Further longitudinal studies might be required to validate this finding.

Studies correlating height with the heart size have been investigated in previous reports with emphasis on the role of body weight as major determinant [12, 30]. Similarly, the current study showed a relationship between height and heart diameter amongst participants only below 40 years, although, the influence of body weight was not captured. The observed relationship could be due to the pubertal growth spurt defined by age, which is approximately linear until maturity, where a further increase in age indicates no further growth [31].

Similar to other studies, sex was shown to be a predictor of heart diameter [27, 29, 30, 32]. The female frame regardless of race is known to be smaller than the male, hence, its reflection on the dimensions of the heart. Bainton et al., 1932, reduced the transverse diameter of the male heart by 0.8cm to derive the female diameter, while Oberman et al., 1967, came up with a 0.5-1.0cm consistent difference between men and women within the same group and matched for height and weight. However, our study, though not matched for height and weight revealed an 11.4% increase in the diameter of males relative to females matched for the same age group.

Deductions from this study

In this study, it was shown that the right palm was bigger than the left palm, although, the difference did not distort the analysis that both palms correlated with the diameter of the heart. The right hand being bigger is attributed to the increased neuronal firing and hypertrophy in right-handed people, due to high usage [33]. Studies involving left-handed individuals in the current study was not feasible due to small sample size. From this study, it could be speculated that the heart diameter ranges from the measurement of the left palm to that of the right palm with the right palmar diameter being the upper limit of normal heart size for the measured individual.

While further considering the general clinical applicability of this method, a pilot study involving the impact of deranged blood pressure on the palm-heart diameter was carried out. The finding drew our attention to an important clinical phenomenon previously observed in the MONICA/KORA Augsburg study, which concluded that persistent prehypertension results in heart tissue remodelling which facilitates the occurrence of ventricular hypertrophy resulting in diastolic dysfunction [34]. This study's observation supports the direct applicability of the palm-heart diameter for the normotensive and the hypertensive population on treatment, but not for the prehypertensive group. The reason though not fully understood but might be attributed to the onset of a possible cardiac tissue remodelling [35] which might have resulted in the "subtle" non-symptomatic enlargement which is similarly seen in the endurance-trained athletes' heart [36]. It is assumed that the prehypertensive period is faced with continuous hemodynamic load causing the ventricular myocardium to stretch (Frank-Starling), triggering the heart to subsequently initiate the process of hypertrophy in response to the stress (Laplace law). The correlation with the hypertensive group suggests that medicating hypertensive patients might be experiencing some therapeutic palliation on pressure load and vascular resistance thus ameliorating the net tissue remodelling effect as opposed to the prehypertensives or simply that the process of hypertrophy might have already been established in this group, hence no noticeable net myocardial stretch. The pathophysiological impact of prehypertension is difficult to define, as it represents over 30% of the adult healthy population [37]. Prehypertension has been linked to an increased long-term cardiovascular risk [38] and may be the starting point in the cardiovascular disease spectrum [39]. The observation from this research further corroborates the Strong heart study of 2006, which has advocated for an increasingly aggressive follow-up on patients with persistent prehypertension [40].

Prospects of current study in diagnosing heart enlargement

The study predicts that the relationship between the palm and heart size may give more direction in anticipating cardiac dimension referencing tool. Thus, a one-time diametric measurement of the palm-size might just be sufficient to determine an individual's normal heart diameter which can then be used as a benchmark for comparison of the heart cavities. This benchmark is essential as it will aid the customisation of the heart cavity dimensions as opposed to using a single range of values for everyone. This is soon necessary since BSA which is currently the main tool has been shown to be deficient in certain cases such as when relating the heart dimensions of a short obese person to a tall, slim person who might have the same BSA [12]. Furthermore, the present reference ranges might also result in delayed diagnosis and treatment of heart enlargement in patients possessing small BSA, due to the underestimation of the heart size [12].

Although not fully substantiated, observations from our study revealed that the applicability of this study to the prehypertensive population must be done with caution as a 1cm increase in heart diameter over the expected upper limit (the right palm diameter) might be seen on sonography. Hence, this tool might also prove effective for the screening and identification of early cardiac remodelling in patients with persistent prehypertension.

The PLAX approach used in this study is easy and captures the essential cavities and valves. Therefore, heart enlargement from any underlying defect would likely reflect on the measurement. It suffices to say that this simple procedure could be an alternative to unnecessary exposure to radiation from plain chest X-rays done to screen for cardiomegaly especially in developing countries. Also, this simple technique can be done by medical students at the patient's bedside to screen for a possible cardiomegaly. Hence, this further supports the on-going advocacy for the introduction of ultrasound education into the medical curriculum across the globe.

Limitations of the study

Though we were limited by the numbers of participants in the age ranges over 26, however, we do not think that would have affected the outcome of our study. However, further studies still need to be carried out to closely investigate the adult population. The study did not account for the impact of other predictors of heart enlargement on the palm-heart relationship, such as participation in sports and hemodynamic conditions like Sickle cell disease amongst others.

In conclusion, the statement linking the sizes of the heart with the size of either hand has been said among few but without scientific backing until now. This study tends to confirm the relationship between details and also offer the prospect on its clinical relevance. This relationship could be employed in the early investigation of heart enlargement, thus reducing the incidence of poor prognosis among patients. However, there is the need for further study to properly understand the relationship between the heart and palm measurements. The study also elucidated on the unclear relationship between the increase in age and the growth of cardiac tissue. With the current trend of attributing more attention to hypertensive blood pressure than prehypertension, the study suggests that both abnormal pressures should receive an equal level of response to avoid cardiac tissue remodelling. It also supports the on-going advocacy for the inclusion of basic ultrasound education in the curriculum for medical students at large.

References

1. Nishimoto S, Logan MP. Subdivision of the lateral plate mesoderm and specification of the forelimb and hindlimb forming domains. In Seminars in cell & developmental biology. Academic Press, 2016; 49:102-108. https://doi.org/10.1016/j.semcdb.2015.11.011

2. Itoh N, Ohta H, Nakayama Y, Konishi M. Roles of FGF signals in heart development, health, and disease. Frontiers in cell and developmental biology. 2016;4. https://doi.org/10.3389/fcell.2016.00110

3. Satou Y, Satoh N. Gene regulatory networks for the development and evolution of the chordate heart. Genes & development. 2006;20(19):2634-8. https://doi.org/10.1101/gad.1485706 PMid:17015427

4. Al-Qattan MM, Yang Y, Kozin SH. Educational Objectives. Journal of Hand Surgery. 2009;7(34):1340-50. https://doi.org/10.1016/j.jhsa.2009.06.013 PMid:19700076

5. Azhar M, Schultz JEJ, Grupp I, Dorn GW, Meneton P, Molin DGM, et al. Transforming growth factor beta in cardiovascular development and function. Cytokine & Growth Factor Reviews [Internet]. Elsevier BV; 2003;14(5):391–407.

6. Schleich J-M, Abdulla T, Summers R, Houyel L. An overview of cardiac morphogenesis. Archives of Cardiovascular Diseases [Internet]. Elsevier BV; 2013;106(11):612–23.

7. Massagué J, Chen YG. Controlling TGF-β signalling. Genes & development. 2000;14(6):627-44. PMid:10733523 PMid:10733523

8. Schneider MD, Gaussin V, Lyons KM. Tempting fate: BMP signals for cardiac morphogenesis. Cytokine & Growth Factor Reviews [Internet]. Elsevier BV; 2003;14(1):1–4.

9. Fielitz J, Hein S, Mitrovic V, Pregla R, Zurbrügg HR, Warnecke C, et al. Activation of the cardiac renin-angiotensin system and increased myocardial collagen expression in human aortic valve disease. Journal of the American College of Cardiology [Internet]. Elsevier BV; 2001;37(5):1443–9.

10. Khan R, Sheppard R. Fibrosis in heart disease: understanding the role of transforming growth factor-beta1 in cardiomyopathy, valvular disease and arrhythmia. Immunology [Internet]. Wiley-Blackwell; 2006;118(1):10–24.

11. Zhao F, Bosserhoff A-K, Buettner R, Moser M. A Heart-Hand Syndrome Gene: Tfap2b Plays a Critical Role in the Development and Remodeling of Mouse Ductus Arteriosus and Limb Patterning. Gaetano C, editor. PLoS ONE [Internet]. Public Library of Science (PLoS); 2011;6(7):e22908.

12. Pfaffenberger S, Bartko P, Graf A, Pernicka E, Babayev J, Lolic E, et al. Size Matters! Impact of Age, Sex, Height, and Weight on the Normal Heart Size. Circulation: Cardiovascular Imaging [Internet]. Ovid Technologies (Wolters Kluwer Health); 2013;6(6):1073–9.

13. Oda M, Womack BF, Tsubouchi K.1971. http://ieeexplore.ieee.org/stamp/stamp.jsp?arnumber=4308270

14. Roman MJ, Devereux RB, Kramer-Fox R, O'Loughlin J. Two-dimensional echocardiographic aortic root dimensions in normal children and adults. The American Journal of Cardiology [Internet]. Elsevier BV; 1989;64(8):507–12.

15. D'Andrea A, Cocchia R, Riegler L, Scarafile R, Salerno G, Gravino R, et al. Aortic Root Dimensions in Elite Athletes. The American Journal of Cardiology [Internet]. Elsevier BV; 2010;105(11):1629–34.

16. Gardin JM, Henry WL, Savage DD, Ware JH, Burn C, Borer JS. Echocardiographic measurements in normal subjects: Evaluation of an adult population without clinically apparent heart disease. Journal of Clinical Ultrasound [Internet]. Wiley-Blackwell; 1979;7(6):439–47.

17. Henry WL, Gardin JM, Ware JH. Echocardiographic measurements in normal subjects from infancy to old age. Circulation [Internet]. Ovid Technologies (Wolters Kluwer Health);

1980;62(5):1054–61.

18. Devereux RB, Lutas EM, Casale PN, Kligfield P, Eisenberg RR, Hammond IW, et al. Standardization of M-mode echocardiographic left ventricular anatomic measurements. Journal of the American College of Cardiology [Internet]. Elsevier BV; 1984;4(6):1222–30.

19. Hammond IW, Devereux RB, Alderman MH, Laragh JH. Relation of blood pressure and body build to left ventricular mass in normotensive and hypertensive employed adults. Journal of the American College of Cardiology [Internet]. Elsevier BV; 1988;12(4):996–1004.

20. Lauer MS. The Impact of Obesity on Left Ventricular Mass and Geometry. JAMA [Internet]. American Medical Association (AMA); 1991;266(2):231.

21. Lauer MS, Larson MG, Levy D. Gender-specific reference M-mode values in adults: Population-derived values with consideration of the impact of height. Journal of the American College of Cardiology [Internet]. Elsevier BV; 1995;26(4):1039–46.

22. George KP, Batterham AM, Jones B.1998. https://insights.ovid.com/pubmed?pmid=9624638

23. Lowry AW, Olabiyi OO, Adachi I, Moodie DS, Knudson JD. Coronary Artery Anatomy in Congenital Heart Disease. Congenital Heart Disease [Internet]. Wiley-Blackwell; 2013;8(3):187–202.

24. WHO. 2016. http://www.who.int/cardiovascular_diseases/en/

25. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. European Heart Journal [Internet]. Oxford University Press (OUP); 2016;37(27):2129–200.

26. Rajangam S, Dhawan S, Tilak P. Congenital Heart Defects and its Types. Indian Journal of Anatomy [Internet]. Red Flower Publication Private, Ltd.; 2015;4(2):85–91.

27. Oberman A, Myers AR, Karunas TM, Epstein FH. Heart Size of Adults in a Natural Population-Tecumseh, Michigan: Variation by Sex, Age, Height, and Weight. Circulation [Internet]. Ovid Technologies (Wolters Kluwer Health); 1967;35(4):724–33. https://doi.org/10.1161/01.CIR.35.4.724

28. Margolies A. Clinical roentgenology of the heart. American Heart Journal [Internet]. Elsevier BV; 1947;33(3):410.

29. Amundsen, P.1959. https://archive.org/details/in.ernet.dli.2015.118812

30. Neuhauser EBD. Roentgen Diagnosis of the Heart and Great Vessels. JAMA: The Journal of the American Medical Association [Internet]. American Medical Association (AMA); 1965;193(11):982.

31. Björk A, Helm S. Prediction of the age of maximum puberal growth in body height. The Angle orthodontist. 1967;37(2):134-43. PMid:4290545 PMid:4290545

32. Bainton JH. The transverse diameter of the heart. American Heart Journal [Internet]. Elsevier BV; 1932;7(3):331–41.

33. Adam A, Luca C, Erim Z.1998. http://jn.physiology.org/content/80/3/1373.long

34. Markus MRP, Stritzke J, Lieb W, Mayer B, Luchner A, Döring A, et al. Implications of persistent prehypertension for ageing-related changes in left ventricular geometry and function: The MONICA/KORA Augsburg study. Journal of Hypertension [Internet]. Ovid Technologies (Wolters Kluwer Health); 2008;26(10):2040–9. https://doi.org/10.1097/HJH.0b013e328308da55

35. Ganau A, Devereux RB, Roman MJ, de Simone G, Pickering TG, Saba PS, et al. Patterns of left ventricular hypertrophy and geometric remodeling in essential hypertension. Journal of the American College of Cardiology [Internet]. Elsevier BV; 1992;19(7):1550–8.

36. Mihl C, Dassen WRM, Kuipers H. 2008. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2300466/pdf/nhj1612900.pdf

37. Qureshi AI, Suri MF, Kirmani JF, Divani AA.2005. http://www.medscimonit.com/download/index/idArt/428446

38. Vasan RS, Larson MG, Leip EP, Evans JC, O'Donnell CJ, Kannel WB, et al. Impact of High-Normal Blood Pressure on the Risk of Cardiovascular Disease. New England Journal of Medicine [Internet]. New England Journal of Medicine (NEJM/MMS); 2001;345(18):1291–7. https://doi.org/10.1056/NEJMoa003417 PMid:11794147

39. Schunkert H. Pharmacotherapy for Prehypertension — Mission Accomplished? New England Journal of Medicine [Internet]. New England Journal of Medicine (NEJM/MMS); 2006;354(16):1742–4. https://doi.org/10.1056/NEJMe068057 PMid:16537661

40. Zhang Y, Lee ET, Devereux RB, Yeh J, Best LG, Fabsitz RR, et al. Prehypertension, Diabetes, and Cardiovascular Disease Risk in a Population-Based Sample: The Strong Heart Study. Hypertension [Internet]. Ovid Technologies (Wolters Kluwer Health); 2006;47(3):410–4. https://doi.org/10.1161/01.HYP.0000205119.19804.087

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):825-830.
https://doi.org/10.3889/oamjms.2017.213
eISSN: 1857-9655
Basic Science

Impact of Size of the Tumour, Persistence of Estrogen Receptors, Progesterone Receptors, HER2Neu Receptors and Ki67 Values on Positivity of Axillary Lymph Nodes in Patients with Early Breast Cancer with Clinically Negative Axillary Examination

Borislav Kondov[1*], Rosalinda Isijanovska[2], Zvonko Milenkovikj[3], Gordana Petrusevska[4], Marija Jovanovski-Srceva[5], Magdalena Bogdanovska-Todorovska[4], Goran Kondov[1]

[1]University Clinic for Thoracic and Vascular Surgery, Clinical Centre "Mother Theresa", Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]Institute for Epidemiology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [3]University Clinic for Infective Diseases and Febrile Conditions, Clinical Centre "Mother Theresa", Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [4]Institute for Pathology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [5]University Clinic for Anesthesia and Reanimation, Clinical Centre "Mother Theresa", Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Kondov B, Isijanovska R, Milenkovikj Z, Petrusevska G, Jovanovski-Srceva M, Bogdanovska-Todorovska M, Kondov G. Impact of Size of the Tumor, Persistence of Estrogen Receptors, Progesterone Receptors, HER2Neu Receptors and Ki67 Values on Positivity of Axillary Lymph Nodes in Patients with Early Breast Cancer with Clinically Negative Axillary Examination. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):825-830. https://doi.org/10.3889/oamjms.2017.213

Keywords: early breast cancer; factors that predict axillary status; tumour size; lymphovascular invasion; Ki67.

***Correspondence:** Borislav Kondov. University Clinic for Thoracic and Vascular Surgery, Clinical Centre "Mother Theresa", Faculty of Medicine, Ss Cyril and the Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: b_kondov@yahoo.com

Received: 16-Sep-2017; **Revised:** 20-Oct-2017; **Accepted:** 24-Oct-2017; **Online first:** 26-Oct-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: The study aimed to identify factors that influence the positivity of axillary lymph nodes in patients with early breast cancer and clinically negative axillary lymph nodes, who were subjected for modified radical mastectomy and axillary lymphadenectomy.

MATERIAL AND METHODS: This study included 81 surgically treated, early breast cancer patients during the period from 08-2015 to 05-2017. All the cases have been analysed by standard histological analysis including macroscopic and microscopic examination by routine H&E staining. For determination of molecular receptors, immunostaining by PT LINK immunoperoxidase has been done for HER2neu, ER, PR, p53 and Ki67.

RESULTS: Patients age ranged between 31-73 years, an average of 56.86 years. The mean size of a primary tumour in the surgically treated patient was 20.33 ± 6.0 mm. Axillary dissection revealed from 5 to 32 lymph nodes, with an average of 14. Metastases have been found in 1 to 7 lymph nodes, with an average 0.7. Only 26 (32.1%) of the patients showed metastases in the axillary lymph nodes. The univariant regression analysis showed that the size of a tumour and presence of HER2neu receptors on cancer cells influence the positivity of the axillary lymph nodes. The presence of the estrogen receptors, progesterone receptors have no influence on the positivity for metastatic deposits of lymph nodes. Multivariant model and logistic regression analysis as significant independent factors or predictors of positivity of the axillary lymph nodes are influenced by the tumour size only.

CONCLUSION: Our study showed that the metastatic involvement of the axillary lymph nodes is mainly influenced by the size of a tumour and presence of HER2neu receptors in the univariant analysis. This point to the important influence of positivity of the axillary lymph nodes but, in multi-variant regressive analysis the lymph node status correlates with the tumour size only.

Introduction

Axillary status (involvement of lymph nodes in axilla with a metastatic tumour from primary breast cancer) together with the size of a primary breast tumour is the main factors that define the stage of breast cancer but also predict prognosis of the breast cancer [1].

Introducing procedure – detection of sentinel node and biopsy, is a minimally invasive procedure that determines first drainage lymph node in the axillar

pit [2]. Examination of this lymph node at the same surgical intervention gives us information about the status of this lymph node but also gives us information about other lymph nodes in the axilla. We suppose that if the sentinel lymph node is negative (not involved with metastases), we suppose that all other lymph nodes in the axillary pit is negative and is not necessary to do lymphadenectomy. Knowing the status of the axillary lymph nodes is very important, for the planning further therapeutic procedure.

The study aimed to analyse which factors that influence the positivity of axillary lymph nodes, with a point to tumour size, expression of estrogen, progesterone and HER2neu receptors on tumour cell surface, values of Ki67, in patients with early breast cancer and clinically negative axillary lymph nodes.

Material and Methods

In the prospective study were analyse 81 surgically treated patients with early breast cancer during 08.2015-05.2017 year, with clinically negative axilla, at which were done a radical surgical intervention of the breast with complete axillary lymphadenectomy. All the cases have been analyzed by standard histological analysis including macroscopic and microscopic analysis of standard H&E staining. For determining of molecular receptors immunostaining by PT, LINK immunoperoxidase has been done for HER2neu, ER, PR, p53 and Ki67.

Statistical analysis was done with statistical program Statistica 7.

Results

Patients age ranged between 31-73 years, an average of 56.86. The stages of the primary breast cancer in our patients are given in Table 1. The biggest frequency was found for stage IIA (41.97%), stage IA (29.63%), and for stage IIB (23.46%). We found stage IIA in 2.47%, the stage 0 in1.23%, and stage IB in 2.23%. We did not find any cases in stages IIIB and IIIC.

Table 1: Stages of the primary breast cancer in our patients

Variable	Total (No = 81) (100.00%)
Stage	
0	1 (1.23%)
IA	24 (29.63%)
IB	1 (1.23%)
IIA	34 (41.97%)
IIB	19 (23.46%)
IIIA	2 (2.47%)
IIIB	0 (0.00%)
IIIC	0 (0.00%)

Characteristics of the primary breast cancer in our patients are shown in Table 2. There were not found statically significant differences between axilla positive and axilla negative patients for tumour size, location, and histology. Nuclear grade was significantly diferent in the patients with axillary negative patients, where patients with G1 were (10.91%) and with G3 were (1.82%) in comparison with the positive axillary patients, where patients with G1 were (0%), and with G3 were (11.54%), ($p < 0.044$). The mean size of a primary tumour in the surgically treated patient was 20.33 $\pm$ 6.0 mm. On dissection from the axillary pits, there were taken out 5 to 32 lymph nodes, an average of 14.0. Metastases have been found in 1 to 7 lymph nodes, an average 0.7. In 32.1% of the patients have been found metastases in the axillary lymph nodes (Table 2).

Table 2: Characteristics of the primary breast cancer in our patients

Variable	Axilla positive (N = 26) (32.1%)	Axilla negative (N = 55) (67.9%)	Total (N = 81) (100.00%)	P
Tumor size				
Tis	0 (0.00%)	1 (1.82%)	1 (8.40%)	
T1a	0 (0.00%)	3 (5.45%)	3 (3.70%)	
T1b	0 (0.00%)	8 (14.54%)	8 (9.87%)	0.540
T1c	5 (19.23%)	16 (29.09%)	21 (25.92%)	
T2	21 (80.76%)	27 (49.09%)	48 (59.25%)	
Location				
Central	5 (19.23%)	10 (18.18%)	15 (18.52%)	
Inner	4 (15.38%)	9 (16.36%)	13 (16.05%)	0.991
Lateral	17 (65.38%)	36 (65.45%)	53 (65.43%)	
Histology				
Ductal	21 (80.77%)	46 (83.64%)	67 (82.72%)	
Lobular	3 (11.54%)	4 (7.28%)	7 (8.64%)	0.807
Other	2 (7.69%)	5 (9.09%)	7 (8.64%)	
Nuclear grade				
1	0 (0.00%)	6 (10.91%)	6 (7.41%)	
2	23 (88.46%)	48 (87.27%)	71 (87.65%)	0.044*
3	3 (11.54%)	1 (1.82%)	4 (4.94%)	

*, Statistically significant differences; N = number.

The univariant regression analysis showed that the size of a tumour ($p = 0.022$) and presence of HER2neu receptors on cancer cell ($p = 0.037$) influence on the positivity of the axillary lymph nodes.

Table 3: Characteristics of receptors in the primary breast cancer in our patients

Variable	Axilla positive (N=26) (32.1%)	Axilla negative (N = 55) (67.9%)	Total (N = 81) (100.00%)	P
Estrogen receptors				
Positive	22 (84.61%)	45 (87.82%)	67 (82.71%)	0.755
Negative	4 (15.38%)	10 (18.18%)	14 (17.28%)	
Progesterone receptors				
Positive	18 (69.23%)	41 (74.54%)	59 (72.84%)	0.615
Negative	8 (30.77%)	14 (25.45%)	22 (27.16%)	
Her 2neu receptors				
Positive	13 (50.00%)	21 (38.18%)	34 (41.97%)	0.314
Negative	13 (50.00%)	34 (61.82%)	47 (58.02%)	
P53				
Positive	11 (42.31%)	26 (47.27%)	37 (45.68%)	0.675
Negative	15 (57.69%)	29 (52.73%)	44 (54.32%)	
LVI				
Positive	15 (57.69%)	5 (9.09%)	20 (24.69%)	< 0.001*
Negative	11 (42.31%)	50 (90.91%)	61 (75.31%)	
Ki67				
< 20	11 (42.31%)	24 (43.64%)	35 (43.21%)	0.910
> 20	15 (57.69%)	31 (56.36%)	46 (56.79%)	

*, Statistically significant differences; N = number.

Characteristics of the receptors in the patients with primary breast cancer are shown in Table 3. The presence of the estrogen receptors, progesterone

receptors showed that they do not have influence on the positivity for metastatic deposits in axillary lymph nodes, except the frequency of LVI positive patients (57.69%) in positive axillary patients in comparison with axillary negative patients (9.09%), ($p < 0.001$) (Table 3).

Multivariant model and logistic regression analysis as significant independent factors or predictors of positivity of the axillary lymph nodes are influenced by the tumour size only ($p = 0.014$).

Discussion

Axilar lymphadenectomy gives us parameters for the axillar status, but at the same time is the therapeutic procedure. On the other hand, axillar lymphadenectomy was followed with many unlike features and complications like as sensation in the arm, reduction of the arm mobility and lymphedema [3]. Use the thesis of the Fisher and Veronesi that breast cancer is the systemic disease at the moment of the diagnosis it, and needs to be treated as a systemic disease with drugs that work in the whole body (chemotherapeutic, antihormonal therapy, immunotherapy) [4, 5]. So axillar status is a first diagnostic tool, and in many instances especially if it is not involved with metastatic disease, which is in 40-70% not involved, it is not necessary to done axillar lymphadenectomy [6].

Prediction of axillary status can be used to predict whole axillar status, predict sentinel node and predict nonsentinel node status if sentinel node is positive [7-10].

Many authors use many standard methods for prediction of the axillar status, as clinical examination, mammography, ultrasonography, but also introduce new methods like ultrasound guide biopsy, CT, NMRI, Pet-CT, SPET-CT, contrast examinations, but in any case they see enlarged lymph nodes, but is impossible to guarantee that all this are metastatic changed (low sensitivity) [11-14]. Use of these methods is possible only to lower rate of falls negative results [15].

In last period we done lot investigations how different factors influent to positivity of axillary lymph nodes, on different groups of patients (patients with early breast cancer, patients with early breast cancer and clinically negative axilla, all surgically treated patients with breast cancer, patients with advanced breast cancer) and in last period we examined how size of tumor, persistence of estrogen receptors, progesterone receptors, HER 2 new receptors and Ki67 values influent to chance of dispersion of the disease in the axilla. Interesting is the fact that in the same institution in the same period, but on different groups of patients according to the stage of the disease were analysed the factors that can predict positivity of axilla. We find that in the whole group where patients with early but also with advantage stage were an as important factor for appearing metastases in axilla were the persistence of lymphovascular invasion and bigger values of Ki67 that were not important in groups with only early breast cancer. The possibility is that with the advantage of the stage are appearing aggressive factors in a tumour that after that influence on spreading the tumour cells in lymph nodes in axilla [16, 17].

Introduction of SLND detection, especially if are used both type of detection, as vital blue due (methylene blue) and radioisotope Technician with colloid particles (radiocoloid) at the end of the last century, give us very successful tool for detection SLND, which histological examination, give us successfully status of SLND but also status of whole axilla [16, 18-22].

In literature, there are many investigations for determination of factors that can predict positivity of axilla, SLND and NSLND if SLND is positive. Factors can be divided into few categories:

- Epidemiological (age, race, side, localisation);
- Clinical (palpability of a tumour, palpability of axillary lymph nodes, location);
- Pathological (histology of a tumour, differentiation of cells, neovascularisation of a tumour, vascular and lymphovascular invasion, extensive intraductal component, the persistence of receptors on the surface of the cells- estrogen, progesterone, Her-2 new, the persistence of p53 proteins, the persistence of factor of proliferation Ki67. Knowing these parameters is possible to determine subtype of breast cancer.);
- Biochemical (CEA, CA 15-3);
- Genetic (BRCA1, BRCA2, VEGFC, MIB1, CCR7, CXCR4) [23-59].

Many of the factors that were examined as a predictor of axillary status, is very well known, known is a biological way of action, and is very well known how is their action to the biology of a tumour, and how they work to spread the disease in the body. So estrogen receptors are on the surface of the cell. The connection of the estrogen and estrogen receptors activate many processes in the cell and favourite raising and dividing the cells, so favourite estrogen rising of a tumour. Giving the drugs that blockade estrogen receptors or drugs that blockade synthesis of the estrogen will stop rising of a tumour. The same situation is with persistence of HER-2 neu receptors. HER2 is a membrane tyrosine kinase and oncogene that is overexpressed and gene amplified in about 20% of breast cancers. When activated it provides the

cell with potent proliferative and anti-apoptosis signals, and it is the major driver of tumour development and progression of breast cancer. Over expression will activate many pathways in the cell, so the cells will raise and divide uncontrolled, so a tumour will raise and can't be under control. Giving the target drug – monoclonal antibody-Trastuzumab (Herceptin), will blockade this receptor, and the tumour will be under control. More, giving chemotherapeutics which interact with all cells which is divide fast; a tumour will be under control. Ki67 is a factor that shows the proliferative activity of the tumour cells. Ki67 is in correlation with S phase of cells and mitotic activity. Normal breast cell has a proliferative activity of 3% (3% of cells are in dividing stage). The Bigger activity of 20 % shows an aggressive tumour with bad prognosis and shorter survival [60-62].

Many investigators analyse many factors, how to allow or in combination can predict the status of the axillar lymph nodes, the status of SLND and in recent time status of NSLND. Postaci, Jiao, Jaime Jans, Ugras, Gangi, Pijnappel, Sawaki, Brenin, Chung, Chadha, Tan, Gajdos, Qiu, Ashturkar, Wu, Tseng, Ko, Li, Ngo, Yoo, Danko, Cabioglu, Capdet, Susini, Wasuthit are part of authors whose in last decade investigate which factors influent to positivity of axillar lymph node or positivity of sentinel node. They investigate all factors that can be investigated like epidemiological, clinical, histopathological, genetic, molecular. Mainly from all these studies dominantly main factors that can influent to positivity of axillar nodes are the size of a tumour, location, histology, grade of differentiation, lymphovascular invasion. But also in many of the investigations, other factors that can influence to positivity of axillar lymph nodes are referred: age, the persistence of estrogen, progesterone and Her two new receptors on the surface of the cells, a subtype of breast cancer, values of Ki67, multifocality, EIC and other. In only a few studies were referred VEGFC, MIB1, CEA, CA 15-3, CCR7, CXCR4 and others [23-46].

It is very interesting how some factors in some studies are important factors that predict axillar involvement with metastases, but in other studies, these factors are not important, and no influence to axillar involvement. For example in studies of Jiao, Pijnappel, Sawaki, Gangi, Qiu one of the essential factors that predict axillar involvement is the persistence of hormonal receptors and HER 2 receptors on the tumour cell, much more is well defined that Luminal and Her enriched tumours lymph nodes are more often involved with metastatic disease. On the other hand, triple negative tumours rarely have involvement of lymph nodes with metastatic disease, never less than this type shows early distant metastasis and worse prognosis. But in many others, studies show that persistence of hormone receptors, HER 2 receptors on the surface of tumour cells, does not influence involvement of axillary lymph nodes with metastases. So it is interesting why the same factor in one study is the main factor, and in other study is not important [24, 27-29, 35].

In our study, the univariant regression analysis showed that the size of a tumour and presence of HER2 neu receptors on the surface of cancer cell influence on the positivity of the axillary lymph nodes. The presence of the estrogen receptors and progesterone receptors showed that they do not have an influence on the positivity for metastatic deposits in axillary lymph nodes. Multivariant model and logistic regression analysis as significant independent factors or predictors of positivity of the axillary lymph nodes are influenced by the tumour size only. It is necessary as a minimum to done detection and biopsy of the sentinel node, which is further histology examined. With detecting status of the sentinel node, we can safely predict the status of other lymph nodes in the axilla.

In conclusion, our study showed that the involving of the axillary lymph nodes is mainly influenced by the size of a tumour and presence of HER2neu receptors in the univariant analysis points the important influence of positivity in the axillary lymph nodes but the only size of a tumour in the multivariate regressive analysis.

References

1. Carter CL, Allen C, Henson DE. Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases. Cancer. 1989;63:181–187. https://doi.org/10.1002/1097-0142(19890101)63:1<181::AID-CNCR2820630129>3.0.CO;2-H

2. Giuliano AE, Kirgan DM, Guenther JM, Morton DL. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. Annals of Surgery. 1994;220(3):391–401. https://doi.org/10.1097/00000658-199409000-00015 PMid:8092905 PMCid:PMC1234400

3. Maunsell E, Brisson J & Deshenes L. Arm problems and psychological distress after surgery for breast cancer. Canadian Journal of Surgery 1993; 36: 315–320. PMid:8370012

4. Fisher B, Jeong JH, Anderson S, et al. Twenty-five-year follow-up of a randomized trial comparing radical mastectomy, total mastectomy and total mastectomy followed by irradiation. N Engl J Med. 2002; 347(8):567–575. https://doi.org/10.1056/NEJMoa020128 PMid:12192016

5. Veronesi U, Saccozzi R, Del Vecchio M, et al. Comparing radical mastectomy with quadrantectomy, axillary dissection, and radiotherapy in patients with small cancer of the breast. N Engl J Med. 1981; 305(1):6–11. https://doi.org/10.1056/NEJM198107023050102 PMid:7015141

6. Cady B, Stone MD, Schuler JG, Thakur R, Wanner MA, Lavin PT. The new era in breast cancer: invasion, size, and nodal involvement dramatically decreasing as a result of mammographic screening. Archives of Surgery. 1996; 131:301–308. https://doi.org/10.1001/archsurg.1996.01430150079015 PMid:8611096

7. Barth A, Craig PH, Silverstein MJ. Predictors of axillary lymph node metastases in patients with T1 breast carcinoma. Cancer. 1997;79:1918–22. https://doi.org/10.1002/(SICI)1097-

0142(19970515)79:10<1918::AID-CNCR12>3.0.CO;2-Y

8. Tan YY, Wu CT, Fan YG, et al. Primary tumor characteristics predict sentinel lymph node macrometastasis in breast cancer. Breast J. 2005;11:338–43. https://doi.org/10.1111/j.1075-122X.2005.00043.x PMid:16174155

9. Ravdin PM, De Laurentiis M, Vendely T, Clark GM. Prediction of axillary lymph node status in breast cancer patients by use of prognostic indicators. J Natl Cancer Inst. 1994;86:1771–75. https://doi.org/10.1093/jnci/86.23.1771 PMid:7966415

10. Hwang RF, Krishnamurthy S, Hunt KK, et al. Clinicopathologic factors predicting involvement of nonsentinel axillary nodes in women with breast cancer. Ann Surg Oncol. 2003;10:248–54. https://doi.org/10.1245/ASO.2003.05.020 PMid:12679309

11. Patani NR, Dwek MV, Douek M.Predictors of axillary lymph node metastasis in breast cancer: a systematic review. Eur J Surg Oncol. 2007; 33(4):409-19. https://doi.org/10.1016/j.ejso.2006.09.003 PMid:17125963

12. Ahmed M, Purushotham AD, Douek M. Novel techniques for sentinel lymph node biopsy in breast cancer: a systematic review. Lancet Oncol. 2014;15(8):e351-62. https://doi.org/10.1016/S1470-2045(13)70590-4

13. Byon W, Kim E, Kwon J, Song BJ, Park C. FDG-PET, MRI and USG in the assesment of axillary lymph node metastases in breast cancer. The Breast. 2015; 24:S1-S67. https://doi.org/10.1016/S0960-9776(15)70165-7

14. Hwang SO, Lee SW, Kim HJ, Kim WW, Park HY, Jung JH. The Comparative Study of Ultrasonography, Contrast-Enhanced MRI, and (18)F-FDG PET/CT for Detecting Axillary Lymph Node Metastasis in T1 Breast Cancer. J Breast Cancer. 2013;16(3):315-21. https://doi.org/10.4048/jbc.2013.16.3.315 PMid:24155761 PMCid:PMC3800728

15. Hyun SJ, Kim EK, Yoon JH, Moon HJ, Kim MJ. Adding MRI to ultrasound and ultrasound-guided fine-needle aspiration reduces the false-negative rate of axillary lymph node metastasis diagnosis in breast cancer patients. Clin Radiol. 2015;70(7):716-22. https://doi.org/10.1016/j.crad.2015.03.004 PMid:25917544

16. Kondov B, Milenkovikj Z, Spirovski Z, Popgjorceva D, Stojanovski S, Petrusevska G, Ivkovski Lj, Jovanovski-Srceva M, Kondov G. Application of "sentinel lymph node detection" in axilla in patients surgically treated for early breast cancer. Physioacta. 2016; 10(2):53-62.

17. Kondov B, Kondov G, Spirovski Z, Milenkovikj Z, Colanceski R, Petrusevska G, Pesevska M. Prognostic factors on the positivity for metastases of the axillary lymph nodes from primary breast cancer-Pril. 2017; 37:1-10.

18. Giuliano AE, Han SH. Local and regional control in breast cancer: role of sentinel node biopsy. Adv Surg. 2011; 45:101-16. https://doi.org/10.1016/j.yasu.2011.03.015

19. Mamounas ET.Optimal Management of the Axilla: A Look at the Evidence. Adv Surg. 2016;50(1):29-40. https://doi.org/10.1016/j.yasu.2016.03.003 PMid:27520860

20. Moncayo VM, Aarsvold JN, Grant SF, Bartley SC, Alazraki NP. Status of sentinel lymph node for breast cancer. Semin Nucl Med. 2013;43(4):281-93. https://doi.org/10.1053/j.semnuclmed.2013.02.004 PMid:23725990

21. Gherghe M, Bordea C, Blidaru A. Sentinel lymph node biopsy (SLNB) vs. axillary lymph node dissection (ALND) in the current surgical treatment of early stage breast cancer. J Med Life. 2015;8(2):176-80. PMid:25866575 PMCid:PMC4392088

22. Zervoudis S, Iatrakis G, Tomara E, Bothou A, Papadopoulos G, Tsakiris G.Main controversies in breast cancer. World J Clin Oncol. 2014;5(3):359-73. https://doi.org/10.5306/wjco.v5.i3.359 PMid:25114851 PMCid:PMC4127607

23. Postacı H, Zengel B, Yararbaş U, Uslu A, Eliyatkın N, Akpınar G, Cengiz F, Durusoy R. Sentinel lymph node biopsy in breast cancer: predictors of axillary and non-sentinel lymph node involvement. Balkan Med J. 2013;30(4):415-21. https://doi.org/10.5152/balkanmedj.2013.9591 PMid:25207151 PMCid:PMC4115951

24. Jiao D, Qiao J, Lu Z, Li L, Zhang H, Liu H, Cui S, Liu Z. [Analysis of predictive factors affecting sentinel lymph node status in early breast cancer patients]. Zhonghua Zhong Liu Za Zhi. 2014;36(3):198-201. PMid:24785280

25. Jans BJ, Escudero MN, Pulgar BD, Acevedo CF, Sánchez RC, Camus AM. Clinicopathologic subtypes and compromise of lymph nodes in patients with breast cancer. eCancer Medical Science. 2014;8.

26. Ugras S, Stempel M, Patil S, Morrow M. Estrogen receptor, progesterone receptor, and HER2 status predict lymphovascular invasion and lymph node involvement. Ann Surg Oncol. 2014;21(12):3780-6. https://doi.org/10.1245/s10434-014-3851-y PMid:24952028 PMCid:PMC4362539

27. Gangi A, Mirocha J, Leong T, Giuliano AE. Triple-negative breast cancer is not associated with increased likelihood of nodal metastases. Ann Surg Oncol. 2014;21(13):4098-103. https://doi.org/10.1245/s10434-014-3989-7 PMid:25155393

28. Pijnappel EN, Bhoo-Pathy N, Suniza J, See MH, Tan GH, Yip CH, Hartman M, Taib NA, Verkooijen HM. Prediction of lymph node involvement in patients with breast tumors measuring 3-5 cm in a middle-income setting: the role of Cancer Math. World J Surg. 2014;38(12):3133-7. https://doi.org/10.1007/s00268-014-2752-3 PMid:25167896

29. Sawaki M, Idota A, Ichikawa M, Gondo N, Horio A, Kondo N, Hattori M, Fujita T, Yatabe Y, Iwata H. Impact of intrinsic subtype on predicting axillary lymph node metastasis in breast cancer. Oncol Lett. 2014;8(4):1707-1712. https://doi.org/10.3892/ol.2014.2333

30. Brenin DR, Manasseh DM, El-Tamer M, Troxel A, Schnabel F, Ditkoff BA, Kinne D. Factors correlating with lymph node metastases in patients with T1 breast cancer. Ann Surg Oncol. 2001; 8(5):432-7. https://doi.org/10.1007/s10434-001-0432-7 PMid:11407518

31. Chung MJ, Lee JH, Kim SH, Suh YJ, Choi HJ.Simple Prediction Model of Axillary Lymph Node Positivity After Analyzing Molecular and Clinical Factors in Early Breast Cancer. Medicine (Baltimore). 2016;95(20):e3689. https://doi.org/10.1097/MD.0000000000003689 PMid:27196477 PMCid:PMC4902419

32. Chadha M, Chabon AB, Friedmann P, Vikram B. Predictors of axillary lymph node metastases in patients with T1 breast cancer. A multivariate analysis. Cancer. 1994;73(2):350-3. https://doi.org/10.1002/1097-0142(19940115)73:2<350::AID-CNCR2820730219>3.0.CO;2-5

33. Tan LG, Tan YY, Heng D, Chan MY. Predictors of axillary lymph node metastases in women with early breast cancer in Singapore. Singapore Med J. 2005;46(12):693-7. PMid:16308642

34. Gajdos C, Tartter PI, Bleiweiss IJ. Lymphatic invasion, tumor size, and age are independent predictors of axillary lymph node metastases in women with T1 breast cancers. Ann Surg. 1999;230(5):692-6. https://doi.org/10.1097/00000658-199911000-00012 PMid:10561094 PMCid:PMC1420924

35. Qiu PF, Liu JJ, Wang YS, Yang GR, Liu YB, Sun X, Wang CJ, Zhang ZP. Risk factors for sentinel lymph node metastasis and validation study of the MSKCC nomogram inbreast cancer patients. Jpn J Clin Oncol. 2012;42(11):1002-7. https://doi.org/10.1093/jjco/hys150 PMid:23100610

36. Ashturkar AV, Pathak GS, Deshmukh SD, Pandave HT. Factors predicting the axillary lymph node metastasis in breast cancer: is axillary node clearance indicated in every breast cancer patient?: factors predicting the axillary lymphnode metastases in breast cancer. Indian J Surg. 2011;73(5):331-5. https://doi.org/10.1007/s12262-011-0315-5 PMid:23024536 PMCid:PMC3208716

37. Wu SG, He ZY, Ren HY, Yang LC, Sun JY, Li FY, Guo L, Lin HX. Use of CEA and CA15-3 to Predict Axillary Lymph Node Metastasis in Patients with Breast Cancer. J Cancer. 2016;7(1):37-41. https://doi.org/10.7150/jca.13090 PMid:26722358 PMCid:PMC4679379

38. Tseng HS, Chen LS, Kuo SJ, Chen ST, Wang YF, Chen DR. Tumor characteristics of breast cancer in predicting axillary lymph node metastasis. Med Sci Monit. 2014;20:1155-61. https://doi.org/10.12659/MSM.890491 PMid:24998473 PMCid:PMC4099209

39. Ko BS, Lim WS, Kim HJ, Yu JH, Lee JW, Kwan SB, Lee YM, Son BH, Gong GY, Ahn SH. Risk factor for axillary lymph node metastases in microinvasive breast cancer. Ann Surg Oncol. 2012;19(1):212-6. https://doi.org/10.1245/s10434-011-1812-2 PMid:21633867

40. Ngô C, Mouttet D, De Rycke Y, Reyal F, Fourchotte V, Hugonnet F, Falcou MC, Bidard FC, Vincent-Salomon A, Fourquet A, Alran S. Validation over time of a nomogram including HER2 status to predict the sentinel node positivity in early breast carcinoma. Eur J Surg Oncol. 2012;38(12):1211-7. https://doi.org/10.1016/j.ejso.2012.08.007 PMid:22954526

41. Yoo SH, Park IA, Chung YR, Kim H, Lee K, Noh DY, Im SA, Han W, Moon HG, Lee KH, Ryu HS. A histomorphologic predictive model for axillary lymph node metastasis in preoperative breastcancer core needle biopsy according to intrinsic subtypes. Hum Pathol. 2015; 46(2):246-54. https://doi.org/10.1016/j.humpath.2014.10.017 PMid:25496835

42. Danko ME, Bennett KM, Zhai J, Marks JR, Olson JA Jr. Improved staging in node-positive breast cancer patients using lymph node ratio: results in 1,788 patients with long-term follow-up. J Am Coll Surg. 2010;210(5):797-805.e1. https://doi.org/10.1016/j.jamcollsurg.2010.02.045 PMid:20421053

43. Cabioglu N, Yazici MS, Arun B, Broglio KR, Hortobagyi GN, Price JE, Sahin A. CCR7 and CXCR4 as novel biomarkers predicting axillary lymph node metastasis in T1 breast cancer. Clin Cancer Res. 2005;11(16):5686-93. https://doi.org/10.1158/1078-0432.CCR-05-0014 PMid:16115904

44. Capdet J, Martel P, Charitansky H, Lim YK, Ferron G, Battle L, Landier A, Mery E, Zerdoub S, Roche H, Querleu D - Factors predicting the sentinel node metastases in T1 breast cancer tumor: an analysis of 1416 cases. Eur J Surg Oncol. 2009; 35(12):1245-9. https://doi.org/10.1016/j.ejso.2009.06.002 PMid:19574018

45. Susini T, Nori J, Olivieri S, Molino C, Marini G, Bianchi S, Vezzosi V, Livi L, Mascalchi M, Scarselli G. Predicting the status of axillary lymph nodes in breast cancer: a multiparameter approach including axillary ultrasound scanning. Breast. 2009;18(2):103-8. https://doi.org/10.1016/j.breast.2009.02.001 PMid:19285396

46. Wasuthit Y, Kongdan Y, Suvikapakornkul R, Lertsithichai P, Chirappapha P. Predictive factors of axillary lymph node metastasis in breast cancer. J Med Assoc Thai. 2011;94(1):65-70. PMid:21425730

47. Giuliano AE, McCall L, Beitsch P, Whitworth PW, Blumencranz P, Leitch AM, Saha S, Hunt KK, Morrow M, Ballman K. Locoregional recurrence after sentinel lymph node dissection with or without axillary dissection in patients with sentinel lymph node metastases: the American College of Surgeons Oncology Group Z0011 randomized trial. Ann Surg. 2010;252(3):426-32. https://doi.org/10.1097/SLA.0b013e3181f08f32

48. Meattini I, Saieva C, Bertocci S, Francolini G, Zei G, De Luca Cardillo C, Scotti V, Greto D, Bonomo P, Orzalesi L, Bianchi S, Livi L. Predictive factors for additional non-sentinel lymph node involvement in breast cancer patients with one positive sentinel node. Tumori. 2015; 101(1):78-83. https://doi.org/10.5301/tj.5000220 PMid:25702658

49. Nadeem RM, Gudur LD, Saidan ZA. An independent assessment of the 7 nomograms for predicting the probability of additional axillary nodal metastases after positive sentinel lymph node biopsy in a cohort of British patients with breastcancer. Clin Breast Cancer. 2014;14(4):272-9. https://doi.org/10.1016/j.clbc.2014.02.006 PMid:25037530

50. van den Hoven I, Kuijt G, Roumen R, Voogd A, Steyerberg EW, Vergouwe Y. A head to head comparison of nine tools predicting non-sentinel lymph node status in sentinel node positive breast cancer women.J Surg Oncol. 2015;112(2):133-8. https://doi.org/10.1002/jso.23992 PMid:26258749

51. van den Hoven I, van Klaveren D, Voogd AC, Vergouwe Y, Tjan-Heijnen V, Roumen RM. A Dutch Prediction Tool to Assess the Risk of Additional Axillary Non-Sentinel Lymph Node Involvement in Sentinel Node-Positive Breast Cancer Patients. Clin Breast Cancer. 2016;16(2):123-30. https://doi.org/10.1016/j.clbc.2015.09.003 PMid:26602438

52. Kuo YL, Chen WC, Yao WJ, Cheng L, Hsu HP, Lai HW, Kuo SJ, Chen DR, Chang TW. Validation of Memorial Sloan-Kettering Cancer Center nomogram for prediction of non-sentinel lymph node metastasis in sentinel lymph node positive breast cancer patients an international comparison. Int J Surg. 2013;11(7):538-43. https://doi.org/10.1016/j.ijsu.2013.05.005 PMid:23707626

53. Cordero JM, Bernet L, Cano R, Bustamante M, Vila R, Ballester B, González PJ. [Study of the sentinel node in breast cancer using lymphoscintigraphy and a fast method for cytokeratin]. Rev Esp Med Nucl. 2004;23(1):9-14. https://doi.org/10.1016/S0212-6982(04)72239-9

54. Gur AS, Unal B, Johnson R, Ahrendt G, Bonaventura M, Gordon P, Soran A. Predictive probability of four different breast cancer nomograms for nonsentinel axillary lymph node metastasis in positive sentinel node biopsy.J Am Coll Surg. 2009;208(2):229-35. https://doi.org/10.1016/j.jamcollsurg.2008.10.029 PMid:19228534

55. Gur AS, Unal B, Ozbek U, Ozmen V, Aydogan F, Gokgoz S, Gulluoglu BM, Aksaz E, Ozbas S, Baskan S, Koyuncu A, Soran A; Turkish Federation of Breast Disease Associations Protocol MF08-01 investigators. Validation of breast cancer nomograms for predicting the non-sentinel lymph node metastases after a positive sentinel lymph node biopsy in a multi-center study. Eur J Surg Oncol. 2010;36(1):30-5. https://doi.org/10.1016/j.ejso.2009.05.007 PMid:19535217

56. Cserni G, Bori R, Maráz R, Leidenius MH, Meretoja TJ, Heikkila PS, Regitnig P, Luschin-Ebengreuth G, Zgajnar J, Perhavec A, Gazic B, Lázár G, Takács T, Vörös A, Audisio RA. Multi-institutional comparison of non-sentinel lymph node predictive tools in breast cancer patients with high predicted risk of further axillary metastasis. Pathol Oncol Res. 2013;19(1):95-101. https://doi.org/10.1007/s12253-012-9553-5 PMid:22798061

57. Cserni G, Boross G, Maráz R, Leidenius MH, Meretoja TJ, Heikkila PS, Regitnig P, Luschin-Ebengreuth G, Zgajnar J, Perhavec A, Gazic B, Lázár G, Takács T, Vörös A, Audisio RA. Multicentre validation of different predictive tools of non-sentinel lymph node involvement in breast cancer. Surg Oncol. 2012;21(2):59-65. https://doi.org/10.1016/j.suronc.2011.12.001 PMid:22197294

58. Freedman GM, Fowble BL, Li T, Hwang ES, Schechter N, Devarajan K, Anderson PR, Sigurdson ER, Goldstein LJ, Bleicher RJ. Risk of positive nonsentinel nodes in women with 1-2 positive sentinel nodes related to age and molecular subtype approximated by receptor status. Breast J. 2014;20(4):358-63. https://doi.org/10.1111/tbj.12276 PMid:24861613 PMCid:PMC4472437

59. Gülben K, Berberoğlu U, Aydoğan O, Kınaş V. Subtype is a predictive factor of nonsentinel lymph node involvement in sentinel node-positive breast cancer patients. J Breast Cancer. 2014;17(4):370-5. https://doi.org/10.4048/jbc.2014.17.4.370 PMid:25548586 PMCid:PMC4278057

60. Williams C, Lin C-Y. Oestrogen receptors in breast cancer: basic mechanisms and clinical implications; Ecancer Medical Science. 2013; 7: 370. PMid:24222786 PMCid:PMC3816846

61. Gutierrez C, Schiff R. HER 2: Biology, Detection, and Clinical Implications. Arch Pathol Lab Med. 2011; 135(1): 55–62. PMid:21204711 PMCid:PMC3242418

62. van Diest PJ, van der Wall E, Baak JPA. Prognostic value of proliferation in invasive breast cancer: a review. J Clin Pathol. 2004; 57(7): 675–681. https://doi.org/10.1136/jcp.2003.010777 PMid:15220356 PMCid:PMC1770351

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):831-835.
https://doi.org/10.3889/oamjms.2017.175
eISSN: 1857-9655
Basic Science

Influence of *Interleukin-6 (174G/C)* Gene Polymorphism on Obesity in Egyptian Children

Ola M. Ibrahim[1], Azza A. Gabre[1], Sara F. Sallam[1], Inas R. El-Alameey[1*], Rania N. Sabry[1], Essam M. Galal[1], Sawsan M. Tawfik[1], Waheba A. Zarouk[2], Rehab M. Mosaad[2], Abeer Ramadan[2]

[1]Child Health Department, Medical Division, National Research Centre, Egypt; [2]Molecular Genetics & Enzymology Department , Human Genetics and Genome Research Division, National Research Centre, Egypt

Citation: Ibrahim OM, Gabre AA, Sallam SF, El-Alameey IR, Sabry RN, Galal EM, Tawfik SM, Zarouk WA, Mosaad RM, Ramadan A. Influence of *Interleukin-6 (174G/C)* Gene Polymorphism on Obesity in Egyptian Children. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):831-835. https://doi.org/10.3889/oamjms.2017.175

Keywords: Obesity; Interleukin-6(174 G/C); Children; polymorphism; Egypt.

***Correspondence:** Inas R. El-Alameey. National Research centre, EGYPT, Child Health department Medical Division, Cairo, Egypt. E-mail: inasno@hotmail.com

Received: 24-Jun-2017; **Revised:** 06-Sep-2017; **Accepted:** 07-Sep-2017; **Online first:** 17-Oct-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Obesity is a multi-factorial chronic disorder. A considerable number of studies have been performed to figure out whether there is an association between obesity and polymorphisms of gene *IL-6 (174G/C)*, but the results are equivocal.

AIM: This study aimed to find out whether the *IL-6 (174G/C)* gene was associated with the risk of developing obesity in Egyptian children.

SUBJECTS AND METHODS: The study included 149 children and adolescents with age ranged between 9.5 – 18 years. Eighty-five of them were obese which BMIZ-score is > 2, and sixty-four children with BMIZ-score ≤ 2 served as control group. Serum level of IL-6 and genetic analysis for *IL-6 (174G/C)* gene polymorphism were done.

RESULTS: Obese children had significantly higher serum levels of IL-6 as compared to those of control children (P = 0.003). A high percentage of *IL-6* polymorphism *GC* was found in obese subjects (93.7%), while the control group had a higher percentage of *IL-6* polymorphism *GG* (70.6 %).

CONCLUSION: Our study showed that carriers of the *C* allele for the *IL-6 (174G/C)* polymorphism have higher BMI. As the *G174C* polymorphism is likely to affect IL-6 expression and its physiological regulation; consequently this polymorphism may affect adiposity.

Introduction

Obesity represents one of the major Public Health problems. Obese children had a shorter life expectancy by 20 years if they remain obese till young adulthood [1]. North African Countries and the region of Middle East just like the other developing countries are not spared from the issue of the world [2, 3]. Genetics, metabolic, social, cultural and environmental factors interact as different cofactors for the development of obesity. The prevalence of obesity in Egyptian children ranged from 13.5 % up to 23.7% [4-5].

Advanced knowledge of human genome variations led to identification of genes susceptible to contribute in obesity and other related diseases; meanwhile few studies focused specifically on the interactions between obesity and genetic polymorphism [6]. Interleukin-6 (IL-6) is an immune-modulator pro-inflammatory cytokine involved in the regulation of the acute phase response [7]. High IL-6 serum level was found in obese patients as well as patients suffering chronic inflammatory conditions and serum lipid concentrations abnormalities [8]. Interleukin-6 production has been shown to be significantly increased by adipose tissue in a state of obesity. The increased IL-6 levels in obese individuals may result in a state of insulin resistance and increased risk of cardiovascular complication [9].

Plasmatic levels of cytokines and its transcriptional regulation is influenced by the

polymorphisms of IL-6 (especially174G/C) as proved in many population groups based genetic studies, that have been performed to figure out whether there is an association between obesity and *IL-6* (*174G/C*) polymorphisms, but results were not conclusive with a lot of controversies [10-11].

Based on this; this study aimed to find out whether *IL6 (174G/C)* polymorphisms designated as rs1800795 as well, is associated with the risk of developing obesity in Egyptian children.

Subjects and Methods

This is a cross-sectional study that included one hundred and forty-nine children and adolescents, 71 males and 78 females. Their age ranged between 9.5 to 18 years (mean 11.72 ± 2.52 yrs). This study started by one thousand children and adolescents chosen randomly from three governmental schools at Giza. According to their anthropometric measures, it was found that one hundred and thirty were obese (BMI-Z score >2). Eighty-five of them (41 males and 44 females) were fulfilling the criteria and gave the consent to share in the study.

Exclusion criteria: were factors that might lead to the misclassification of a child's weight status, that are: (1) an acute or chronic illness affecting weight; (2) genetic conditions associated with obesity or failure to thrive; and/or (3) use of medications associated with weight gain or loss.

Inclusion criteria: apart from obesity; all obese subjects and non-obese controls were in good health. This study was a part of project that approved by the Medical Ethical Committee of the National Research Center with registration number 14046. All participants were informed about the objectives of the study and volunteered to participate and the parents of all subjects provided a written informed consent.

Anthropometric assessments

Anthropometric assessments were performed according to techniques described in the Anthropometric Standardization Reference Manual [12]. Weight was measured using a calibrated Seca scale to the nearest 0.1 kg (Seca, Hamburg, Germany), whereas height (in cm) was measured using a Seca 225 stadiometer to the nearest 0.1 cm, with the children dressed in minimal clothes and without shoes. Each measurement was taken as the mean of three consecutive readings following the recommendations of the International Biological Program [13]. Weight for height, weight for age, height for age, and BMI for age was recorded according to WHO standards using Anthro Plus software for personal computers [3]. Measurements were expressed as weight-for-age Z-score (WAZ), height for age Z-score (HAZ), and BMI Z-score (BAZ).The participants were then grouped according to the WHO Global Database on Child Growth and Malnutrition using a Z-score cut-off point [14]: Overweight and obese children with BAZ >2 and control children with BAZ ≤ 2.

Laboratory Investigations

Fasting blood sample was drawn from each participant into vacutainer tube containing EDTA as an anticoagulant. The samples were immediately transferred to the laboratory, centrifuged immediately for 10 min at 4000 and stored at −80°C.

- Serum interleukin-6 level measuring using the quantitative Enzyme-Linked Immune-Sorbent Assay (ELISA) with a commercial kit provided by DIA source, Belgium [15].

- DNA extraction: Blood samples were collected on Na_2EDTA as an anticoagulant.Genomic DNA was purified from 200 µl whole blood with the QIAamp® DNA Blood Mini Kit (Qiagen) according to the Blood and Body Fluid Spin Protocol in the accompanying handbook. DNA was eluted in 200 µl elution buffer and stored at –20°C.

- Detection of *IL6 (-174G/C)* polymorphism: DNA was amplified with primers specific for *-174G/C* (rs1800795) in a 25 µl reaction mixture containing ; 50 ng µL genomic DNA, ddH_2O 15.7 µL, 10 x buffer 2.5 µL, dNTP (10 mmol) 0.5 µL, each of primer sequences (10 µmol) 0.5 µL, $MgCL_2$ (25 mmol) 2 µL and 1U Taq enzyme. The PCR was performed in 30 cycles; (95°C for 30 sec, annealing at 55°C for 30 s, and extension at 72°C for 1 min), with an initial denaturation at 95°C for 5 min and a final extension at 72°C for 10 min. Followed by final extension 72°C for 5 min. All PCR products were analyzed by 2% agarose gel electrophoresis.

- Enzyme digestion: Post PCR–RFLP was done by incubating five µl of SNP-PCR product with the restriction enzyme *NIa III* at 37 °C for 30 min. The digested SNP-PCR product was electrophoresed on 3% agarose gel [16].

Statistical analysis

The data were analyzed using statistical package for social sciences (SPSS) version 16. Results were presented as mean ± SD, except where otherwise indicated. Comparisons between groups were done by independent samples t-test. Chi-square test was used to estimate differences in qualitative variables. Pearson correlation test was used to determine the relationship between some numerical variables. For all tests, probability values (P) of less than 0.05 were regarded as statistically significant.

Results

The characteristics of the studied groups are shown in Table 1. The mean serum level of IL-6 in obese children was significantly higher as compared to those of control children (7.7 ± 0.46 pg/mL and 5.46 ± 0.40 pg/mL respectively (P = 0.003). Serum IL-6 level had a positive correlation with age (r = 0.28 and p = 0.001) and with BAZ (r = 0.188 and p = 0.03).

Table 1: Characteristics of the studied groups

Parameter	Controls	Obese	P
Number	64	85	
Males: females	30:34	41:44	ns
Mean age (years)	10.9 ± 2.3	12.3 ± 2.4	ns
Z-score BMI (kg/m^2)	o.13 ± 0.16	2.8 ± 0.62	0.00
IL-6 (pg/ml)	5.46 ± 0.40	7.7 ± 0.46	

P value ≤ 0.05 is significant.

Result of genotyping analysis of IL6-174G/C polymorphism

The Nla III restriction enzyme digest the 500 bp applicant of the IL6-174; where *G* allele yielded three bands (of 264, 207 and 29bp); the -174 *C* allele yielded four bands (of 264, 122, 85 and 29 bp) (Figure 1A & 1B).

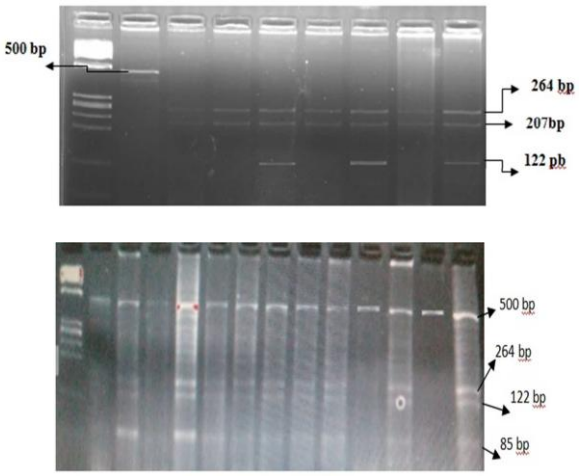


Figure 1: A) Agarose gel (3%) photographs of PCR-RFLP products (amplicons digested with restriction enzymes) depicting the genotypes of IL-6 polymorphisms PCR and digestion products run on 3% agarose gel electrophoresis. Lane 1: ΦX 174 marker/Hae III digest. Lane 2: The PCR amplified product (500 bp). Lanes 3, 4, 6 and 8: G/G genotype (264, 207 and 29 bp). Lanes 5, 7, and 9: G/C genotype (264, 207 and 122 bp); B) Fig (2): PCR and digestion products run on 3% agarose gel electrophoresis. Lane 1: ΦX 174 marker / Hae III digest. Lane 2: The PCR amplified product (500 bp). Lanes 3, 4, 6, 7, 8, 9, 10, 12: G/C genotype (500, 264, 122 and 85 bp)

Table 2 shows the distribution of genotype for *IL6 (-174G/C)* polymorphism: The homozygote genotype *(G/G)* was 57.05 %, the heterozygote *(G/C)* 42.95 % and none of our patients was carrying the homozygote genotype *(C/C)*.

Table 2: The frequency of genotype of *IL-6* polymorphism

	Frequency	Percent
Heterozygous *GC*	64	42.95
Homozygous *GG*	85	57.05
Total	149	100

No significant difference in the serum level of *IL-6* between subjects with IL-6 (174G/C) polymorphism and those with IL6 (174G/G) polymorphism (Table 3).

Table 3: The mean serum level of *IL-6* in both groups of polymorphism

Parameter	GC	GG	P
IL-6 (pg/ml) Mean ± SD	6.53 ± 4.97	6.99 ± 4.52	ns

Table 4 shows that the mean of BMI Z score was significantly higher in *IL-6* polymorphism *GC* as compared to that of polymorphism *GG*. Our results showed that 95.3 % of *IL-6* polymorphism *GC* was found in obese subjects as compared to 4.7 % in normal weight children.

Table 4: The mean ±SD of BMIZ-score (BAZ) in both types of *IL-6* polymorphism

Parameter	GC	GG	P
BMIZ-score, Mean ± SD	2.76 ± 0.68	0.86 ± 1.6	0.00

P value ≤ 0.05 is significant.

On the other hand, 71.8 of *IL-6 (174G/G)* polymorphism were found in controls as compared to 28.2% in obese children (Table 5). It was noted in the results that, none of our children were carrying *C/C* genotyping.

Table 5: The percentage of *IL-6* polymorphism in obese and control groups

IL-6 polymorphism	Controls		Obese		P
	(n)	%	(n)	%	
GG (n=85)	61	71.8	24	28.2	0.00
GC (n=64)	3	4.7	61	95.3	0.00

Discussion

This study revealed a significantly higher serum level of IL-6 in obese children as compared to those of controls. The Same finding was previously reported in a study of Filippo et al. [11]. Our data showed a positive correlation of IL-6 with both age and BAZ.

These results are by the results of Diego et al. [17] and Paltoglou et al. [18]. In this work, we studied whether the *IL6(174G/C)* gene is associated with the risk of developing obesity in obese Egyptian children. The analysed polymorphism *(174G/C)* is localised in the promoter region of the *IL-6* gene, where transcription factors frequently exert their functions through its influence on the energy expenditure

processes by different ways. The results revealed that the *GG* homozygote genotype is predominated in the control group (95.3 %) with a statistically significant difference compared to that of obese children (28.2 %). If we considered the *GC* genotypes as *C* carrier; so, the study showed a prevalence 71.8 % (61 of 85), of this C allele among the obese children as compared with 4.7% (3 of 64) in the normal weight children. So, the examined polymorphisms of the pro-inflammatory cytokine *IL-6 (174G/C)* could play a role in the regulation of body mass. According to these aspects, the results of this study seem to confirm the hypothetical relation between the *IL6 (174G/C)* genotype and the risk of developing obesity among Egyptian children.

Our data match and is replicated with previously published data; as some authors have investigated the relation between polymorphisms in *IL-6* gene and obesity based on experimental evidence on mice suggested an influence on fat mass, fat metabolism, and body mass and on the development of obesity [19]. Zhangbin et al., [20] realized that *IL-6 (174 G/C)* polymorphism was also associated with obesity by covering 48 studies with outcome similar to the results in our study.

In a study of Popko et al., [21] the detected *C* allele in both *C/C* homozygotes and G/C heterozygotes of *IL6 (174 G/C)* gene was related to a significant increase in the sum of 10 skinfold thickness measurements in obese girls; which is consistent with the results of our study. A study enrolling Greek school children had shown an association of the IL-6 variant rs1800795 with parameters related to obesity [22]. Oana et al. [23] described the *G* allele at *C-174* as being more common in lean subjects and observed the *C* allele to be associated with indices of obesity. These findings are in concordance with the results of our study, in which the *GC* heterozygote genotype predominated in the obese group, and the *GG* genotype was less pronounced in this group than in the normal weight group. In this study, none of our children was carrying *C/C* genotyping. This is because it is a pilot study and our sample size was small. It was previously reported by Saxena et al. [24] that *CC* genotype was very rare in their study.

Contrary to our findings, data reported in an Iranian study among 242 persons, sustained more frequent *G* alleles in the obese but was statistically insignificant [25]. Another meta-analysis didn't find significant associations of *IL6 (174G/C)* genotypes with waist-to-hip ratio, waist circumference, or central obesity and did not support a role for this polymorphism in adiposity [26].

In conclusion, our study showed that carriers of the *C* allele for the *IL-6* polymorphism *G174C* have higher BMI. As the *G174C* gene polymorphism is likely to affect *IL-6* expression and its physiological regulation; consequently this polymorphism may affect adiposity and insulin sensitivity. It is recommended in future studies for the *IL-6* polymorphism *G174C* to be done in a wider scale of children and adolescents and in different demographic areas as our study enrolled relatively small number of obese and normal children which decreased the power of the group in statistic results.

References

1. Gabbert C, Donohue M, Arnold J, Schwimmer JB. Adenovirus 36 and obesity in children and adolescents. Pediatrics. 2010; 126(4): 721-726. https://doi.org/10.1542/peds.2009-3362 PMid:20855385

2. Remla N, Hadjidj Z, Ghezzaz K, Moulessehoul S, Aribi M. Increased gustatory response score in obesity and association levels with IL-6 and Leptin. Journal of Nutrition and Metabolism. 2016; 2016:Article ID 7924052.

3. WHO. Anthro Plus for personal computers, manual software for assessing growth of the world's children and adolescents. Geneva: department of nutrition for health; 2009. Available at: ttp://www.who.int/growthref/tools/en

4. Badawi NES, Barakat AA, El Sherbini SA, Fawzy HM. Prevalence of overweight and obesity in primary school children in Port Said city. Egyptian Pediatric Association Gazette. 2013; 61: 31-36. https://doi.org/10.1016/j.epag.2013.04.007

5. Taha AA, Marawan HM. Socio-behavioral determinants of overweight and obesity in Egyptian primary school children. J Child Adolesc Behav. 2015; 3:236.

6. Qi L, Zhang C, van Dam RM, Hu FB. Interleukin-6 genetic variability and adiposity associations in two prospective cohorts and systematic review in 26,944 individuals. J Clin Endocrinol Metab. 2007; 92(9): 3618–3625. https://doi.org/10.1210/jc.2007-0877 PMid:17623760

7. Senn JJ, Klover Pj, NowakI A, Zimmers TA, Koniaris LG, Furlanetto RW, Mooney RA. Suppressor of cytokine signaling-3(socs-3), a potential mediator for interleukin-6 dependent insulin resistance in hepatocytes. J Biol Chem. 2003;278(16) :13740-6 https://doi.org/10.1074/jbc.M210689200 PMid:12560330

8. Galcheva S V ,Violeta M L, Yoto T Y, Sergio B Maria E S. Circulating proinflammatory peptides related to abdominal adiposity and cardiometabolic risk factors in healthy prepubertal children. Eur J Endocrinol. 2011; 164(4):553-8. https://doi.org/10.1530/EJE-10-1124 PMid:21224406

9. Raynald T, Sarah MW, Hesti L. Interleukin-6 and insulin resistance in obese adolescents. Paediatr Indones. 2013;53(5):268-72. https://doi.org/10.14238/pi53.5.2013.06

10. Popko K, Gorska E, Pyrzak B, Telmaszczyk-Emmel A, Wisniewska A, Majcher A, Wasik M, Demkow U. Influence of proinflammatory cytokine gene polymorphism on childhood obesity. Eur J Med Res. 2009; 14(Suppl4):59–62. PMid:20156727 PMCid:PMC3521345

11. Filippo DeG, Rendina D, Moccia F., Rocco V., Campanozzi A. Interleukin-6, Soluble interleukin-6 receptor/interleukin-6 complex and insulin resistance in obese children and adolescents. Journal of Endocrinological Investigation. 2015; 38 (3) 339–343. https://doi.org/10.1007/s40618-014-0176-4 PMid:25245339

12. Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Publishers, 1988. PMCid:PMC279682

13. Tanner JM, Hiernaux J, Jarman S, Weiner JS, Lourie JA. Growth, and physique studies. Human biology: a guidance to fields methods. In: WeinerJS, Lourie SA, editors. London: Blackwell Scientific Publications, 1969:273–275.

14. WHO/EMRO: Regional data on non-communicable diseases risk factors World Health Organization, Regional Office of East

Mediterranean. Non- Communicable diseases. Website: http// www.emr.who.int.ncd. Accessed April 2010.

15. DIA source IL-6 EASIA Kit. Catalogue No: KAP1261. DIA source Immuno Assays S.A. Rue Du Bosquet, 2, B-1348-Louvain0La-Neuve, Belgium.

16. Martinez JC, Etxagibel A, Calleja SC. Saez-torres C, M. Sedeno M, Julia R, Muncunill J, Matamoros N, Gaya A. Allelic frequencies of polymorphic variants of cytokine genes (IL1A, IL1B, IL1RN, IL6, IL10, IL12p40, and IFNG) in a Spanish population. Immunología. 2002; 21(2):76-86.

17. Diego Y, Joan V, Rosangela T, Montse B, Miquel G, Ana M, Antonio C. Interleukin-6 in obese children and adolescents with and without glucose intolerance. Diabetes Care. 2007; 30 (7): 1892-1894. https://doi.org/10.2337/dc06-2289 PMid:17416792

18. Paltoglou G, Maria S, George V, Alexandra A, Athanasios C. The Morbid connection among obesity, aseptic inflammation, pro-oxidation and markers of insulin resistance in pre- and early-pubertal boys before and after an acute bout of treadmill exercise. Endocrine Society's 97th. Annual Meeting and Expo, March 5–8: San Diego, 2015

19. Chida D, Osaka T, Hashimoto O, Iwakura Y. Combined interleukin-6 and interleukin-1 deficiency causes obesity in young mice. Diabetes. 2006; 55:971–977. https://doi.org/10.2337/diabetes.55.04.06.db05-1250 PMid:16567518

20. Zhangbin Y, Shuping H, Xingguo C, Chun Z, Xuejie W, Xirong G. Genetic polymorphisms in adipokine genes and the risk of obesity: a systematic review and metaanalysis. Obesity. 2011; 20:396–406.

21. Popko K, Gorska E, Pyrzak B, Telmaszczyk-Emmel A, Wisniewska A, Majcher A, Wasik M, Demkow U. Influence of proinflammatory cytokine gene polymorphism on childhood obesity. Eur J Med Res. 2009; 14(Suppl4):59–62. PMid:20156727 PMCid:PMC3521345

22. Dedoussi GVZ, ManiosY, Choumerianou DM, Yiannakouris N, Panagiotakos DB, Skenderi K and Zampelas A. The IL-6 Gene G-174C polymorphism related to health indices in Greek primary school children. Obes Res. 2004; 12: 1037-1041. https://doi.org/10.1038/oby.2004.130 PMid:15292466

23. Oana M, Claudia B, Carmen D, Maria P, Septimiu V, Claudiu M. The role of IL-6 572 C/G, 190 C/T, and 174 G/C gene polymorphisms in children's obesity. European Journal of Pediatrics. 2014;173 (10) 1285–1296. https://doi.org/10.1007/s00431-014-2315-5 PMid:24740880

24. Saxena M, Agrawal C, Srivastava N, and Banerjee M. Interleukin-6 (IL-6)597 A/G (rs1800797) & 174 G/C (rs1800795) gene polymorphisms in type 2 diabetes. Indian J Med Res. 2014; 140 (1): 60–68. PMid:25222779 PMCid:PMC4181162

25. Rostami F, Haj Hosseini R, Sharifi K, Daneshpour M, Azizi F,and Hedayati M. Association of G174C polymorphism of the interleukin-6 Gene promoter with obesity in Iranian population. World Acad Sci Technol. 2010; 45:99–102.

26. Todendi P, Klinger E, Ferreira M, Reuter C, Burgos M, Possuelo L, Valim A. Association of IL-6 and CRP gene polymorphisms with obesity and metabolic disorders in children and adolescents. An Acad Bras Cienc. 2015;87 (2) 1678-2690. https://doi.org/10.1590/0001-3765201520140364 PMid:25993353

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):836-843.
https://doi.org/10.3889/oamjms.2017.093
eISSN: 1857-9655
Basic Science

Genistein Ameliorates Cyclophosphamide - Induced Hepatotoxicity by Modulation of Oxidative Stress and Inflammatory Mediators

Dina F. Mansour, Dalia O. Saleh*, Rasha E. Mostafa

Pharmacology Department, Medical Division, National Research Centre, (ID: 60014618), Dokki, Giza, Egypt

Citation: Mansour DF, Saleh DO, Mostafa RE. Genistein Ameliorates Cyclophosphamide-Induced Hepatotoxicity by Modulation of Oxidative Stress and Inflammatory Mediators. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):836-843. https://doi.org/10.3889/oamjms.2017.093

Keywords: Cyclophosphamide; genistein; interleukin-1β; Myeloperoxidase; Oxidative stress.

***Correspondence:** Dalia O. Saleh, Pharmacology Department, Medical Division, National Research Centre, (ID: 60014618), Dokki, Giza, Egypt. E-mail: doabdelfattah@yahoo.com

Received: 30-Mar-2017; **Revised:** 07-May-2017; **Accepted:** 16-May-2017; **Online first:** 01-Nov-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: The present study investigated the protective effect of the phytoestrogen, genistein (GEN), against (CP)-induced acute hepatotoxicity in rats.

MATERIAL AND METHODS: Male adult rats were randomly assigned into five groups. Normal control group received the vehicles; CP group received a single dose of CP (200 mg/kg, i.p). The other three groups received subcutaneous GEN at doses of 0.5, 1 and 2 mg/kg/day, respectively, for 15 consecutive days prior CP injection. Sera and liver tissues were collected forty-eight hours after CP injection for assessment of liver function enzymes (ALT and AST) in rat sera, the hepatic oxidative/nitrosative biomarkers (GSH, MDA and NOx), hepatic interleukin-1β, and myeloperoxidase activity. Immunohistochemistry of cyclooxygenase-2 and histopathological examination of liver tissues were also conducted.

RESULTS: The CP-induced acute liver damage was evidenced by elevated serum ALT and AST accompanied by increased hepatic oxidative stress and inflammatory biomarkers. Immunohistochemical outcomes revealed hepatic cyclooxygenase-2 expression in CP group with distortion of liver architecture. GEN-pretreatment significantly ameliorated the deterioration of liver function and exerted significant anti-oxidant and anti-inflammatory activity with a marked decline in hepatic cyclooxygenase-2 expression in a dose dependent-manner.

CONCLUSION: The present study demonstrated that the antioxidant and anti-inflammatory activities of GEN might contribute to its protective effects against CP-induced liver damage.

Introduction

Cyclophosphamide (CP) is an oxazaphosphorine derivative of the traditional alkylating agent nitrogen mustard [1]. Cyclophosphamide is widely used in the treatment of several human cancers [2] including solid tumours, lymphomas and leukaemia [3], as well as many non-neoplastic diseases, such as systemic lupus erythematosus and rheumatoid arthritis [4, 5]. The therapeutic dose of CP has been shown to cause liver toxicity; which often restricts its clinical use [6-8]. Growing evidence suggests that oxidative stress plays a major role in CP-induced hepatotoxicity [9, 10] via causing severe cellular damage accompanied by lipid peroxidation and changes in cellular nucleic acids [11, 12]. CP is metabolised by the hepatic microsomal cytochrome P450 (CYP450), giving rise to phosphoramide mustard and acrolein [13]. Acrolein; a highly reactive metabolite of CP with short biological half-life; inhibits CYP450 by alkylating the reduced glutathione (GSH) sulfhydryl groups thus inducing oxidative stress and causing hepatotoxicity [14, 15]. Furthermore, CP damages mitochondria and impairs cellular respiration [16]. Thus it interferes with hepatic intracellular oxidant/antioxidant balance leading to reactive oxygen species (ROS) accumulation [9]. ROS then induces lipid peroxidation and activates multiple signalling cytotoxicity pathways including nuclear transcription factor kappa-B (NF-κB) and mitogen-activated protein kinases (MAPKs), thus initiating different steps in the inflammatory pathway [17]. Thus, introducing some anti-oxidant agents may be useful in alleviating the toxic side effect of anti-cancer drugs. Earlier studies have shown that some plant extracts with antioxidant activity protect against CP hepatotoxicity [18-20]. Accordingly, chemotherapeutic-

treatment protocol combined with potent and safe antioxidants could be a desirable tool to mitigate CP-induced hepatotoxicity.

Genistein (GEN), a phytoestrogen present in soy products, possesses structural similarity to estrogen. GEN acts like human estrogen and binds to estrogen receptors [21] which justifies its use for treatment of symptoms of menopause [22]. Previous studies suggested that GEN possesses anti-osteoporotic, anti-neoplastic, cardio-protective, anti-proliferative, anti-apoptotic, anti-necrotic, antioxidant and anti-inflammatory actions [23].

It has been well-established the link between the estrogenic activity of GEN and its antioxidant effect via decreasing oxidative stress as a consequence of increased expression of antioxidant defence genes [24]. Recently, GEN has been shown to improve the endothelium-dependent relaxation in insulin-resistant ovariectomized rats with further modulation of the elevated blood pressure [25]. Previous studies reported GEN-hepatoprotective activity against several models of hepatotoxicity [26, 27].

Therefore, the current study aims at exploring the potential protective effects of GEN against cyclophosphamide-induced hepatotoxicity in rats.

Materials and Methods

Animals

Forty adult male Sprague-Dawley rats weighing 180-200 g were utilised in the present study. Standard food pellets and tap water were supplied ad libitum. Animals and food pellets were obtained from the animal house colony of the National Research Center (NRC, Egypt). The study was conducted by ethical procedures and policies outlined in the Canadian Council of Animal Care guidelines (NAC 2011) and was approved by the Medical Research Ethics Committee (MREC) of the National Research Centre (approval no. 16/434).

Drugs and chemicals

Genistein (LC Laboratories, Woburn, Massachusetts, USA; purity > 99%) and cyclophosphamide (CP, Endoxan®, Baxter Oncology GmbH, Germany) were used in the study. GEN was dissolved in 5 ml DMSO (1.25%) and divided into equal aliquots [28]. Every day, an aliquot of the drug was freshly diluted by distilled water and injected subcutaneously. The concentration was adjusted so that each 100 g animal body weight received 0.25 ml, 0.5 ml and 1 ml for doses 0.5, 1 and 2 mg/kg, respectively [29]. CP was injected intraperitoneally in a single hepatotoxic dose of 200 mg/kg [7]. All of the other chemicals were of highest analytical grade available.

Experimental design and treatment protocol

Animals were randomly allocated into five groups (8 rats each). Rats of the 1st group received subcutaneous injections of the corresponding vehicle (DMSO) and served as normal control group. Acute hepatotoxicity was induced in the remaining four groups by single intraperitoneal injection of cyclophosphamide (CP; 200 mg/kg) on the 15th day of treatment. Group 2 received only the vehicle (saline) subcutaneously for 15 days before CP treatment and served as CP control group. Groups 3, 4 and 5 received genistein (GEN; 0.5, 1 and 2 mg/kg/day, s.c.) respectively for 15 days before CP treatment on day 15. All animals were sacrificed 48 h after CP injection.

Serum biochemical analysis

Forty-eight hours after CP injection, rats were anaesthetised with diethyl ether, and blood samples were withdrawn from the retro-orbital venous plexus. Collected blood samples were allowed to stand for 10 min at room temperature then centrifuged at 4^{o}C using cooling centrifuge (Laborezentrifugen, 2k15, Sigma, Germany) at 3000 r.p.m for 10 min and sera were separated for the assessment of levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) according to the methods of Reitman and Frankel (1957) using commercially available colorimetric assay kits (Biodiagnostic, Egypt) [30].

Hepatic Tissue biochemical analysis

Directly after collecting the last blood sample in the experiment, rats were sacrificed by cervical dislocation and liver tissues were collected, washed with normal saline and then divided into 3 parts: one part was homogenized (using MPW–120 homogenizer, Med instruments, Poland); the homogenate was centrifuged using a cooling centrifuge (Laborezentrifugen, 2k15, Sigma, Germany) at 3000 r.p.m for 10 min; the supernatant was assessed for hepatic levels of reduced glutathione (GSH), lipid peroxides as malondialdehyde (MDA) [32], and nitric oxide (NO) metabolites [33]. Moreover, inflammatory markers such as myeloperoxidase (MPO) [34], interleukin-1β (IL-1β) were assessed using enzyme-linked immunosorbent assay (ELISA) kits (Hycult Biotech, Netherlands) and (R&D Systems, USA), respectively, according to the manufacturer's instructions.

Immunohistochemistry of Cyclooxygenase-2 (COX-2)

Liver COX-2 was assessed by immunohistochemical staining. Liver sections on polylysine-coated slides obtained were fixed in neutral buffered formalin, and embedded in paraffin and were treated for COX-2 antibodies for immunohistochemical analysis. Following deparaffinization and rehydration, sections were irradiated in 0.1 mol/L sodium citrate buffer [pH 6.0] in a microwave oven [medium low temperature] for 20 min. Then the sections were exposed to 3% H_2O_2 for 10 min to bleach endogenous per-oxidases, followed by rinsing three times in Tris buffer (pH 7.4) for 10 min. Sections were selectively incubated under humid conditions using an anti-COX-2 antibody [1:200; Santacruz Biotechnology, Inc., USA] [10].

Histopathological examination

The other parts of the livers were fixed in 10% neutral buffered formalin and embedded in paraffin wax. 4 µm thick sections were stained with Hematoxylin and Eosin (H&E) and examined using binocular Olympus CX31 microscope [35].

Statistical analysis

All the values are presented as means ± standard error of the means (SEM) of eight experiments. Comparisons between different groups were carried out using one-way analysis of variance (ANOVA) followed by *Tukey's* multiple comparison post hoc tests. The difference was considered significant when $P < 0.05$. GraphPad prism® software (version 6 for Windows, San Diego, California, USA) was used to carry out these statistical tests.

Results

Effects of GEN on serum liver microsomal enzymes in CP-induced hepatotoxicity in rats

Cyclophosphamide (CP, 200 mg/kg, i.p.) resulted in acute liver damage in rats as evidenced by the significant elevation of serum alanine trans-aminase (ALT) and aspartate transaminase (AST) to 477% and 285%, respectively, as compared to the normal control group. Pretreatment of rats with GEN (0.5, 1 or 2 mg/kg/day, s.c.) significantly decreased the elevated serum AST and ALT in a dose-dependent manner as compared to the CP-control group. Genistein treatment at 2 mg/kg recorded normal levels of ALT and improved both ALT and AST significantly compared to the other dose levels (Figure 1).

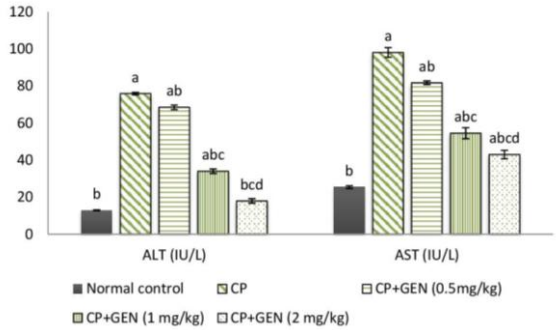


Figure 1: Effects of genistein on serum alanine transaminase (ALT) and aspartate transaminase (AST) in cyclophosphamide-induced hepatotoxicity in rats. Rats of the normal control group received s.c. Injections of DMSO. Acute hepatotoxicity was induced in the remaining four groups by single intraperitoneal injection of cyclophosphamide (CP; 200 mg/kg) on the 15th day of treatment. Group 2 received only saline s.c. for 15 days and served as CP control group. Groups 3, 4 and 5 received genistein (GEN; 0.5, 1 and two mg/kg/day, s.c.) respectively for 15 days before CP treatment. All animals were sacrificed 48 h after CP injection, blood samples were collected, and sera were separated. Data are presented as mean ± SEM (n = 8). a, Significantly different from Normal control group at p < 0.05 (Tukey's post hoc test). b, significantly different from CP control group at p < 0.05 (Tukey's post hoc test). c, Significantly different from CP+GEN (0.5 mg/kg) group at p < 0.05 (Tukey 's post hoc test). d Significantly different from CP+GEN (l mg/kg) group at p < 0.05 (Tukey's post hoc test)

Effects of GEN on hepatic oxidative/nitrosative stress parameters in CP-induced hepatotoxicity in rats.

Cyclophosphamide (CP, 200 mg/kg, i.p.) resulted in acute liver damage in rats as evidenced by a significant decrease in hepatic GSH by 77%, with a marked increase in hepatic MDA and NO as compared to the normal control group.

Pretreatment of rats with GEN, at 1 and 2 mg/kg, significantly elevated the decreased liver GSH while GEN at 0.5 mg/kg failed to exert significant increase in GSH as compared to the CP-control group. On the other hand, GEN-pretreatment (0.5, 1 and 2 mg/kg/day, s.c.) for 15 days prior CP injection significantly reduced the elevation in hepatic MDA and NO in a dose-dependent manner compared to the CP-control group, recording normal levels of NO at GEN dose of 2 mg/kg/day with insignificant difference from normal control group (Table 1).

Effects of GEN on hepatic inflammatory marker (MPO) and the cytokine (IL-1β) in CP-induced hepatotoxicity in rats

Cyclophosphamide (CP, 200 mg/kg, i.p.) resulted in acute liver damage in rats as evidenced by an increase in hepatic MPO and IL-1β content as compared to the normal control group. Pretreatment of rats with GEN (0.5, 1 and 2 mg/kg/day, s.c.) for 15 days prior CP injection significantly reduced the elevation in hepatic MPO and IL-1β in a dose-

dependent manner compared to the CP-control group, recording normal levels of MPO and IL-1β at GEN dose of 2 mg/kg/day with insignificant difference from normal control group (Table 1).

Table 1: Effects of genistein on reduced hepatic glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO) metabolites, myeloperoxidase (MPO), and interleukin one beta (Dtp) contents in cyclophosphamide-induced hepatotoxicity in rats

Groups	Liver tissue oxidative/ nitrosative stress Parameters			Liver inflammatory marker	Liver tissue Cytokine
	GSH (μmol/g)	MDA (nmol/g)	NO (μmol/gl)	MPO (U/g)	IL-1β (pg/g)
Normal Control	4.00 ^{b} ± 0.14	12.03 ^{b} ± 0.48	2.04 ^{b} ± 0.05	1.88^{b} ± 0.085	8.11 ^{b} ± 0.44
CP-Control (200 mg/kg, i.p.)	0.93 ^{a} ± 0.01	36.32^{a} ± 0.28	24.10 ^{a} ± 0.67	12.2 ^{a} ± 0.7	47.15^{a} ± 0.45
CP + GEN (0.5 mg/kg, s.c.)	1.31 ^{a} ±0.05	28.43 ^{ab} ± 0.21	11.7 ^{ab} ± 0.30	5.73 ^{ab} ± 0.26	27.25 ^{ab} ± 0.91
CP + GEN (1 mg/kg, s.c.)	2.64 ^{abc} ± 0.04	23.9 ^{abc} ± 0.61	5.8 ^{abc} ± 0.40	2.66 ^{bc} ± 0.15	13.48 ^{abc} ± 0.56
CP + GEN (2 mg/kg, s.c.)	2.90 ^{abc} ± 0.08	16.85 ^{abcd} ± 0.43	3.8 ^{bcd} ± 0.05	1.48 ^{bc} ± 0.056	8.26 ^{bcd} ± 0.43

Rats of the normal control group received s.c. Injections of DMSO. Acute hepatotoxicity was induced in the remaining four groups by single intraperitoneal injection of cyclophosphamide (CP; 200 mg/kg) on the 15th day of treatment. Group 2 received only saline s.c. for 15 days and served as CP control group. Groups 3, 4 and 5 received genistein (GEN; 0.5, 1 and 2 mg/kg/day, s.c.) respectively for 15 days before CP treatment. All animals were sacrificed 48 h after CP injection. The livers were removed, homogenized, and the homogenate was obtained. Data are presented as mean ± SEM (n = 8). a, Significantly different from Normal control group at p < 0.05 *(Tukey's* post hoc test). b, Significantly different from CP control group at p < 0.05 *(Tukey's* post hoc test). C, Significantly different from CP+GEN (0.5 mg/kg) group at p < 0.05 *(Tukey's* post hoc test). d Significantly different from CP+GEN (I mg/kg) group at p < 0.05 *(Tukey's* post hoc test)

Effect of GEN on Cyclooxygenase-2 (COX-2) expression in liver tissue

Pretreatment with genistein for 15 days before CP injection showed a dose-dependent reduction in hepatic COX-2 expression ranging from mild, moderate and negative COX-2 immunostaining of the kupffer cells and the hepatocytes at 0.5, 1 and 2 mg/kg of GEN, respectively, compared to CP-treated liver tissues.

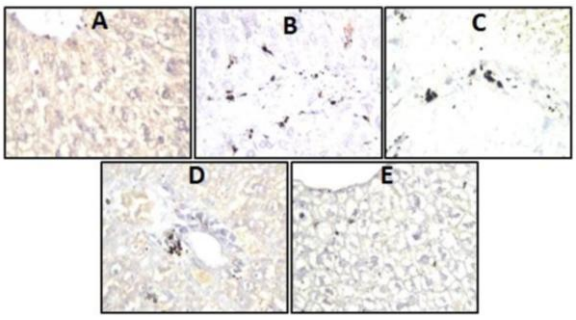


Figure 2: A. Photomicrograph of liver sections prepared from a normal control rat showing negative. COX-2 immunostaining of the kupffer cells and the hepatocytes (COX-2 immunohistochemistry H&E X 80). B. Photomicrograph of liver sections prepared from a cyclophosphamide-control rat showing severe COX-2 immunostaining of the kupffer cells and the hepatocytes (COX-2 immunohistochemistry H&E X 80). C. Photomicrograph of liver sections prepared from a rat treated with genistein (0.5 mg/kg/day, s.c.) for 15 days before CP treatment showing moderate COX-2 immunostaining of the kupffer cells and the hepatocytes (COX-2 immunohistochemistry H&E X 80). D. Photomicrograph of liver sections prepared from a rat treated with genistein (1 m///lkg/day, s.c.) for 15 days before CP treatment showing mild COX-2 immunostaining of the kupffer cells and the hepatocytes (COX-2 immunohistochemistry H&E X 80). E. Photomicrograph of liver sections prepared from a rat treated with genistein (2 m/l:g/day, s.c.) for 15 days before CP treatment showing negative COX-2 immunostaining of the kupffer cells and the hepatocytes (COX-2 immunohistochemistry H&E X 80)

Cyclophosphamide caused severe COX-2 immunostaining of the kupffer cells and the hepatocytes compared to normal tissues (Figure 2).

Effect of GEN on in liver histopathological picture

Cyclophosphamide exerted severe distortion in the hepatic architecture showing severe congestion and dilatation of the central and portal veins associated with degeneration in the hepatocytes surrounding the central vein and oedema in the portal area after 48 hours of injection (Figure 3). While GEN-pretreatment restored the histopathological picture of the liver causing mild to a moderate improvement compared to CP-treated liver tissues (Figure 3).

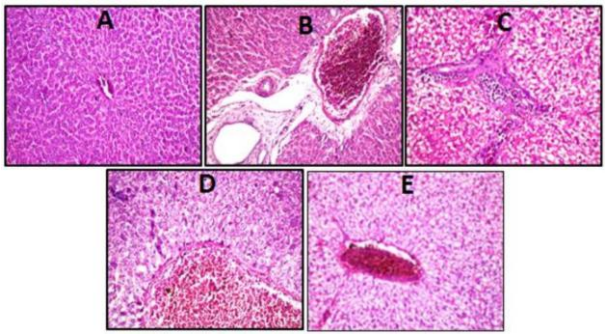


Figure 3: Photomicrograph of a liver section of a rat from a normal control group (H&E X 40) showing no histopathological alteration and the normal histological structure of the central vein and surrounding hepatocytes were recorded (Figure 3A). Photomicrograph of a liver section of a rat from the cyclophosphamide-control group (H&E X 40) showing severe congestion and dilatation of the central and portal veins associated with degeneration in the hepatocytes surrounding the central vein and oedema in the portal area (Figure 3B). Photomicrograph of a liver section of a rat that received genistein (0.5 mg/kg/day, s.c.) for 15 days before CP treatment (H&E X 40) showing mild improvement in the overall histopathological picture. Inflammatory cells infiltration was apparent in the portal area while the hepatocytes showed degenerative change (Figure 3C). Photomicrograph of a liver section of a rat that received genistein (1 mg/kg/day, s.c.) for 15 days before CP treatment (H&E X 40) showing minimal improvement in the overall histopathological picture. Dilatation and congestion in the central veins associated with degeneration in the surrounding adjacent hepatocytes were apparent (Figure 3D). Photomicrograph of a liver section of a rat that received genistein (2 mg/kg/day, s.c.) for 15 days before CP treatment (H&E X 40) showing moderate improvement in the overall histopathological picture. Congestion in the central and portal veins along with degeneration in the surrounding hepatocytes was also apparent (Figure 3E)

Discussion

Chemotherapy-induced oxidative stress causes severe cellular and tissue damage and usually results in many undesirable side effects [36]. Cyclophosphamide, a widely known chemotherapeutic agent used in the treatment of several human cancers, is well known to induce prominent oxidative

stress in the liver [13]. Even low doses of CP can produce significant hepatotoxicity in humans which often stands as a barrier against its clinical use [4, 37].

In the present study, CP-treated animals showed significant elevation of liver function enzymes AST and ALT, important markers for the evaluation of liver injury since their leakage into serum defines the severity of liver damage [38]. Liver tissue oxidative stress parameters; MDA and NO, inflammatory enzymes; MPO and liver tissue cytokine IL-1β were significantly elevated while liver tissue GSH content was significantly decreased when compared to normal counterparts. Cyclophosphamide administration exerted severe expression of hepatic cyclooxygenase-2 (COX-2) as well. Moreover, CP caused severe deterioration of the overall histopathological picture of the liver tissue when compared to normal control group. Our results are in line with previous reports that stated severe hepatotoxicity associated with elevated AST, ALT [4] and lactate dehydrogenase (LDH) [10, 39] with a significant elevation in oxidative stress marker; MDA and a significant decrease in GSH, along with deteriorated histopathological picture in CP-treated rats [9]. Linking oxidative stress to inflammation, myeloperoxidase (MPO) was found to exert a primary role in chronic inflammation [40] and demonstrates an essential mechanistic link between oxidation and inflammation [41]. Increased levels of MPO and the pro-inflammatory cytokine; IL-1β after CP administration was reported after 48 hours in the present study. In a recent study, COX-2 expression in liver of rats received CP was increased along with significant elevation in serum ALT, liver NF-κB p65, TNF-α, IL-1β, MDA, NO, Bax/Bcl-2 ratio, inducible nitric oxide synthetase (iNOS), caspases 3 and 9 activities while decreased hepatic total antioxidant capacity [42]. Furthermore, TNF-α and IL-1β play crucial roles in stimulation of iNOS and COX-2 [23, 43], which further explains the increased levels of NO after CP administration. Others reported elevation in pro-inflammatory mediators like TNF-α, IL-1β, IL-6 and COX-2 along with increased ROS, LPO, decreased GSH and antioxidant enzymes (SOD, CAT, GST, and GSH-Px) in liver tissues of CP-treated animals [44]. These further support current data of increased oxidation and inflammation in CP hepatotoxicity.

Since its discovery in 1987, genistein has been investigated in a myriad of research fields, especially after reporting no side effects or toxicity with high doses of genistein in vivo [45]. GEN; a phytoestrogen found abundantly in soy and widely used an alternative to estrogens; possesses immune-modulatory effects of estrogen without exhibiting its side effects [21]. GEN mainly undergoes hepatic metabolism through cytochrome P450 system to give monohydroxyl and dihydroxyl metabolites [46]. Exhibiting well-established antioxidant and anti-inflammatory properties against different models of hepatotoxicity, pretreatment of rats with GEN (0.5, 1 and 2 mg/kg/day, s.c.) for 15 days significantly protected against CP-induced deterioration of liver function and showed marked anti-oxidant and anti-inflammatory properties that were demonstrated by the reduction of serum AST and ALT, hepatic MDA, NO, MPO and IL-1β while elevation of hepatic GSH content when compared to CP group. Previous studies revealed the potent hepatoprotective effect of GEN through elevation of liver GSH and reduction of liver MDA in thioacetamide- [29] and CCl4- [26, 47] induced hepatotoxicity. GEN exhibited protective effects against oxidative stress hepatic injury as manifested by increased activity of hepatic SOD, catalase, glutathione peroxidase and reduction of MDA level [48, 49]. Meanwhile, GEN exerted ameliorative effects on hepatic injury and fibrosis induced by chronic alcohol in rats [50]. Some researchers attributed the anti-inflammatory effects of GEN to its antioxidant effects [51] where significant low levels of plasma TNF-α was reported in non-alcoholic steatohepatitis by GEN antioxidant properties [52]. A Recent study revealed protective effects of GEN against CP-induced ovarian toxicity by mitigating oxidative stress and inflammation through elevating GSH, SOD while reducing MDA along with modulation of IL-1β and iNOS, respectively [29].

Moreover, GEN was shown to inhibit NO production from activated macrophages by LPS and IFN-γ through inhibition of iNOS expression [53] by suppression of STAT-1 and NF-κB activation, which are important transcription factors for iNOS [54] that further supports our current observations. The anti-inflammatory effects of GEN were demonstrated earlier [55], which was in line with current results that revealed reduced hepatic levels of MPO and IL-1β after CP administration. In a rat model of D-galactosamine-induced fulminant hepatic failure, GEN supplementation exerted an anti-inflammatory activity via modulation of iNOS and COX-2, thereby reducing NO and prostaglandin-E2 (PGE2) levels, respectively. Also, pro-inflammatory cytokines; TNF-α and IL-1β are reduced with the suppression of NF-κB activation, IKKα/β and mitogen-activated protein kinase (MAPK) with a significant reduction in the serum AST and ALT levels as well as serum and liver tissue MDA accompanied by significant elevation of liver tissue GSH concentration [23].

Being contributed to the pathogenesis of inflammation-related diseases, a recent study demonstrated the relation between COX-2 and IL-1β as COX-2 catalyzes the synthesis of PGE2 and increases IL-1β by increasing NF-κB activation and enhancing caspase-1 activation through triggering mitochondrial damage, mitochondrial reactive oxygen species production and subsequent release of mitochondrial DNA into cytosol via pyrin domain-containing 3 (NLRP3) inflammasome, a reactive oxygen species-sensitive multiprotein complex, that regulates IL-1β maturation in the spleen and liver of LPS-challenged mice [56]. Hence, linking decreased

level of hepatic IL-1β and COX-2 expression by GEN-pretreatment in a dose-dependent manner in CP-treated liver tissues, providing a new insight of GEN as a potential protective agent in inflammatory liver disease. Eventually, GEN caused significant improvement of the overall histopathological picture of the liver when compared to CP-control group.

From the current study, it can be concluded that GEN possesses potent ameliorative effects against hepatotoxic actions of CP from various aspects. GEN significantly decreased the elevated serum levels of hepatic microsomal enzymes, reduced oxidative stress parameters as MDA and NO, augmented anti-oxidation via elevating GSH along with decreased inflammatory mediators; MPO, IL-1β and COX-2 expression. Hence, it may provide effective protection against liver damage via anti-oxidative and anti-inflammatory activities.

Acknowledgments

The authors are thankful to Prof. Adel Bekir, Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt, for the kind help in histopathology and immunohistochemistry.

References

1. Moignet A, Hasanali Z, Zambello R, Pavan L, Bareau B, Tournilhac O, Roussel M, Fest T, Awwad A, Baab K, Semenzato G, Houot R, Loughran TP, Lamy T. Cyclophosphamide as a first-line therapy in LGL leukemia. Leukemia. 2014;28:1134-1136. https://doi.org/10.1038/leu.2013.359 PMid:24280867 PMCid:PMC4017255

2. Jurado JM, Sanchez A, Pajares B, Perez E, Alonso L, Alba E. Combined oral cyclophosphamide and bevacizumab in heavily pre-treated ovarian cancer. Clinical & translational oncology. 2008;10: 583-586. https://doi.org/10.1007/s12094-008-0254-7

3. Kim SH, Lee IC, Ko JW, Moon C, Kim SH, Shin IS, Seo YW, Kim HC, Kim JC. Diallyl Disulfide Prevents Cyclophosphamide-Induced Hemorrhagic Cystitis in Rats through the Inhibition of Oxidative Damage, MAPKs, and NF-kappaB Pathways. Biomolecules & therapeutics. 2015; 23: 180-188. https://doi.org/10.4062/biomolther.2014.126 PMid:25767687 PMCid:PMC4354320

4. Cuce G, Cetinkaya S, Koc T, Esen HH, Limandal C, Balci T, Kalkan S, Akoz M. Chemoprotective effect of vitamin E in cyclophosphamide-induced hepatotoxicity in rats. Chemico-biological interactions. 2015; 232: 7-11. https://doi.org/10.1016/j.cbi.2015.02.016 PMid:25779342

5. Sinanoglu O, Yener AN, Ekici S, Midi A, Aksungar FB. The protective effects of spirulina in cyclophosphamide induced nephrotoxicity and urotoxicity in rats. Urology. 2012;80(6):1392.e1-6. https://doi.org/10.1016/j.urology.2012.06.053 PMid:22951000

6. De Jonge ME, Huitema AD, Beijnen JH, Rodenhuis S. High exposures to bioactivated cyclophosphamide are related to the occurrence of veno-occlusive disease of the liver following high-dose chemotherapy. British journal of cancer. 2006;94(9):1226-30. https://doi.org/10.1038/sj.bjc.6603097 PMid:16622453 PMCid:PMC2361415

7. Habibi E, Shokrzadeh M, Chabra A, Naghshvar F, Keshavarz-Maleki R, Ahmadi A. Protective effects of Origanum vulgare ethanol extract against cyclophosphamide-induced liver toxicity in mice. Pharmaceutical biology. 2015;53(1):10-5. https://doi.org/10.3109/13880209.2014.908399 PMid:25026348

8. Tsai-Turton M, Luong BT, Tan Y, Luderer U. Cyclophosphamide-induced apoptosis in COV434 human granulosa cells involves oxidative stress and glutathione depletion. Toxicological sciences. 2007;98(1):216-30. https://doi.org/10.1093/toxsci/kfm087 PMid:17434952

9. Fahmy SR, Amien AI, Abd-Elgleel FM, Elaskalany SM. Antihepatotoxic efficacy of Mangifera indica L. polysaccharides against cyclophosphamide in rats. Chemico-biological interactions. 2016;244:113-20. https://doi.org/10.1016/j.cbi.2015.11.009 PMid:26615121

10. Nafees S, Rashid S, Ali N, Hasan SK, Sultana S. Rutin ameliorates cyclophosphamide induced oxidative stress and inflammation in Wistar rats: role of NFκB/MAPK pathway. Chemico-biological interactions. 2015;231:98-107. https://doi.org/10.1016/j.cbi.2015.02.021 PMid:25753322

11. El-Sheikh AA, Rifaai RA. Peroxisome proliferator activator receptor (PPAR)-γ ligand, but not PPAR-α, ameliorates cyclophosphamide-induced oxidative stress and inflammation in rat liver. PPAR research. 2014;2014.

12. Lata S, Singh S, NathTiwari K, Upadhyay R. Evaluation of the antioxidant and hepatoprotective effect of Phyllanthus fraternus against a chemotherapeutic drug cyclophosphamide. Applied biochemistry and biotechnology. 2014;173(8):2163-73. https://doi.org/10.1007/s12010-014-1018-8 PMid:24993488

13. Zarei M, Shivanandappa T. Amelioration of cyclophosphamide-induced hepatotoxicity by the root extract of Decalepis hamiltonii in mice. Food and chemical toxicology. 2013;57:179-84. https://doi.org/10.1016/j.fct.2013.03.028 PMid:23542512

14. Marinello AJ, Gurtoo HL, Struck RF, Paul B. Denaturation of cytochrome P-450 by cyclophosphamide metabolites. Biochemical and biophysical research communications. 1978;83(4):1347-53. https://doi.org/10.1016/0006-291X(78)91369-4

15. Zhu H, Long MH, Wu J, Wang MM, Li XY, Shen H, Xu JD, Zhou L, Fang ZJ, Luo Y, Li SL. Ginseng alleviates cyclophosphamide-induced hepatotoxicity via reversing disordered homeostasis of glutathione and bile acid. Scientific reports. 2015;5:17536. https://doi.org/10.1038/srep17536 PMid:26625948 PMCid:PMC4667192

16. Souid AK, Tacka KA, Galvan KA, Penefsky HS. Immediate effects of anticancer drugs on mitochondrial oxygen consumption. Biochemical pharmacology. 2003;66(6):977-87. https://doi.org/10.1016/S0006-2952(03)00418-0

17. Kim HG, Yoon DH, Lee WH, Han SK, Shrestha B, Kim CH, Lim MH, Chang W, Lim S, Choi S, Song WO. Phellinus linteus inhibits inflammatory mediators by suppressing redox-based NF-κB and MAPKs activation in lipopolysaccharide-induced RAW 264.7 macrophage. Journal of ethnopharmacology. 2007;114(3):307-15. https://doi.org/10.1016/j.jep.2007.08.011 PMid:17936530

18. Haque R, Bin-Hafeez B, Ahmad I, Parvez S, Pandey S, Raisuddin S. Protective effects of Emblica officinalis Gaertn. in cyclophosphamide-treated mice. Human & experimental toxicology. 2001;20(12):643-50. https://doi.org/10.1191/096032701718890568 PMid:11936579

19. Kumar KB, Kuttan R. Chemoprotective activity of an extract of Phyllanthus amarus against cyclophosphamide induced toxicity in mice. Phytomedicine. 2005;12(6):494-500. https://doi.org/10.1016/j.phymed.2004.03.009 PMid:16008127

20. Sharma N, Trikha P, Athar M, Raisuddin S. Inhibitory effect of Emblica officinals on the in vivo clastogenicity of benzo alpyrene and acyclophosphamide in mice. Human & experimental toxicology. 2000;19(6):377-84. https://doi.org/10.1191/096032700678815945 PMid:10962512

21. Verdrengh M, Jonsson IM, Holmdahl R, Tarkowski A. Genistein as an anti-inflammatory agent. Inflammation Research. 2003;52(8):341-6. https://doi.org/10.1007/s00011-003-1182-8 PMid:14504672

22. Ogbuewu IP, Uchegbu MC, Emenalom OO, Okoli IC, Iloeje MU. Overview of the chemistry of soy isoflavones, potential threats and potential therapeutic benefits. Electronic Journal of Environmental, Agricultural & Food Chemistry. 2010;9(4).

23. Ganai AA, Khan AA, Malik ZA, Farooqi H. Genistein modulates the expression of NF-κB and MAPK (p-38 and ERK1/2), thereby attenuating d-Galactosamine induced fulminant hepatic failure in Wistar rats. Toxicology and applied pharmacology. 2015;283(2):139-46. https://doi.org/10.1016/j.taap.2015.01.012 PMid:25620059

24. Borrás C, Gambini J, Gómez-Cabrera MC, Sastre J, Pallardó FV, Mann GE, Vi-a J. Genistein, a soy isoflavone, up-regulates expression of antioxidant genes: involvement of estrogen receptors, ERK1/2, and NFκB. The FASEB Journal. 2006;20(12):2136-8. https://doi.org/10.1096/fj.05-5522fje PMid:16966488

25. Saleh DO, El-Awdan SA, Nofel SM, El-Eraky WI, El-Khatib AS, Kenawy SA. Estrogens improve the cardiovascular alterations in fructose-induced insulin resistant ovariectomized rats. International Journal of Pharmacy and Pharmaceutical Sciences. 2015;7(7):241-7.

26. Kuzu N, Metin K, Dagli AF, Akdemir F, Orhan C, Yalniz M, Ozercan IH, Sahin K, Bahcecioglu IH. Protective role of genistein in acute liver damage induced by carbon tetrachloride. Mediators of inflammation. 2007;2007.

27. Saleh DO, Abdel Jaleel GA, El-Awdan SA, Oraby F, Badawi M. Thioacetamide-induced liver injury: protective role of genistein. Canadian journal of physiology and pharmacology. 2014;92(11):965-73. https://doi.org/10.1139/cjpp-2014-0192 PMid:25358106

28. Vera R, Galisteo M, Villar IC, Sanchez M, Zarzuelo A, Pérez-Vizcaíno F, Duarte J. Soy isoflavones improve endothelial function in spontaneously hypertensive rats in an estrogen-independent manner: role of nitric-oxide synthase, superoxide, and cyclooxygenase metabolites. Journal of Pharmacology and Experimental Therapeutics. 2005;314(3):1300-9. https://doi.org/10.1124/jpet.105.085530 PMid:15958720

29. Saleh DO, Mansour DF. Ovario-protective effects of genistein against cyclophosphamide toxicity in rats: Role of anti-müllerian hormone and oestradiol. European journal of pharmacology. 2016;789:163-71. https://doi.org/10.1016/j.ejphar.2016.07.026 PMid:27448503

30. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology. 1957;28(1):56-63. https://doi.org/10.1093/ajcp/28.1.56 PMid:13458125

31. Beutler E. Improved method for determination of blood glutathione. J Lab Clin Med. 1963;61(5):882-8. PMid:13967893

32. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Analytical biochemistry. 1978;86(1):271-8. https://doi.org/10.1016/0003-2697(78)90342-1

33. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric oxide. 2001;5(1):62-71. https://doi.org/10.1006/niox.2000.0319 PMid:11178938

34. Roelofs JJ, Rouschop KM, Leemans JC, Claessen N, de Boer AM, Frederiks WM, Lijnen HR, Weening JJ, Florquin S. Tissue-type plasminogen activator modulates inflammatory responses and renal function in ischemia reperfusion injury. Journal of the American society of nephrology. 2006;17(1):131-40. https://doi.org/10.1681/ASN.2005010089 PMid:16291841

35. Bancroft J, Stevens A, Turner D. Theory and practice of histological techniques: Churchill Livingstone New York. the text, 1996:766.

36. Weijl N, Cleton F, Osanto S. Free radicals and antioxidants in chemotherapyinduced toxicity. Cancer treatment reviews. 1997; 23:209-240. https://doi.org/10.1016/S0305-7372(97)90012-8

37. Subramaniam SR, Cader RA, Mohd R, Yen KW, Ghafor HA. Low-dose cyclophosphamide-induced acute hepatotoxicity. The American journal of case reports. 2013;14:345. https://doi.org/10.12659/AJCR.889401 PMid:24023976 PMCid:PMC3767583

38. Yogalakshmi B, Viswanathan P, Anuradha CV. Investigation of antioxidant, anti-inflammatory and DNA-protective properties of eugenol in thioacetamide-induced liver injury in rats. Toxicology. 2010;268(3):204-12. https://doi.org/10.1016/j.tox.2009.12.018 PMid:20036707

39. Senthilkumar S, Devaki T, Manohar BM, Babu MS. Effect of squalene on cyclophosphamide-induced toxicity. Clinica Chimica Acta. 2006;364(1):335-42. https://doi.org/10.1016/j.cca.2005.07.032 PMid:16150433

40. Podrez EA, Abu-Soud HM, Hazen SL. Myeloperoxidase-generated oxidants and atherosclerosis. Free Radical Biology and Medicine. 2000;28(12):1717-25. https://doi.org/10.1016/S0891-5849(00)00229-X

41. Rao AM, Anand U, Anand CV. Myeloperoxidase in chronic kidney disease. Indian Journal of Clinical Biochemistry. 2011;26(1):28-31. https://doi.org/10.1007/s12291-010-0075-1 PMid:22211010 PMCid:PMC3068762

42. Fouad AA, Qutub HO, Al-Melhim WN. Punicalagin alleviates hepatotoxicity in rats challenged with cyclophosphamide. Environmental toxicology and pharmacology. 2016;45:158-62. https://doi.org/10.1016/j.etap.2016.05.031 PMid:27310207

43. Ghosh S, Hayden MS. New regulators of NF-κB in inflammation. Nature Reviews Immunology. 2008;8(11):837-48. https://doi.org/10.1038/nri2423 PMid:18927578

44. Jnaneshwari S, Hemshekhar M, Thushara RM, Shanmuga Sundaram M, Sebastin Santhosh M, Sunitha K, Shankar RL, Kemparaju K, Girish KS. Sesamol ameliorates cyclophosphamide-induced hepatotoxicity by modulating oxidative stress and inflammatory mediators. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 2014;14(7):975-83.

45. Lau DT, Luxon BA, Xiao SY, Beard MR, Lemon SM. Intrahepatic gene expression profiles and alpha-smooth muscle actin patterns in hepatitis C virus induced fibrosis. Hepatology. 2005;42(2):273-81. https://doi.org/10.1002/hep.20767 PMid:15986378

46. Rimbach G, De Pascual-Teresa S, Ewins BA, Matsugo S, Uchida Y, Minihane AM, Turner R, Vafei Adou K, Weinberg PD. Antioxidant and free radical scavenging activity of isoflavone metabolites. Xenobiotica. 2003;33(9):913-25. https://doi.org/10.1080/0049825031000150444 PMid:14514441

47. Aneja R, Upadhyaya G, Prakash S, Dass SK, Chandra R. Ameliorating effect of phytoestrogens on CCl4-induced oxidative stress in the livers of male Wistar rats. Artificial cells, blood substitutes, and biotechnology. 2005;33(2):201-13. https://doi.org/10.1081/BIO-200055908

48. Lee JS. Effects of soy protein and genistein on blood glucose, antioxidant enzyme activities, and lipid profile in streptozotocin-induced diabetic rats. Life sciences. 2006;79(16):1578-84. https://doi.org/10.1016/j.lfs.2006.06.030 PMid:16831449

49. Wei H, Bowen R, Cai Q, Barnes S, Wang Y. Antioxidant and antipromotional effects of the soybean isoflavone genistein. Proceedings of the Society for Experimental Biology and Medicine. 1995;208(1):124-30. https://doi.org/10.3181/00379727-208-43844 PMid:7892286

50. Huang Q, Huang R, Zhang S, Lin J, Wei L, He M, Zhuo L, Lin X. Protective effect of genistein isolated from Hydrocotyle sibthorpioides on hepatic injury and fibrosis induced by chronic alcohol in rats. Toxicology letters. 2013;217(2):102-10. https://doi.org/10.1016/j.toxlet.2012.12.014 PMid:23274713

51. Mizutani K, Ikeda K, Nishikata T, Yamori Y. Phytoestrogens attenuate oxidative DNA damage in vascular smooth muscle cells from stroke-prone spontaneously hypertensive rats. Journal of hypertension. 2000;18(12):1833-40. https://doi.org/10.1097/00004872-200018120-00018 PMid:11132608

52. Yalniz M, Bahcecioglu IH, Kuzu N, Poyrazoglu OK, Bulmus O, Celebi S, Ustundag B, Ozercan IH, Sahin K. Preventive role of genistein in an experimental non-alcoholic steatohepatitis model. Journal of gastroenterology and hepatology. 2007;22(11):2009-14. https://doi.org/10.1111/j.1440-1746.2006.04681.x PMid:17914984

53. Wang J, Mazza G. Inhibitory effects of anthocyanins and other phenolic compounds on nitric oxide production in LPS/IFN-γ-activated RAW 264.7 macrophages. Journal of Agricultural and Food Chemistry. 2002;50(4):850-7. https://doi.org/10.1021/jf010976a PMid:11829656

54. Hämäläinen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E. Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-κB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-κB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. Mediators of inflammation. 2007;2007.

55. Guo Q, Rimbach G, Packer L. Nitric oxide formation in macrophages detected by spin trapping with iron-dithiocarbamate complex: effect of purified flavonoids and plant extracts. Methods in enzymology. 2001;335:273-82. https://doi.org/10.1016/S0076-6879(01)35250-3

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):844-847.
https://doi.org/10.3889/oamjms.2017.197
eISSN: 1857-9655
Basic Science

A Comparative Study of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism and Fungal Culture for the Evaluation of Fungal Species in Patients with *Tinea Cruris*

Cut Hazlianda*, Kamaliah Muis, Isma Lubis

Universitas Sumatera Utara, Fakultas Kedokteran, Dermatology and Venereology, Medan, Indonesia

Citation: Hazlianda C, Muis K, Lubis I. A Comparative Study of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism and Fungal Culture for the Evaluation of Fungal Species in Patients with Tinea Cruris. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):844-847. https://doi.org/10.3889/oamjms.2017.197

Keywords: Tinea cruris; Dermatophyte; Culture; PCR-RFLP; Fungal species.

***Correspondence:** Cut Hazlianda. Universitas Sumatera Utara Fakultas Kedokteran, Dermatology and Venereology, Medan, Indonesia. E-mail: cutputrihazlianda@yahoo.com

Received: 05-Sep-2017; **Revised:** 20-Oct-2017; **Accepted:** 21-Oct-2017; **Online first:** 21-Nov-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Tinea cruris is the second most common dermatophytosis in the world and the most common in Indonesia. The conventional laboratory tests for dermatophyte infection are slow and less specific. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) is a PCR method with the addition of enzyme after amplification, therefore enabling for more specific results.

AIM: This study aimed to find whether the PCR-RFLP test could yield the same fungal species result as a fungal culture.

METHODS: The specimens were skin scrapings from thirty-one patients suspected *tinea cruris*. The tools and materials that were used were Sabaroud's dextrose agar media, primer ITS 1 and ITS 4 and MvaI.

RESULTS: The equation percentage of the test result species between PCR-RFLP and fungal culture was 50% of 12 subjects whose the test results were both positive from the fungal culture and PCR-RFLP. The percentage of the test result with fungal culture the fungal species were found, but in the PCR-RFLP test which the fungal species was not found, the percentage was 50% of 12 subjects which the test results were both positive as fungi from the culture and PCR-RFLP test.

CONCLUSIONS: The species from PCR-RFLP examination was the same with the fungal culture.

Introduction

Dermatophytes are a group of keratinophilic fungi that can grow on humans' and animals' keratinous tissues such as skin, hair, and nails causing dermatophytosis [1-4]. *Tinea kruris* is a dermatophytosis that may be found on groins, genitals, pubic area, perineal and perianal skins. It's the second most common dermatophytosis globally and also the most common in Indonesia [3, 5-9]. A study by Hajar (1999), found *tinea kruris* as the most common dermatophytosis in Pirngadi General Hospital, Medan [10]. Other studies by Bilkes, 2005 and Nasution, 2005 also found *tinea kruris* as the most common dermatophytosis in several Puskesmas (community health centre) at 40% of all dermatophytosis cases [11, 12].

The conventional laboratory tests for dermatophyte infection are direct microscopic examination with 10% potassium hydroxide (KOH) and fungal culture [1, 3, 13]. These procedures are rather slow. Thus, a faster diagnostic method is needed. Dermatophytes identification can be made in a fast and specific manner by using nucleic acid amplification technology [13, 14]. Molecular techniques such as the polymerase chain reaction (PCR) method has a high sensitivity and specificity rate and can be used to diagnose myriads of microorganism including pathogenic fungi [1, 14, 15].

PCR is an in vitro method for synthesising and amplifying dermatophyte deoxyribonucleic acid (DNA) [16, 17]. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) may produce an even more specific outcome by adding post-amplification enzymes [18]. A study by

Elavarashi, et al. 2013 suggests using Internal Transcribed Spacer (ITS) primer, MvaI and DdeI enzyme for the PCR-RFLP to have a satisfactory outcome [19]. Species identification, however, can be different from the PCR and culture. A study by Irime, et al. 2011, found different species between the real-time PCR and the culture on four samples [20].

Species identification becomes important as a study by Paramata, et al. in Makassar, found 28% dermatophytosis agents on glabrous skins to be itraconazole-resistant [21].

This study aimed to find whether the PCR-RFLP test could yield the same fungal species result as a fungal culture.

Methods

This is a descriptive study using a cross-sectional design. Samples were collected starting in September 2013 in the mycology division of dermatovenereology outpatient unit of RSUP. H. Adam Malik, Medan. Fungal culture and PCR-RFLP were done in the integrated laboratory of Sumatera Utara University, Faculty of Medicine.

The specimens were skin scrapings from thirty-one patients suspected of having *tinea cruris* from history and dermatological examination. The tools and materials that were used were Sabaroud's dextrose agar media (the gold standard for fungal species identification) added with Cycloheximide (0.5 g/l) and Chloramphenicol (0.05 g/l), thermocycler (applied biosystem Verity 96-type well thermal cycler, Singapore), DNA extraction kit (Promega), PCR kit (Promega), ITS 1 primer (forward) and ITS 4 (reverse) (1st Base), and MvaI restriction enzyme (Fermentas). Preheat (94^{o}C, 10 minutes); denaturation (94^{o}C, 1 minutes); annealing (58^{o}C, 1 minute); extension (72^{o}C, 1 minute (35 cycles)) and final extension (72^{o}C, 7 minute) were done using the thermocycler. This study uses the same basepair used in previous studies by Elavarashi E. and Mirzahoseini H., et al. [19]. No positive controls are being used.

The data from the basic information, history, dermatological examination and specimens were then collected. The scraped specimens were then separated into different envelopes and divided into two groups. The first group were being used for fungal culture and another for the PCR-RFLP. The results were then presented in frequency distribution tables and then analysed in a descriptive manner using other literature as comparisons.

Results

Skin scrapings from 31 subjects were collected in this study.

Table 1: Subject characteristics based on gender

Gender	n	%
Male	16	51.6
Female	15	48.4
Total	31	100.0

According to Table 1, out of 31 subjects, fifteen were identified as female (48.4%), and sixteen were identified as male (51.6%).

Table 2: Subject characteristics based on age groups

Age (y.o.)	n	%
12-21	12	38.7
22-31	3	9.7
32-41	2	6.5
42-51	4	12.9
52-61	5	16.1
62-71	3	9.7
72-81	2	6.5
Total	31	100

According to Table 2, most subjects were part of 12-21 years old group at 38.7% of all subjects.

Table 3: Skin scraping analysis using culture and PCR-RFLP

Sample	Culture	Species detected	PCR-RFLP	PCR-RFLP species
1	Negative	NCG	Negative	-
2	Negative	NCG	Positive	*T. mentagrophytes*
3	Negative	NCG	Negative	-
4	Positive	*T. rubrum*	Positive	*T. rubrum*
5	Negative	NCG/*Paecilomyces*	Negative	-
6	Negative	NCG/*Aspergillus flavus*	Positive	*T. mentagrophytes*
7	Positive	*T. rubrum*	Positive	*T. rubrum*
8	Positive	*T. violaseum*	Positive	O
9	Negative	NCG/*Cladosporium*	Negative	-
10	Positive	*M. rivalieri*	Negative	-
11	Negative	NCG	Negative	-
12	Positive	*T. tonsured*	Negative	-
13	Negative	NCG/*Aspergillus flavus*	Positive	*E. floccosum*
14	Positive	*M. rivalieri*	Positive	O
15	Negative	NCG/*Aspergillus flavus*	Negative	-
16	Negative	NCG/*Aspergillus fumigatus*	Negative	-
17	Negative	NCG/*Aspegillus niger*	Negative	-
18	Negative	NCG/*Aspergillus flavus*	Negative	-
19	Positive	*T. rubrum*	Positive	O
20	Negative	NCG/*Aspergillus fumigatus*	Positive	*T. verrucosum*
21	Positive	*T. tonsuran*	Positive	*T. tonsuran*
22	Positive	*T. ericinae*	Negative	-
23	Positive	*T. tonsuran*	Positive	O
24	Positive	*T. rubrum*	Positive	*T. rubrum*
25	Negative	NCG/*Aspergillus flavus*	Negative	-
26	Positive	*T. rubrum*	Negative	-
27	Positive	*T. rubrum*	Positive	*T. rubrum*
28	Negative	NCG/*Paecilomyces*	Positive	*T. verrucosum*
29	Positive	*T. rubrum*	Positive	*T. rubrum*
30	Positive	*T. rubrum*	Positive	O
31	Positive	*T. schoenleinii*	Positive	O

NCG: no culture growth; o: not detected.

According to Table 3, 16 positive subjects were found on the culture, and 17 positive subjects were found using PCR-RFLP.

According to Table 4, *T. rubrum* is the most common species found in the culture out of all subjects at eight subjects (25.8% of all subjects).

Table 4: Fungal species distribution based on culture result

Species	n	%
M. rivalieri	2	6.5
T. ericinae	1	3.2
T. rubrum	8	25.8
T. schoenleinii	1	3.2
T. tonsuran	3	9.7
T. violaseum	1	3.2
NCG	4	12.9
NCG/*Aspergillus niger*	1	3.2
NCG/*Aspergillus flavus*	5	16.1
NCG/*Aspergillus fumigatus*	2	6.5
NCG/*Cladosporium*	1	3.2
NCG/*Paecilomyces*	2	6.5
Total	31	100.0

NCG: no culture growth.

According to Table 5, *T. rubrum* is the most common species found using PCR-RFLP out of 31 subjects in five subjects (16.1% out of all subjects). It could be concluded from the cultures and PCR-RFLP that *T. rubrum* was the most common fungal species found.

Table 5: Fungal species distribution based on PCR-RFLP

Species Jamur	n	%
E. floccosum	1	3.2
T. mentagrophytes	2	6.5
T. rubrum	5	16.1
T. tonsuran	1	3.2
T. verrucosum	2	6.5
-	14	45.2
O	6	19.4
Total	31	100.0

o: species not detected.

According to Table 6, out of 31 subjects, twelve (38.71%) were found positive both in the culture and PCR-RFLP. Four (12.90%) were found positive on the culture and negative on the PCR-RFLP. Five (16.13%) were found positive on the PCR-RFLP and negative on the culture. Out of twelve subjects that were found positive both on the culture and PCR-RFLP, six (50%) yield the same species and six (50%) were found on the culture but not found on the PCR-RFLP.

Table 6: Fungal species distribution based on fungal culture and PCR-RFLP

Species	Culture		PCR-RFLP	
	n	%	n	%
M. rivalieri	2	6.5	-	-
T. ericinae	1	3.2	-	-
T. rubrum	8	25.8	5	16.1
T. schoenleinii	1	3.2		
T. tonsuran	3	9.7	1	3.2
T. violaseum	1	3.2		
E. floccosum	-	-	1	3.2
T. mentagrophytes	-	-	2	6.5
T. verrucosum	-	-	2	6.5
NCG	4	12.9	-	-
NCG/*Aspegillus niger*	1	3.2	-	-
NCG/*Aspergillus flavus*	5	16.1	-	-
NCG/*Aspergillus fumigatus*	2	6.5	-	-
NCG/*Cladosporium*	1	3.2	-	-
NCG/*Paecilomyces*	2	6.5	-	-
Negative	-	-	14	45.2
O			6	19.4
Total	31	100.0	31	100.0

NCG: no culture growth; o: not detected.

Discussion

There were more male subjects to female in this study. Hajar, 1999 also found more male subjects to female in his study which is around 26.67% [10]. Gupta, et al. 2003 and Daili, et al. 2005 conclude that *tinea cruris* affect more male to female [8, 22]. It's suggested that the preference was caused because scrotal areas on males make a warm and humid condition [4]. Menswear also tends to have more coverings for women, and this also contributes to the humid condition [23].

Patel, et al. 2009 and Fernandes, et al. 2001 found higher incidences of *tinea cruris* in young-adult and adolescent males [24, 25]. Andrews, et al. 2008 also found *tinea cruris* are mostly seen in young-adult males [26] In the current study, *tinea cruris* is mostly seen on the 12- 21 years old age group (38.7%). According to Patel, et al. 2009, increase of obesity cases are seen among children and adolescents, and this may contribute to the rising number of *tinea cruris* cases in those age groups [24]. Children who are using tight shirts or underwear may sweat profusely or causing immune disorder thus rising the risk of contracting *tinea cruris* [27].

This study found *T. rubrum* as the most common fungal species found from the fungal culture and PCR-RFLP. Hajar, 1999 and Nasution, 2005 found *T. rubrum* and *T. mentagrophytes* as the most common aetiology for *tinea cruris* [10, 12]. Schieke et al., 2012 and Wiederkehr, et al found *T. rubrum* dan *E. floccosum* as the most common causative agent of *tinea cruris* followed by *T. mentagrophytes* and *T. verrucosum* [3, 6].

Out of twelve subjects that were found positive by using culture and also PCR-RFLP, six (50%) belong to the same species and on another six (50%) fungal species were found on the culture but the PCR-RFLP yield otherwise result. The thinness of the base pair from the PCR may contribute to the result by causing the splicing enzyme used unable to detect the base pair. A study by Irime, et al., 2011, found difference between the identification done using culture and the real time PCR on four samples. Two samples were identified as *T. rubrum* using the culture but identified as *T. interdigitale* using the PCR. Two other samples were identified as *T. Interdigitale* using the culture but identified as *T. rubrum* using the PCR [20]. A study by Wissenlik, et al., 2011 were using real-time PCR for dermatophytes identification. Four different samples yield different species result between using the fungal culture and the real-time PCR [28]. Another study by Girgis, et al., 2006 found seven samples that yield different species result between using the fungal culture and the real-time PCR [29].

Species identification becomes necessary to plan the therapy since *Epidermophyton* and *Trichophyton* were sensitive to terbinafine but *Microsporum* is less sensitive. Thus, a clear and concise way to identify the species is integral in order to be able to give a correct treatment, so that it may speed up the patients' recovery [30].

References

1. Garg J, Tilak R, Garg A, Prakash P, Gulati AK, Nath G. Rapid detection of dermatophytes from skin and hair. BMC Res Notes. 2009;2:1-6. https://doi.org/10.1186/1756-0500-2-60 PMid:19374765 PMCid:PMC2678142

2. Hay RJ, Moore J. Mycology. In: Burn T, Breathnach S, Cox N, Griffiths C, editor. Rook's Text Book of Dermatology. 7th Ed. United State: Black-Well, 2004:1425-7.

3. Schieke SM, Garg A. Superficial fungal infection. In: Goldsmith LA, Katz SI, Gilchesrt BA, Paller AS, Leffell DJ, Wolff Klaus, editor. Fitzpatrick's Dermatology In General Medicine. 8th Ed. New York: McGraw-Hill Companies Inc., 2012:2277-97.

4. Havlickova B, Czaika VA, Friedrich M. Epidemiological trends in skin mycoses worldwide. Mycoses. 2008;51:2-15. https://doi.org/10.1111/j.1439-0507.2008.01606.x PMid:18783559

5. Nadalo D, Montoya C. What is the best way to treat tinea cruris?. The Journal of Family Practice. 2006;55(3):256-8. PMid:16510062

6. Wiederkehr M, Schwartz RA. Tinea cruris. Available at: http://emedicine.medscape.com/article/1091806-overview#showall. Accesced on 28 March 2013.

7. Lakshmipathy DT, Kannabiran K. Riview on dermatomycosis: pathogenesis and treatment. Natural Science. 2010;2(7):726-31. https://doi.org/10.4236/ns.2010.27090

8. Daili ESS, Menaldi SL, Wisnu IM. Penyakit Kulit yang Umum di Indonesia Sebuah Panduan Bergambar. Jakarta Pusat: PT Medical Multimedia Indonesia, 2005:30.

9. Nasution MA. Mikologi dan mikologi kedokteran beberapa pandangan dermatologis [Pidato Pengukuhan Jabatan Guru Besar Tetap]. Medan: Universitas Sumatera Utara, 2005. PMCid:PMC1764680

10. Hajar S. Penyebab infeksi jamur dermatofitosis di rumah sakit umum daerah dr. Pirngadi Medan [Thesis]. Medan: Universitas Sumatera Utara, 1999.

11. Bilkes. Spektrum klinis dan faktor predisposisi dermatofitosis di beberapa Puskesmas wilayah kota Medan [Thesis]. Medan: Universitas Sumatera Utara, 2005.

12. Nasution EI. Spektrum dermatofita penyebab dermatofitosis di beberapa Puskesmas wilayah kota Medan [Tesis]. Medan: Universitas Sumatera Utara, 2005. PMCid:PMC1764680

13. Liu D, Coloe S, Baird R, Pedersen J. Application of PCR to the indentification of dermatophyte fungi. J Med Microbiol. 2000;49:493-7. https://doi.org/10.1099/0022-1317-49-6-493 PMid:10847201

14. Alexander CL, Shankland GS, Carman W, Williams C. Introduction of a dermatophyte polymerase chain reaction assay to the diagnostic mycology service in Scontland. Br J Dermatol. 2011; 164: 966-72. https://doi.org/10.1111/j.1365-2133.2010.10186.x PMid:21166658

15. Gutzmer R, Mommert S, Küttler U, Werlfel T, Kapp A. Rapid identification and differentiation of fungal DNA in dermatological specimens by LightCycler PCR. J Med Microbiol. 2004;53:1207-14. https://doi.org/10.1099/jmm.0.45779-0 PMid:15585499

16. Handoyono D, Rudiretna A. Prinsip umum dan pelaksanaan polymerase chain reaction (PCR). Unitas. 2001;9(1):17-29.

17. Aryani A, Kusumawaty D. Prinsip-prinsip polymerase chain reaction (PCR) dan aplikasinya. Kursus Singkat Isolasi dan Amplifikasi DNA, 2007:71-4.

18. Mirzahoseini H, Omidinia E, Shams-Ghahfarokhi M, Sadeghi G, Razzaghi-Abyaneh M. Application of PCR-RFLP to rapid identification of the main pathogenic dermatophytes from clinical specimens. Irian J Publ Health. 2009;38(1):18-24.

19. Elavarashi E, Kindo AJ, Kalyani J. Optimization of PCR-RFLP directly from the skin and nails in cases of dermatophytosis, targeting the ITS and the 18 S ribosomal DNA regions. JCDR. 2013:1-6.

20. Irimie M, Tătaru A, Oanţă A. Evaluation of real time polymerase chain reaction assay for idetification of common dermatophyte species. Bulletin of the Transilvania University of Braşov. 2011;4(53):65-72.

21. Paramata NR, Maidin A, Massi N. Perbandingan uji kepekaan itrakonazol terhadap agen penyebab dermatofitosis pada kulit glabrous di Makassar [Thesis]. Makassar: Universitas Hasanuddin, 2012.

22. Gupta AK, Chaudhry M, Elewski B. Tinea corporis, tinea cruris, tinea nigra, and piedra. Dermatol Clin. 2003;21:395-400. https://doi.org/10.1016/S0733-8635(03)00031-7

23. Hainer BL. Dermatophyte infections. American Family Physician. 2003;67(1):101-8. PMid:12537173

24. Patel GA, Wiedderkehr M, Schwartz RA. Tinea cruris in children. Pediatric Dermatology. 2009;84:133-7.

25. Fernandes NC, Akiti T, Barreiros MDGC. Dermatophytoses in children: study of 137 cases. Rev Inst Med Trop S Paulo. 2001;43(2):83-5. https://doi.org/10.1590/S0036-46652001000200006 PMid:11340481

26. Andrews MD, Burns M. Common tinea infections in children. American Family Physician. 2008;77(10):1415-20. PMid:18533375

27. Howell N. Jock itch in children. Available at: http://www.livestrong.com/article/525292-jock-itch-in-children/. Accesced on 9 Mei 2014.

28. Wisselink GJ, Zanten EV, Kooistra-smid AMD. Trapped in keratin; a comparison of dermatophyte detection in nail, skin and hair samples directly from clinical samples using culture and real-time PCR. J. Microbiol. Methods. 2011:1-5. https://doi.org/10.1016/j.mimet.2011.01.023

29. Girgis SA, El-Fakkar NMZ, Bard H, Shaker OA, Metwally FE, Bassim HH. Genotypic identification and antifungal susceptibility pattern of dermatophytes isolated from clinical specimens of dermatophytosis in Egyptian patients. Egyptian Dermatology Online Journal. 2006;2(2):1-23.

30. Kim JY, Choe YB, Ahn KJ, Lee YW. Identification of dermatophytes using multiplex polymerase chain reaction. Ann Dermatol. 2011;23(3):304-12. https://doi.org/10.5021/ad.2011.23.3.304 PMid:21909200 PMCid:PMC3162259

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):848-851.
https://doi.org/10.3889/oamjms.2017.203
eISSN: 1857-9655
Basic Science

Rapid Identification of *Aspergillus Fumigatus* Using *Beta-Tubulin* and *RodletA* Genes

Majid Zarrin[1,2*], Zeinab Rashidnia[2], Sama Faramarzi[2], Lida Harooni[2]

[1]Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; [2]Department of Medical Mycology, Medical School, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Citation: Zarrin M, Rashidnia Z, Faramarzi S, Harooni L. Rapid Identification of *Aspergillus Fumigatus* Using *Beta-tubulin* and *rodletA* Genes. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):848-851. https://doi.org/10.3889/oamjms.2017.203

Keywords: *β-tubulin; RodletA; Aspergillus fumigatus*.

***Correspondence:** Majid Zarrin. Department of Medical Mycology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran: mjzarrin@yahoo.co.uk

Received: 26-Aug-2017; **Revised:** 24-Sep-2017; **Accepted:** 29-Sep-2017; **Online first:** 22-Nov-2017

Funding: The present study was supported by the Health Research Institute (Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran) (grant no. 94110).

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: The main purpose of the present study was to test the β-tubulin and *rodletA* genes for rapid identification of *Aspergillus fumigatus*.

MATERIALS AND METHODS: Fifty-one *A. fumigatus* strains including environmental, clinical and reference isolates were tested in this research. PCR was carried out based on βtub and *rodA* partial gene sequences.

RESULTS: A 198 bp DNA fragment was obtained using ***βtub*** gene. PCR amplification of the *rodA* gene resulted in a 313 bp band. The ***βtub*** and *rodA* genes PCR products exhibited a 100% homology with the associated sequences in the GenBank.

CONCLUSION: In the present study, we used a PCR approach that was able to discriminate *A. fumigatus* from other related species within the section Fumigati.

Introduction

Aspergillus fumigatus is an environmental filamentous fungus and is the main causal agent of the aspergillosis. Increasing the use of immunosuppressive therapy for treating many human diseases, the incidence of mortality rate of invasive aspergillosis (IA) is raised between 30–95% [1].

Teleomorphic species of the *Aspergillus* section *Fumigati* (AsF) belong to the Neosartorya genus. *Aspergillus* section *Fumigati* (AsF) is an economically important fungus and teleomorphic species of this section belong to the genus *Neosartorya*. Seventeen species of *Neosartorya* and 8 strictly mitotic species are typically recognized [2].

Aspergillus section Fumigati and also its teleomorph Neosartorya are significantly important in clinic because they are able to pathogenic or allergenic to human [3-5], responsible for food spoilage and producing mycotoxins [6, 7].

The clinical isolates of *Aspergillus* species are not necessary morphological the same and wrong recognitions of species with morphological characteristics have frequently happened. With the intention of development the diagnostic method, including DNA detection it is important to elucidate intra and interspecies variety in *A. fumigatus* and closely related species.

Misrecognition of species within the section Fumigati has been frequently reported by clinical laboratories. Species, for example, *Aspergillus viridinutans, Aspergillus lentulus, Aspergillus fumisynnematus, Aspergillus fumigatiaffinis, Neosartorya pseudofischeri, Neosartorya udagawae* and *Neosartorya hiratsukae*, are commonly reported as *A. fumigatus* [8, 9].

A number of biochemical and molecular approaches have been performed for identification of *A. fumigstus* and related species. Sequencing of genes, for example, *ITS, calmodulin, actin, βtubulin (βtub)* and *rodlet A (rodA),* has been used to discrimination *A. fumigatus* from related species [10, 11]. In the current study, the βtub and rodA gene were tested for identification of Aspergillus section Fumigati.

Materials and Methods

Microorganisms

A total of 51 *A. fumigatus* strains including environmental, clinical and reference isolates were used in this study. The following strains of *A. fumigatus* were used as a reference: IBRC-M 30033, IBRC-M 30040, IBRC-M 30048. Eight clinical isolates were kindly provided by Dr Mojtaba Taghizadeh (Mazandaran University of Medical Sciences, Mazandaran, Iran). The environmental isolates were recovered from soil or air. The strains were incubated on Sabouraud dextrose agar (Merck KGaA, Darmstadt, Germany) at 37°C. All *A. fumigatus* isolates were recognized by morphology. For getting a pure culture, the isolates were subcultured three times and then stained with lactophenol aniline blue. Phenotypic techniques including colony morphology, conidial arrangement, philiades, vesicles and conidiophores were considerated for identification.

DNA extraction

One ml thick spore suspension from each isolate was transferred to an Erlenmeyer flask containig 50 ml yeast extract peptone dextrose medium (Merck KGaA).

The flasks were incubated at 200 rpm under agitation at 27˚C for 72 h to allow for mycelium growth.

The harvested mycelia were washed with 0.5 M ethylenediamine tetraacetic acid (EDTA) and sterile distilled water (dH2O) and freeze-dried at -70˚C for DNA extraction. Then, the mycelia were ground into a fine powder with a pestle and mortar. The DNA was extracted using the GF-1 Plant DNA Extraction Kit (vivantis, Malysia).

PCR amplification

In our study, PCR was performed based on *βtub* and *rodA* partial gene sequences. The primer sets, βtub-F (5'- TGACGGGTGATTGGGATCTC-3') and βtub-R (5'- CGTCCGCTTCTTCCTTGTTT-3') was used to amplify a 198bp DNA fragment of the *βtub* gene. The primer sets, *rodA* -F (5'-ACATTGACGAGGGCATCCTT -3') and *rodA* -R (5'-ATGAGGGAACCGCTCTGATG -3') was used to amplify a 313bp DNA fragment of the *βtub* gene. The PCR reactions were prepared to a final volume of 30 µl, comprised of 3 µl 10X reaction buffer, 2.2 mM MgCl2, 200 µM of each dNTP, 2.5 unit of Taq DNA polymerase (CinnaGen, Karaj, Iran), a 30 ng DNA template and 50 pmol of each primer.

An initial denaturation for 5 min at 94˚C was followed by 35 cycles of denaturation at 94˚C for 1 min, annealing at 68˚C for 1 min and extension at 68˚C for 2 min. The PCR products were electrophoresed on 2% agarose gel in TAE buffer at 50 V for 2 h and stained with ethidium bromide.

Sequencing

A number of *βtub* and *rodA* genes amplicons were submitted for sequencing (Bioneer Corporation, Daejeon, South Korea). Searching in the NCBI database (http://www.ncbi.nlm.nih.gov/) showed that sequences had 100% identity with *A. fumigatus* β*tub* and *rodA* genes. The MEGA5 software package (http://www.megasoftware.net) was employed for alignment of sequences.

Results

Fifty moulds, all formerly recognized as *A. fumigatus* by morphology, were screened by the PCR method to identify *A. fumigatus* isolates. The *βtub* and *rodA* genes were considered as genes markers.

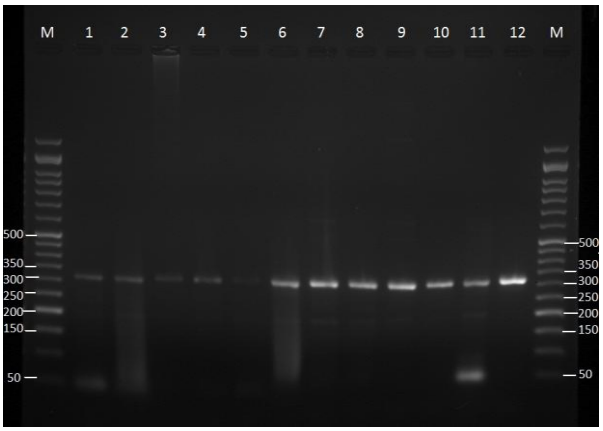


Figure 1: Agarose gel electrophoresis of rodA gene products (313 bp) of the Aspergillus fumigatus (lane 1, 2 references strains; lanes 3-5, clinical isolates; lane 6-12, environmental isolates. Lane M, 50-bp ladder; lane 1, IBRC-M 30040 lane 2, IBRC-M 30048; lane 3, MF6; lane 4, MF30; lane 5, MF34; lane 6, MF13; lane 7, MF17; lane 8, MF35; lane 9, MF39; line 10, MF42; line 11, MF46; line 12, MF53

PCR amplification of the *rodA* gene for all 51 isolates with primers *rodA* -F and *rodA* -R resulted in a 313 bp band (Fig. 1). PCR amplification of the *rodA* gene for all 51 isolates with primers βtub-F and βtub-R resulted in a 198 bp band (Fig. 2).

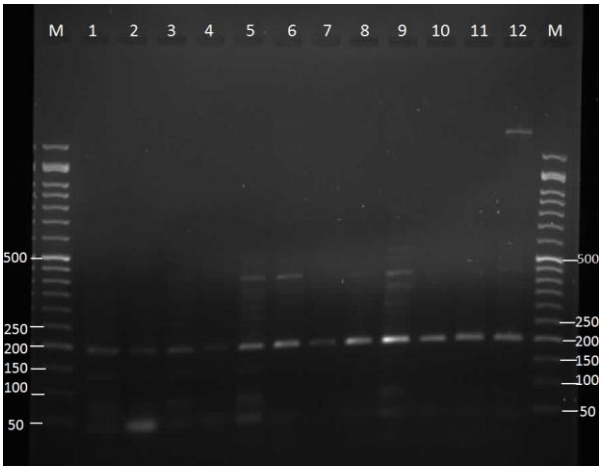


Figure 2: Agarose gel electrophoresis of βtub gene products (198 bp) of the Aspergillus fumigatus (lane 1, 2 references strains; lanes 3-5, clinical isolates; lane 6-12, environmental isolates. Lane M, 50-bp ladder; lane 1, IBRC-M 30040 lane 2, IBRC-M 30033; lane 3, MF6; lane 4, MF30; lane 5, MF34; lane 6, MF13; lane 7, MF17; lane 8, MF35; lane 9, MF39; line 10, MF42; line 11, MF46; line 12, MF53

The *βtub* and *rodA* genes products were sequenced for several isolates, including the reference strains. A Basic Local Alignment Search Tool (BLAST) search demonstrated that the *βtub* and *rodA* genes PCR products exhibited a 100% homology with the associated sequences in the GenBank.

Discussion

Identification of filamentous fungi dissimilar to bacteria, rely mostly on morphological characterizations, however, limitations of phenotypic typing of pathogenic fungi are being progressively more recognized. The new fungal species have recently been recognized within the section Fumigati, Some of them have been associated in severe cases of aspergillosis including pulmonary, cerebral, liver, cutaneous and trabecular bone invasions.

In regard to the *A. fumigatus* may shows a significant part of all aspergillosis clinical cases, molecular description is important for the accurate detection of species within the section of Fumigati.

Hong et al (2005) reported the variability within *A. fumigatus* section in Korea by morphology, growth temperature, extrolite patterns and DNA analyses of the partial *β*-tubulin, actin and calmidulin gene and they suggested two new species which were *A. fumigatiaffinis*, *A. novofumigatus* [12].

The phylogenetic associations between *A. fumigatus* and related species has also been analysed by partial sequencing of cytochrome b gene [13].

Identification of *A. fumigatus* is vital because this fungus is one of the most significant fungal pathogens. The recognition of *Aspergillus* spp. isolated from clinical samples depends mostly on morphological features. However, morphology is not enough for the detection of some clinical isolates because of the occurrence of polymorphism and the deprived development of reproductive structure. Consequently, several additional techniques have been used performed in the study of *A. fumigatus* [10, 14-16]. Burnie et al. employed restriction fragment length polymorphism analysis (RFLP) to discriminate the clinical isolates of *A. fumigatus*. They were succeeded classify 21 isolates into six types with *Xba*I digestion [17].

In the present study, we used a PCR approach that was able to discriminate *A. fumigatus* from other related species within the section Fumigati.

Sequence analysis of PCR products the *benA* and *rodA* genes of several isolates revealed that this approach accurately differentiated the non-*A.fumigatus* from the *A. fumigatus* isolates.

Acknowledgments

The present study was supported by the Health Research Institute (Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran) (grant no. 94110).

References

1. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. Sci Transl Med. 2012; 4(165):165rv13. https://doi.org/10.1126/scitranslmed.3004404 PMCid:PMC3627207

2. Samson RA, Hong SB, Frisvad JC. 2006. Old and new concepts of species differentiation in Aspergillus. Med Mycol. 2006; 44:S133-48. https://doi.org/10.1080/13693780600913224

3. Brakhage AA, Langfelder K. Menacing mold: the molecular biology of Aspergillus fumigatus. Annu Rev Microbiol. 2002; 56:533–455. https://doi.org/10.1146/annurev.micro.56.012302.160625 PMid:12142473

4. Frisvad JC, Larsen TO. Extrolites of Aspergillus fumigatus and Other Pathogenic Species in Aspergillus Section Fumigati. Front Microbiol. 2016; 6:1485. https://doi.org/10.3389/fmicb.2015.01485 PMid:26779142 PMCid:PMC4703822

5. Lamoth F. Aspergillus fumigatus-Related Species in Clinical Practice. Front Microbiol. 2016;7:683. https://doi.org/10.3389/fmicb.2016.00683 PMid:27242710 PMCid:PMC4868848

6. Cole RJ, Cox RH. Handbook of toxic fungal metabolites. Academic, New York, 1981.

7. Larsen TO, Smedsgaard J, Nielsen KF, Hansen MA, Samson RA, Frisvad JC. Production of mycotoxins by Aspergillus lentulus and other medically important and closely related species in section Fumigati. Med Mycol. 2007;45(3):225-32. https://doi.org/10.1080/13693780601185939 PMid:17464844

8. Balajee SA, Nickle D, Varga J, Marr KA. Molecular studies reveal frequent misidentification of Aspergillus fumigatus by morphotyping. Eukaryot Cell. 2006 5(10):1705-12. https://doi.org/10.1128/EC.00162-06 PMid:17030996 PMCid:PMC1595351

9. Hong SB, Shin HD, Hong J, Frisvad JC, Nielsen PV, Varga J, Samson RA. New taxa of Neosartorya and Aspergillus in Aspergillus section Fumigati. Antonie Van Leeuwenhoek. 2008;93(1-2):87-98. https://doi.org/10.1007/s10482-007-9183-1 PMid:17610141 PMCid:PMC2140094

10. Samson R A , Hong S , Peterson S W , Frisvad J C, Varga. Polyphasic taxonomy of Aspergillus section Fumigati and its teleomorph Neosartorya. Stud Mycol. 2007; 59: 147–203. https://doi.org/10.3114/sim.2007.59.14 PMid:18490953 PMCid:PMC2275200

11. Balajee SA, Houbraken J, Verweij PE, Hong SB, Yaghuchi T, Varga J, Samson RA.Aspergillus species identification in the clinical setting. Stud Mycol. 2007; 59: 39–46. https://doi.org/10.3114/sim.2007.59.05 PMid:18490954 PMCid:PMC2275201

12. Hong SB, Go SJ, Shin HD, Frisvad JC, Samson RA. Polyphasic taxonomy of Aspergillus fumigatus and related species. Mycologia. 2005;97(6):1316-29. https://doi.org/10.1080/15572536.2006.11832738 PMid:16722222

13. Wang L, Yokoyama K, Miyaji M, Nishimura K. Mitochondrial cytochrome b gene analysis of Aspergillus fumigatus and related species. J Clin Microbiol. 2000;38(4):1352-8. PMid:10747106 PMCid:PMC86444

14. Ricardo Araujo, António Amorim, Leonor Gusmão. Diversity and specificity of microsatellites within Aspergillus section Fumigati. BMC Microbiol. 2012; 12: 154. https://doi.org/10.1186/1471-2180-12-154 PMid:22838495 PMCid:PMC3438126

15. Etienne KA, Gade L, Lockhart SR, Diekema DJ, Messer SA, Pfaller MA, Balajee SA. Screening of a Large Global Aspergillus fumigatus Species Complex Collection by Using a Species-Specific Microsphere-Based Luminex Assay. J Clin Microbiol. 2009; 47(12): 4171–4172. https://doi.org/10.1128/JCM.01415-09 PMid:19794043 PMCid:PMC2786642

16. Fernandez-Molina JV, Abad-Diaz-de-Cerio A, Sueiro-Olivares M, Pellon A, Ramirez-Garcia A, Garaizar J, Pemán J, Hernando FL, Rementeria A. Rapid and specific detection of section Fumigati and Aspergillus fumigatus in human samples using a new multiplex real-time PCR. Diagn Microbiol Infect Dis. 2014;80(2):111-8. https://doi.org/10.1016/j.diagmicrobio.2014.06.003 PMid:25063549

17. Burnie JP, Coke A, Matthews RC. Restriction endonuclease analysis of Aspergillus fumigatus DNA. J Clin Pathol. 1992; 45:324–327. https://doi.org/10.1136/jcp.45.4.324 PMid:1349614 PMCid:PMC495273

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):852-858.
https://doi.org/10.3889/oamjms.2017.158
eISSN: 1857-9655
Basic Science

Hepatoprotective Effect of Camel Milk on Poloxamer 407 Induced Hyperlipidaemic Wistar Rats

Jibril Zuberu[1]*, Malajiya I. A. Saleh[1], Abdul Wahab Alhassan[2], Bello Y. Adamu[2], Munira Aliyu[1], Bilkisu T. Iliya[1]

[1]Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria; [2]Department of Human Physiology, Faculty of Basic Medical Sciences, Bauchi State University, Gadau, Nigeria

Citation: Zuberu J, Saleh MIA, Alhassan AW, Adamu BY, Aliyu M, Iliya BT. Hepatoprotective Effect of Camel Milk on Poloxamer 407 Induced Hyperlipidaemic Wistar Rats. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):852-858. https://doi.org/10.3889/oamjms.2017.158

Keywords: Camel Milk; Poloxamer 407; Hyperlipidaemia; Liver enzymes; Total Protein.

***Correspondence:** Jibril Zuberu. Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria. E-mail: abdullahizubairu13@gmail.com

Received: 28-Apr-2017; **Revised:** 08-Sep-2017; **Accepted:** 24-Oct-2017; **Online first:** 30-Nov-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: To investigate the effect of oral administration of camel milk on liver enzymes, total proteins and histology of poloxamer 407 induced hyperlipidaemic wistar rats.

MATERIAL AND METHODS: Thirty male wistar rats weighing between 150-200 g were randomly assigned into six groups of five each; group I: administered distilled water, group II: induced with P407, group III: induced with P407 and treated with atorvastatin (20 mg/kg) and groups IV, V and VI: induced with P407 and treated with camel milk 250 mg/kg, 500 mg/kg and 1000 mg/kg respectively. After three weeks, blood samples and liver tissues were collected for the determination of alkaline phospatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, globulin, albumin/globulin ratio and histological studies respectively.

RESULTS: All camel milk treated groups showed significant ($p < 0.05$) decrease in ALT and AST. Camel milk treated groups; 250 mg/kg and 1000mg/kg showed significant ($p < 0.05$) decrease in total protein, globulin with all camel milk treated groups having significant ($p < 0.05$) increase in A/G ratio. Histological examination of liver tissues showed that camel milk at a dose of 250 mg/kg had slight adipocytes infiltration.

CONCLUSION: The results of our findings highlight the hepatoprotective effect of camel milk in poloxamer 407 induced hyperlipidaemic wistar rats.

Introduction

Dyslipidaemia has been implicated as the major risk factor of cardiovascular diseases. The World Health Organization holds it responsible for more than four million deaths annually [1]. Accumulation of triglycerides and other fats in the hepatocytes, if not treated results in inflammatory conditions of the liver. It is characterized by varying degree of liver injury ranging from steatosis to steatohepatitis, fibrosis and necrosis [2]. Poloxamer 407 (P407) is a ubiquitous manmade surfactant and non-ionic detergent with a molecular weight of 12,600 and liquid at room temperature. It assembles into micelles at body temperature and then aggregate into a gel. These temperature-dependent micellization and gelation properties have led to its widespread use in mouthwashes, deodorants, and skin care products and also as an excipient in a variety of pharmaceutical preparations [3]. P407 has a major hyperlipidaemic effect as observed in experimental animals [4-6]. Induction of hyperlipidaemia using P407 has contributed towards understanding the impact of hyperlipidaemia on several tissue markers. The advantage with the P407 model is the production of hyperlipidaemia of the hereditary type [7]. Recent reports have shown the ability of P407 induced hyperlipidaemia to produce early and late stages of atherosclerosis [7-9] and alterations of liver transaminases and plasma proteins in rodents [7-11]. Consumption of synthetic hypolipidaemic drugs have been reported to cause hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function [12].

The unique composition and nutritional

values of camel milk are well known from ancient times for its beneficial health effects. Camel milk is a good substitute for human milk as it does not contain β-lacto globulin. Many research findings have proven that camel milk is easily digested by lactase deficient individuals. It contains disease-fighting immunoglobulin's which are small in size, thus allowing penetration of antigens and boosting the effectiveness of the immune system [13]. Recently, researchers have reported the presence of vitamins, proteins, insulin like peptides, minerals and glycosides in camel milk [13-16]. Several works have displayed the ameliorative effect of camel milk on clinical and experimental animal models of diabetes, hyperlipidaemia and hepatic damage [14, 16-18].

Hence the aim of the current study is to investigate the effect of oral administration of camel milk on serum liver enzymes, total protein, albumin/globulin ratio and histology of the liver tissues in poloxamer 407 induced hyperlipidaemic wistar rats.

Materials and Methods

Experimental Animals

This study was conducted in the department of pharmacology, Ahmadu Bello University Zaria, Nigeria. Thirty healthy adult male wistar rats weighing 150-200 gm were obtained from the animal house of faculty of pharmaceutical sciences, Ahmadu Bello University Zaria. All the rats were kept in the same animal house. Housing was in well ventilated steel wire cages (5 rats per cage) with normal photoperiod of 12 h light/dark cycle and constant temperature of 25-27°C. The animals were maintained on standard animal feed (growers mash from vital feeds company Kaduna-Nigeria) and allowed access to water ad libitum. Experimental protocols were in accordance with the guidelines for animal research, as stated in the NIH guidelines for the care and use of laboratory animals (National Academy of Sciences and National Institute of Health Publications, 2011). The rats were allowed to acclimatize under laboratory conditions for two weeks before commencement of the experiment.

Camel milk collection

Milk collection was done every day from camel herds (Camelus dromedaries) in Kaura Namoda farms, Zamfara state Nigeria. The milk was stored in screwed bottles under ice. The milk was transferred and refrigerated at the department of pharmacology laboratory Ahmadu Bello University Zaria.

Administration of camel milk

Camel milk was administered orally at doses 250 mg/kg, 500 mg/kg and 1000 mg/kg respectively according to Zuberu et al., [16].

Induction of hyperlipidaemia

Poloxamer 407 (Lutrol F127; BASF, Ludwigshafen, Germany) was used to induce hyperlipidaemia. Administration of poloxamer 407 was at a dose of 500mg/kg intra peritoneally twice a week for 3 weeks [5, 11, 19]. Prior to the administration, poloxamer 407 was dissolved in distilled water and refrigerated overnight to facilitate its dissolution. Needles and syringes used for administration were cooled to prevent gelation within the syringe during injection as described by Johnston and Palmer [5].

Preparation of Standard Drug

Atorvastatin was purchased in a tablet form at strength 20 mg (Strovas Tablet 20 mg/kg, Ranbaxy Laboratory Ltd, Paonta Sahib Distribution, Sirmour H.P. 173025 India). Tablets were dissolved in distilled water and administered orally once daily [11].

Groupings of Animals

Group I: Normal control animals fed with a standard diet and orally administered 1 ml/kg distilled water for 21 days.

Group II: Hyperlipidaemic control animals induced with 500 mg/kg of poloxamer 407 intraperitoneally twice a week without treatment for 21 days

Group III: Induced with 500 mg/kg of poloxamer 407 intraperitoneally twice a week and treated with atorvastatin tablet (AT) orally at 20 mg/kg body for 21 days.

Groups IV: Induced with 500 mg/kg of poloxamer 407 intraperitoneally twice a week and co supplemented with oral administration of 250 mg/kg of camel milk once daily for 21 days.

Group V: Induced with 500 mg/kg of poloxamer 407 intraperitoneally twice a week and co supplemented with oral administration of 500 mg/kg of camel milk once daily for 21 days.

Group VI: Induced with 500 mg/kg of poloxamer 407 intraperitoneally twice a week and treated with oral administration of 1000 mg/kg of camel milk once daily for 21 days.

Biochemical Estimations

At the end of the 21-day experimental period, the animals were fasted overnight. All the animals were anaesthetized under chloroform vapor in an

anesthetic box with lid cover. Blood samples were collected via cardiac puncture into anticoagulant free tubes, centrifuged at a speed of 3000 rpm for 15 minutes.

The resultant serum was harvested into plain sample bottles for biochemical analysis. Serum total protein and albumin were measured by biuret reaction and colorimetric estimation respectively by using Agappe diagnostics total protein and albumin kits (Agappe Diagnostics Switzerland, GmbH). Globulin was measured from the difference between total protein and albumin. Serum alkaline phosphatase (ALP) activity was determined using Biolabo ALP reagent (Biolabo SA, 02160, Maizy, France). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using ALT and AST assay kits of Randox Laboratories Limited United Kingdom. Assay was performed according to manufacturer's manual.

Histopathological Studies

The Liver of the experimental animals were harvested and fixed in 10% formal saline solution containing plain sample bottles. This is followed by embedding of the liver tissue sections in melted paraffin wax and staining using hematoxilin and eosin. Histopathological assessment and photomicrography of the prepared slides was done by using an Olympus light microscope with attached Kodak digital camera.

Statistical Analysis

Data obtained was expressed as mean (± SEM). The result was analysed using one way analysis of variance(ANOVA), followed by Tukeys post hoc test to compare the level of significance between groups using statcato software version 0.9.12. Values of $p < 0.05$ were considered significant.

Results

The effect of camel milk on serum ALP on P407 induced hyperlipidaemic wistar rats is presented in Figure 1. Serum ALT activity showed a significant difference ($p < 0.05$) between the normal control group (4.0 ± 0.70U/L) and hyperlipidaemic untreated group (42.50 ± 4.54 U/L). There was significant difference ($p < 0.05$) between the normal control group and camel milk treatment groups 500 mg/kg and 1000 mg/kg (10.20 ± 2.26U/L and 11.20 ± 1.39 U/L) respectively. There was no significant difference ($p > 0.05$) between the normal control group and camel milk treatment group 250 mg/kg (6.40 ± 1.03 U/L).

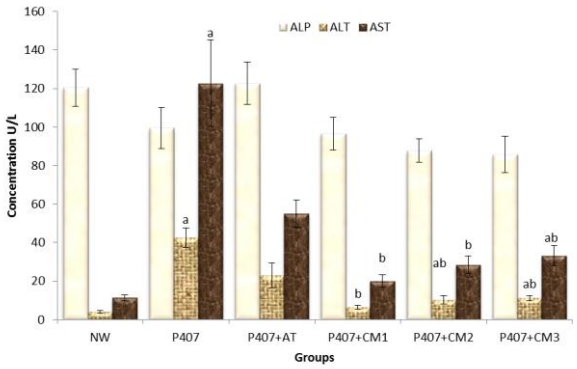


*Figure 1: Effect of camel milk on serum liver enzymes of poloxamer 407 induced hyperlipidemic Wistar rats. *Results are expressed as mean ± standard error of mean n = 5, Group DW: Normal control (1 ml/kg of distilled water) Group P407: P407 (500 mg/kg), Group P407+AT: P407 (500 mg/kg) + AT (20 mg/kg), Group P407+CM1: P407 (500 mg/kg) + CM (250 mg/kg), Group P407+CM2: P407 (500 mg/kg) + CM 500 mg/kg, Group P407+CM3: P407 (500 mg/k) + CM (1000 mg/kg).Values with superscripts are statistically significant (P < 0.05); a = when compared to Group NW, b = when compared to Group P407, c = when compared to Group P407+AT*

Serum concentration of total protein (TP) is shown in Figure 2. There was significant ($p < 0.05$) increase in the concentrations of serum TP between normal control (5.56 ± 0.26 g/dL) and hyperlipidaemic untreated group (11.23 ± 1.25 g/dL). There was significant ($p < 0.05$) decrease between hyperlipidaemic untreated group and camel milk treated groups 250 mg/kg (5.55 ± 0.56 g/dL) and 1000 mg/kg (6.42 ± 0.53 g/dL) respectively.

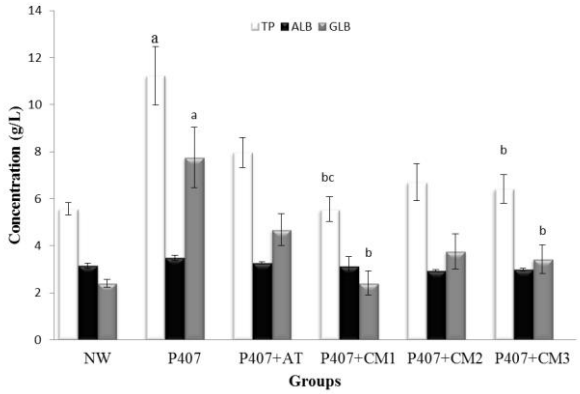


*Figure 2: Effect of Camel Milk on Serum Total Protein, Albumin and Globulin of Poloxamer 407 Induced Hyperlipidemic Wistar Rats. *Results are expressed as mean ± standard error of mean n = 5, Group DW: Normal control (1 ml/kg of distilled water) GroupP407: P407 (500 mg/kg), Group P407+AT: P407 (500 mg/kg) + AT(20 mg/kg), GroupP407+CM1: P407 (500 mg/kg) + CM(250 mg/kg), GroupP407 + CM2: P407 (500 mg/kg) + CM500 mg/kg, Group P407+CM3: P407 (500 mg/k) + CM (1000 mg/kg).Values with superscripts are statistically significant (P < 0.05); a = when compared to Group NW, b = when compared to Group P407, c = when compared to Group P407+AT*

There was significant difference ($p < 0.05$) between all camel milk treated groups when compared to the hyperlipidaemic control group. In AST activity, there was significant difference ($p < 0.05$) between normal control group (11.40 ± 1.05

U/L), hyperlipidaemic untreated group (122.75 ± 22.45 U/L) and camel milk treated group 1000 mg/kg (33.20 ± 5.31U/L) as shown in Figure 1. There was significant difference ($p < 0.05$) between all camel milk treatment group (250 mg/kg, 500 mg/kg and 1000 mg/kg) (20.00 ± 3.17 U/L, 28.40 ± 4.51 U/L and 33.20 ± 5.51 U/L) respectively, when compared to the hyperlipidaemic untreated group.

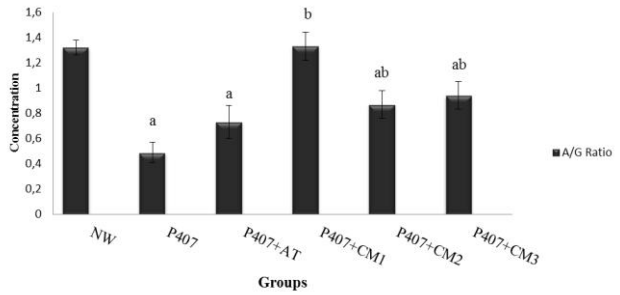


*Figure 3: Effect of Camel Milk on Serum Albumin/Globulin Ratio of Poloxamer 407 Induced Hyperlipidemic Wistar Rats. *Results are expressed as mean ± standard error of mean n = 5, Group DW: Normal control (1 ml/kg of distilled water) GroupP407: P407 (500 mg/kg), Group P407+AT: P407 (500 mg/kg) + AT (20 mg/kg), GroupP407 + CM1: P407 (500 mg/kg) + CM (250 mg/kg), GroupP407 + CM2: P407 (500 mg/kg) + CM500 mg/kg, Group P407 + CM3: P407 (500mg/k) + CM (1000 mg/kg). Values with superscripts are statistically significant ($P < 0.05$); a = when compared to Group NW, b = when compared to Group P407, c = when compared to Group P407+AT*

Camel milk at a dose of 250 mg/kg also showed significant ($p < 0.05$) decrease when compared to atorvastatin treated group (7.96 ± 0.64 g/dL).There was significant ($p < 0.05$) increase in the concentrations of serum globulin between normal control group (2.41 ± 0.17 g/dL) and Hyperlipidemic untreated group (7.75 ± 1.28 g/dL).

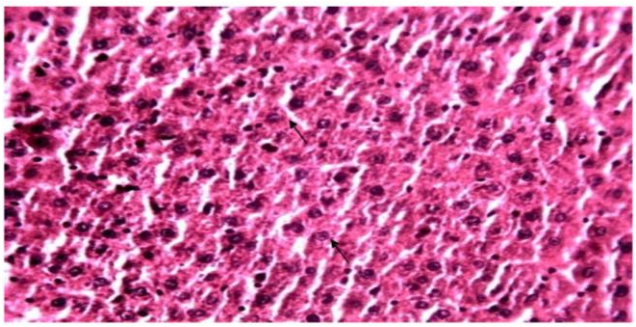


Figure 1: Photomicrograph of Liver Group I showing normal Hepatocytes (Longitudinal Section, H&E x250)

There was significant ($p < 0.05$) difference between Hyperlipidemic untreated group when compared to camel milk groups 1000 mg/k (3.43 ± 0.60 g/dL) and 250 mg/kg (2.41 ± 0.51 mg/dL) respectively. Serum Albumin/Globulin Ratio is shown in Figure 3. There was significant ($p < 0.05$) decrease in the Albumin/Globulin Ratio between normal control group (1.32 ± 0.06), Hyperlipidemic untreated group (0.49 ± 0.08), atorvastatin-treated group (0.73 ± 0.13 g/dL) and Camel milk treated groups (500 mg/kg and 1000 mg/kg) (0.87 ± 0.11 and 0.94 ± 0.11). All Camel milk treated groups showed significant ($p < 0.05$) increase when compared to the hyperlipidemic untreated group.

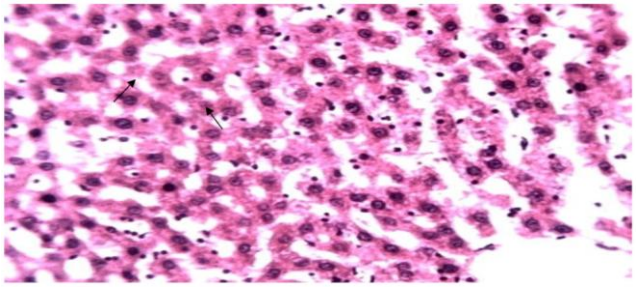


Figure 2: Photomicrograph of Liver of Group II with Intense Adipocytes Infiltration and Necrosis of Hepatocytes (Black Arrows) (Longitudinal Section, H&E x250)

Discussion

Poloxamer 407 (P-407) has been utilized as a model to induce hyperlipidaemia in rodents due to its convenience, reproducibility, low cost and the lack of undesirable underlying pathological conditions [20, 21]. P-407-induced hyperlipidaemia is associated with alterations in the activities of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, lecithin cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), hepatic lipase (HL) and lipoprotein lipase (LPL). It directly inhibits the heparin releasable fraction of LPL and HL and increases the biologic activity of CETP and LCAT indirectly [5, 6].

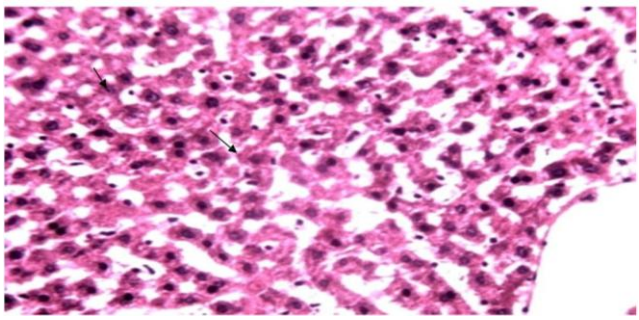


Figure 3: Photomicrograph of Liver Group III showing Moderate Adipocytes Infiltration and Necrosis of Hepatocytes (Black Arrows) (Longitudinal Section, H&E x250)

Fatty liver is an accumulation of triglycerides and other fats in the hepatocytes, if not treated leads to inflammation of the liver. It is characterized by varying degree of liver injury from steatosis to steatohepatitis, fibrosis and necrosis [2]. An elevated serum level of ALT and AST is indicative of liver disease as this enzymes are present in large quantities in the liver [14]. Serum ALT is thought to be more specific for hepatic degeneration [22].

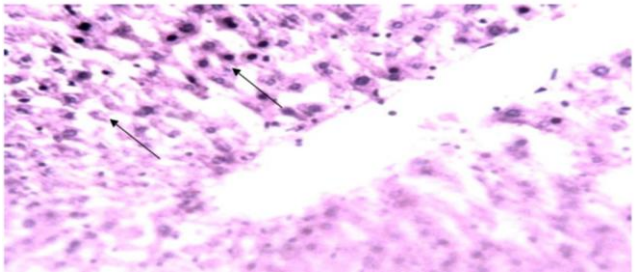


Figure 4: Photomicrograph of Liver Group IV showing Slight Adipocytes Infiltration of Hepatocytes (Black Arrows) (Longitudinal Section, H&E x250)

Following hepatocellular damage, hepatocytes alter their transport functions and membrane permeability thus leading to the leakage of these enzymes from their cells [11, 14]. Because it is a membrane-bound enzyme related to the transport of various metabolites, ALP is a sensitive biomarker of liver disease that is dependent on energy metabolism. A decrease in its activity may indicate impaired cellular energy processing [14].

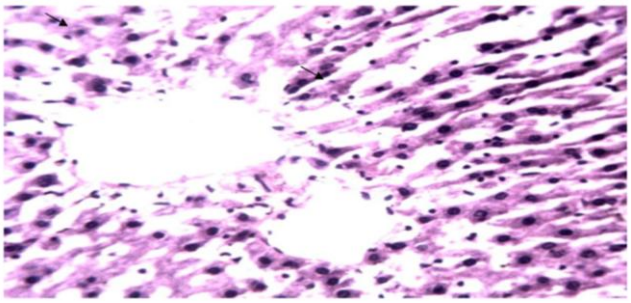


Figure 5: Photomicrograph of Liver Group V showing Intense Adipocytes Infiltration and Necrosis of Hepatocytes (Black Arrows) (Longitudinal Section, H&E x250)

There have been conflicting reports on the effect of poloxamer 407 induced hyperlipidaemia on liver enzymes (ALP, AST and ALT). Report on the effects of poloxamer 407 induced hyperlipidaemia on serum levels of the above enzymes showed that hyperlipidaemia elevated serum levels of ALT and AST [10].

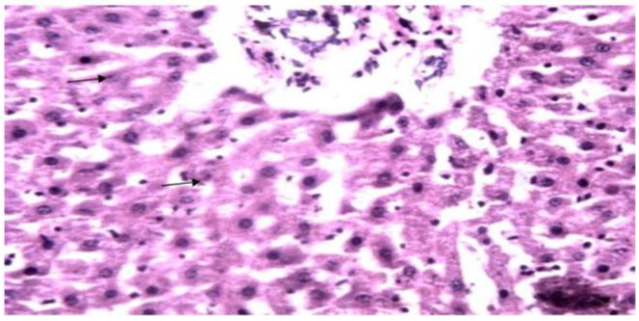


Figure 6: Photomicrograph of Liver Group VI showing Intense Adipocytes Infiltration and Necrosis of Hepatocytes (Black Arrows) (Longitudinal Section, H&E x250)

However, Ameh et al., [23] found no effect on ALT except on AST, while Johnston et al., [24] reported that P407 does not cause hepatic injury or damage.

Moreover, Victor et al., [11] showed elevation of all the three liver enzymes after induction of Wistar rats with P407. The discrepancies in the serum levels of the enzymes was attributed to the levels and duration of hyperlipidaemia [25]. In the present study, there was significant elevation in the serum ALT and AST but not ALP of hyperlipidaemic control group when compared to the normal control group. This may be due to injuries inflicted to the liver secondary to the accumulation of triglycerides and other fats in the liver cells, these findings conform with the work of Hyeung et al., [10]. There was a significant decrease in ALT and AST level of all the camel milk treated groups which is consistent with findings of Abbas et al., [26] and Al Hashem [14] but differs with the work of Helal et al., [27]. The reversal of these liver enzymes towards normal by the milk as observed in this study, maybe due to the prevention of the leakage of intracellular enzymes and increase membrane stabilizing activity. This is in agreement with the commonly accepted view that serum transaminases return to normal level upon healing of hepatic parenchyma and hepatocytes regeneration [28]. It therefore manifests the hepatoprotective effect of camel milk on P407 induced hepatic damage.

Evaluation of total protein or albumin status may be helpful in the assessment of disease progression [29]. Total protein is the measurement of all proteins in the blood serum. Majority of the proteins that are found in the blood are produced by the liver. By calculating the total amount of protein in the blood, performance of the liver in generating proteins can be understood. Total protein produced by the liver includes mainly globulin and albumin. Elevation of total proteins may be caused by dehydration and a decrease from overloading with water. The increase in disease arise mainly from an increase in total globulin, the albumin remaining normal or being reduced to a lesser extent. A decrease in total protein concentration is almost always the result of a drop in the level of albumin while the globulins do not change or increase by a smaller amount [26]. The results of our findings showed significant ($p < 0.05$) increase in total protein of hyperlipidaemic untreated group when compared to the normal control group. The increase total protein from our results differ from the works of Victor et al., [11] and Olorunnisola et al., [30] but are consistent with the works of Korolenko et al., [7]; who reported increased total protein in hyperlipidaemia. Camel milk significantly ($p < 0.05$) reduced the level of total protein of P407 induced hyperlipidaemic wistar rats which is in agreement with the work of Abbas et al., [26]. Albumin is important as nonspecific transport mechanism for many physiologic substances as well as drugs, antibiotics, various ions, amino acids and hormones. Albumin also serves as a precursor for

tissue proteins and in nutrition, proteins play a small part in maintaining the plasma pH. They are negatively charged at body pH and so act as bases, accepting hydrogen ions. Albumin is also an important component of plasma antioxidant activity that primarily binds free fatty acids, divalentcations and hydrogen oxychloride (HOCl) [30]. In chronic liver disease, levels of albumin are usually normal until a lot of liver is damaged [26, 31]. There was no significant ($p > 0.05$) change between all the groups. Globulin is a part of total protein and its raised level is noticed during chronic infection and liver disease such as liver necrosis [26]. Our findings showed significant increase ($p < 0.05$) in globulin level of hyperlipidaemic untreated group when compared to the normal control group. Increase in serum globulin level may have resulted from inflammatory or necrosis effect of P407 on the liver [7]. Camel milk significantly ($p < 0.05$) reduced the level of globulin of P407 induced hyperlipidaemic wistar rats which is in agreement with the work of Abbas et al., [26]. There was significant ($p < 0.05$) decrease of serum A/G ratio all treated groups except the group administered camel milk at a dose of 250 mg/kg. However all camel milk treated group showed significant ($p < 0.05$) increase in A/G ratio when compared to the hyperlipidaemic untreated group. This protective effect may be attributed to the high level of antioxidant vitamins, proteins and immunoglobulin present in camel milk [13, 14, 16, 26] or healing process which may have occurred in the liver.

Tissue sections from the hyperlipidaemic untreated group showed adipocyte infiltration and hepatocyte necrosis in the liver. This implies that the liver is a direct target for P407 as reported by Cogger et al., [32], whose findings showed that P407 has a marked effect on the ultrastructure of the liver sinusoidal endothelial cells (LSECs). Necrosis observed in liver tissues also agrees with the findings of Korolenko et al., [7, 9]. The liver of atorvastatin treated groups showed moderate infiltration and necrosis. This suggests that tissue repair occurred upon treatment with atorvastatin treatment. Group treated with camel milk (250 mg/kg) restored hepatocytes to normal. Thus, camel milk may be capable of ameliorating tissue damage such as hepatocytes injury that may occur in association with hyperlipidaemia.

In conclusion, camel milk showed hepatoprotective effect in poloxamer 407 induced hyperlipidaemic wistar rats. Drinking Camel milk may be helpful in ameliorating hazards caused by toxic substances.

References

1. Oguejiofor OO, Onwukwe CH, Odenigbo CU. Dyslipidemia in Nigeria, Prevalence and Pattern, Annals of African Medicine. 2012; 11(4):197-202. https://doi.org/10.4103/1596-3519.102846 PMid:23103917

2. Day CP, James OF. Steatohepatitis: a tale of two "hits" Gastroenterology. 1998;114(4): 842-845. https://doi.org/10.1016/S0016-5085(98)70599-2

3. Dumortier G, Grossiord JL, Agnely F, Chaumeil JC. A review of poloxamer 407 pharmaceutical and pharmacological characteristics. Pharmaceutical Research. 2006.23: 2709–2.

4. Wout ZG, Pec EA, Maggiore J, et al. Poloxamer 407-mediated changes in plasma cholesterol and triglycerides following intraperitoneal injection to rats. Journal of Parenteral Science Technology. 1992; 46:192–200. PMid:1474430

5. Johnston TP, Palmer WK. Mechanism of poloxamer 407-induced hypertriglyceridemia in rats. Biochemical Pharmacology. 1993; 46:1037–42. https://doi.org/10.1016/0006-2952(93)90668-M

6. Johnston TP. The P-407-induced murine model of dose-controlled hyperlipidemia and atherosclerosis: A review of findings to date. Journal of Cardiovascular Pharmacology. 2004; 43: 595–606. https://doi.org/10.1097/00005344-200404000-00016 PMid:15085072

7. Korolenko TA, Johnston TP, Tuzikov FV, Tuzikova NA, Pupyshev AB, Spiridonov VK, Goncharova NV, Maiborodin IV, Zhukova NA. Early-stage atherosclerosis in poloxamer 407-induced hyperlipidemic mice:pathological features and changes in the lipid composition of serum lipoprotein fractions and subfractions. Lipids in Health and Disease. 2016; 15(16): 1-13.

8. Palmer WK, Emeson EE, Johnston TP. Poloxamer 407-induced atherogenesis in the C57BL/6 mouse. Atherosclerosis. 1998; 136:115–23. https://doi.org/10.1016/S0021-9150(97)00193-7

9. Korolenko TA, Johnston TP, Dubrovina NI, Kisarova YA, Zhanaeva SY, Cherkanova MS, Filjushina EE, Alexeenko TV, Machova E, Zhukova NA. Effect of poloxamer 407 administration on the serum lipids profile, anxiety level and protease activity in the heart and liver of mice. Interdisciplinary Toxicology. 2013;6(1): 18–25. https://doi.org/10.2478/intox-2013-0004 PMid:24170975 PMCid:PMC3795317

10. Hyeung SL, Hyo CA, Sae KK. Hypolipemic effect of water extracts of Picrorrhizarhizoma in PX-407 induced hyperlipemic ICR mouse model with hepatoprotective effects: A prevention study. Journal of Ethnopharmacology. 2006;105:380–386. https://doi.org/10.1016/j.jep.2005.11.010 PMid:16406704

11. Victor DS, Dorcas BJ, Sunday EA. Ethanolic extracts of Vitex doniana possesses hepatocuractive property in poloxamer induced hyperlipidemia, Science Research. 2014; 2(3): 49-54. https://doi.org/10.11648/j.sr.20140203.14

12. Dhaliya SA, Surya AS, Dawn VT, Betty C, Arun K, Sunil C. A review of hyperlipidemia and medicinal plants. Int. JA PS. BMS. 2013;2(4):219-37.

13. Gizachew A, Teha J, Birhanu T. Review on Medicinal and Nutritional Values of Camel Milk. Nature and Science. 2014; 12(12):35-41.

14. Al Hashem F. Camel milk protects against aluminium chloride-induced toxicity in the liver and kidney of white albino rats. Am J Biochem Biotechnol. 2009; 5: 98-108. https://doi.org/10.3844/ajbbsp.2009.98.108

15. Ajamaluddin M, Abdulrahman A, Ewa S, Jerzy J. A study of the anti-diabetic agents of camel milk. International Journal of Molecular Medicine. 2012; 30:585-92. https://doi.org/10.3892/ijmm.2012.1051 PMid:22751901

16. Zuberu J, Saleh MIA, Alhassan AW, Adamu BY, Aliyu M, Iliya BT. Hypolipidemic and Antioxidant Activity of Camel Milk on Poloxamer-Induced Hyperlipidemia in Rats. Journal of African Association of Physiological Science. 2017; 5 (1): 58-66.

17. Agrawal RP, Swami SC, Beniwal R, Kochar DK, Kothari RP. Effect of camel milk on glycemic control, risk factors and diabetes quality of life in type 1 diabetes: A randomized prospective controlled study. International Journal of Diabetes in Developing Countries. 2002; 22: 70-74.

18. Al-Numair K.S. Type II diabetic rats and the hypolipidemic effect of camel milk. Journal of Food, Agriculture and Environment.

2010; 8 (2): 77-81.

19. Woo J, Jae HR, Han JO. The influence of Sam-Chil-Geun (Panax Notoginseng) on the Serum lipid levels and inflammations of rats with hyperlipidemia induced by poloxamer-407. Yonsei Medical Journal. 2010; 51(4):504-510. https://doi.org/10.3349/ymj.2010.51.4.504 PMid:20499414 PMCid:PMC2880261

20. Wout ZG, Pec EA, Maggiore J, et al. Poloxamer 407-mediated changes in plasma cholesterol and triglycerides following intraperitoneal injection to rats. Journal of Parenteral Science Technology.1992; 46:192–200. PMid:1474430

21. Hor SY, Farsi E, Yam MF, Nuyah NM, Asmawi MZ. Lipid-lowering effects of Coriolus versicolor extract in poloxamer 407-induced hypercholesterolaemic rats and high cholesterol-fed rats. Journal of Medicinal Plants Research. 2011;5(11):2261-2266.

22. Gibony PT. Mildly elevated liver transaminase in asymptomatic patients. American Family Physician. 2005; 71(6): 1105-1110.

23. Ameh DA, Adejo EB, James DB. Effect of Garcinal kola biflavonoid fractions on some biochemical parameters of P407-induced hyperlipidemia rats: A phytopreventive and phytotheraupeutic studies.Comparative Clinical Pathology. 2013; 24(1): 109-115. https://doi.org/10.1007/s00580-013-1868-4

24. Johnston TP, Baker JC, Jamal AS, Hall DD, Emeson EE, Palmer WK. Potential down regulation of HMG-CoA reductase after prolonged administration of P-407 in C57BL/6 mice. Journal of Cardiovascular Pharmacology. 1999; 34: 831–842. https://doi.org/10.1097/00005344-199912000-00010 PMid:10598127

25. Lu LS, Wu CC, Hung LM, Chiang MT, Lin CT, Lin CW, Su MJ. Apocynin alleviated hepatic oxidative burden and reduced liver injury in hypercholesterolaemia. Liver International. 2007;27(4):529-37. https://doi.org/10.1111/j.1478-3231.2007.01451.x PMid:17403193

26. Abbas S, Imran R, Nazir A, Qamar MF, Sarfraz L. Effect of camel milk supplementation on blood parameters and liver function of hepatitis patients, American Journal of Ethnomedicine. 2014; 3:129-146.

27. Helal EGE, Abd-Elwahab SM, Mohammad AA. Effect of camel milk on Alloxan-induced diabetic rats. The Egyptian Journal of Hospital Medicine. 2012; 49: 539–55.

28. Chavan SD, Patil SB, Naikwade NS. Biochemical And Histopathological Studies of Butea Monosperma (Lam) Taub leaves on Paracetamol induced hepatotoxicity in Albino rats, Journal of Pharmacy Research. 2012; 5(8):4006-4008.

29. Saad B, Azaizeh H, Abu-Hijleh G, Said S. Safety of traditional arab herbal medicine. Evidence Based Complement. Alternative Medicine. 2006; 3: 433–439. https://doi.org/10.1093/ecam/nel058 PMid:17173106 PMCid:PMC1697757

30. Olorunnisola SO, Graeme B, Anthony JA. Protective Effect of T. violacea Rhizome extract against hypercholesterolemia induced oxidative stress in wistar rats. Molecules. 2012; 6033-6045. https://doi.org/10.3390/molecules17056033 PMid:22614858

31. Kaneko JJ, Harvey JW, Bruss ML. Clinical biochemistry of domestic animals. 6th Edition, Elsevier Academic, Amsterdam, 2008: Pp 356-365.

32. Cogger VC, Hilmer SN, Sullivan D, Muller M, Fraser R, Le Couteur DG. Hyperlipidemia and surfactants: The liver sieve is a link, Atherosclerosis. 2006; 189:273–81. https://doi.org/10.1016/j.atherosclerosis.2005.12.025 PMid:16458315

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):859-865.
https://doi.org/10.3889/oamjms.2017.179
eISSN: 1857-9655
Basic Science

A Comparative Study of Rat Lung Decellularization by Chemical Detergents for Lung Tissue Engineering

Hamid Tebyanian[1,2], Ali Karami[1,2], Ebrahim Motavallian[3], Jafar Aslani[3], Ali Samadikuchaksaraei[4], Babak Arjmand[5], Mohammad Reza Nourani[1*]

[1]Division of Tissue Engineering and Regenerative Medicine, Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran; [2]Research Center for Prevention of Oral and Dental Disease, Baqiyatallah University of Medical Sciences, Tehran, Iran; [3]Department of General Surgery, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran; [4]Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran; Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran; [5]Cell Therapy and Regenerative Medicine Research Center, Endocrinology and Metabolism Cellular-Molecular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

Citation: Tebyanian H, Karami A, Motavallian E, Aslani J, Samadikuchaksaraei A, Arjomand B, Reza Nourani M. A Comparative Study of Rat Lung Decellularization by Chemical Detergents for Lung Tissue Engineering. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):859-865. https://doi.org/10.3889/oamjms.2017.179

Keywords: Decellularization; CHAPS; SDS; SDC and Triton X-100.

***Correspondence:** Mohammad Reza Nourani. Division of Tissue Engineering and Regenerative Medicine, Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran. E-mail: r.nourani@yahoo.com

Received: 21-Apr-2017; **Revised:** 25-Sep-2017; **Accepted:** 26-Sep-2017; **Online first:** 06-Dec-2017

Funding: This work was supported by the Iran National Science Foundation (INSF).

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Lung disease is the most common cause of death in the world. The last stage of pulmonary diseases is lung transplantation. Limitation and shortage of donor organs cause to appear tissue engineering field. Decellularization is a hope for producing intact ECM in the development of engineered organs.

AIM: The goal of the decellularization process is to remove cellular and nuclear material while retaining lung three-dimensional and molecular proteins. Different concentration of detergents was used for finding the best approach in lung decellularization.

MATERIAL AND METHODS: In this study, three-time approaches (24, 48 and 96 h) with four detergents (CHAPS, SDS, SDC and Triton X-100) were used for decellularizing rat lungs for maintaining of three-dimensional lung architecture and ECM protein composition which have significant roles in differentiation and migration of stem cells. This comparative study determined that variable decellularization approaches can cause significantly different effects on decellularized lungs.

RESULTS: Results showed that destruction was increased with increasing the detergent concentration. Single detergent showed a significant reduction in maintaining of three-dimensional of lung and ECM proteins (Collagen and Elastin). But, the best methods were mixed detergents of SDC and CHAPS in low concentration in 48 and 96 h decellularization.

CONCLUSION: Decellularized lung tissue can be used in the laboratory to study various aspects of pulmonary biology and physiology and also, these results can be used in the continued improvement of engineered lung tissue.

Introduction

The mortality of pulmonary diseases is increasing but mortality of other diseases such as heart disease, cancer and so on is decreasing for example 400 thousand of American people die every year [1, 2]. Lung transplantation is the only definitive treatment for some lung diseases such as CF, COPD and IPF. But, patient survival is only 50% during 5 years and 24% during 10 years after lung transplantation [3]. On the other hand, unfortunately, many patients on the waiting list for lung transplant pass out before suitable donor organ get found because of the critical shortage of transplantable donor lungs. Moreover, immunosuppression is another lifelong drawback for transplant recipients [4]. Due to this problem in transplantation, lung tissue engineering research is increasing and demand for engineered lung tissue has been increased for transplantation. There are advantages of engineered lung tissue such as using a patient's own cells, so

avoiding the need for immunosuppression which causes many problems including neurologic disorders and infection and so on [5, 6]. Tissue engineering is a multidisciplinary field which tries to combine cellular, molecular, technological and medical advances to make replacement tissues for implantation [7-10]. However, the lung is a complex tissue and ECM scaffolds are complex bio-structures constructed of a variety of structural and functional compositions that are specially organized and suitable for the intended tissue [11]. One of lung tissue engineering strategies is that to make appropriate scaffolds for lung tissue engineering [12]. Decellularization is a new technique for removing all cellular materials and produces an intact whole lung, which is an important characteristic of a three-dimensional matrix for cellular differentiation and migration [13, 14]. Decellularization methods have been used for producing scaffolds for a variety of tissue engineering applications such as heart valves, trachea, liver and heart and so on [7, 15-19]. Decellularized organs have several advantages as s tissue engineering scaffold. The decellularized scaffold maintains the suitable 3D organization essential for tissue function, such as a vascular system and airway network in the complex structure of lung [20]. Native ECM has the optimal substrate for cell attachment, spreading, growth and differentiation. The aim of the decellularization method is to remove cellular and nuclear material while maintaining 3D and ECM protein compositions and also, diminishing any damage to lung ECM. There are various types of decellularization approaches such as physical, chemical and enzymatic methods [16, 17]. Physical approaches can cause the destruction of the delicate lung ECM which this method includes freezing, pressure, sonication and agitation, while enzymatic approaches cannot be cost-effective due to large volumes of liquid would be needed for decellularization of a whole organ such as proteases, nucleases, and calcium chelators [21]. Trypsin has been used in the enzymatic method which it can remove fibronectin, laminin, and elastin. Because of the importance of these compositions, enzymatic approaches have not been mostly used in decellularization. But, chemical approaches are cost-effective and have several of techniques such as alkaline or acidic solutions, detergents (SDS, SDC, CHAPS and …) hypotonic or hypertonic solutions, and chelators [16, 17, 21]. A hypertonic NaCl solution can efficiently lyse cells, while it cannot help in removing cellular components from the tissue. CHAPS are a zwitterionic detergent which can be an effective solubilization for removing of cellular material [21]. The objectives of this research are to demonstrate that decellularized lung is promising substrates for producing an engineered lung scaffold and as the noted 3D and ECM protein compositions are important characteristics for cellular differentiation and migration.

Material and Methods

Animals and lung extraction

Fourteen male healthy rats (250–300 gr) were purchased from animal researcher centre of Baqiyatallah University of Medical Sciences and housed in this centre. Rats were controlled in a standard environment (humidity and temperature) on a 12/12 hours light/dark cycle with standard food and water, following experimental procedures approved by the Ethical Committee for Animal Research of Baqiyatallah University of Medical Sciences. The rats were anaesthetized with intraperitoneal with Ketamine and Xylazine (100 mg/kg and 10 mg/kg). A midline incision was made in the throat, and the trachea was exposed. Right ventricle perfusion was perfused with 250cc phosphate buffered saline (PBS) containing Heparin 5000 u/ml and 1% penicillin and streptomycin (P/S) (Figure 1). Lung was sliced to use in decellularization processes.

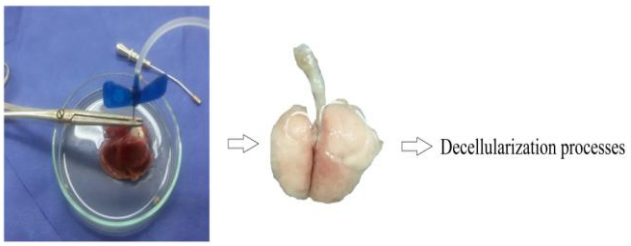


Figure 1: Perfusion of PBS, Heparin and P/S

Twenty four hour decellularization approaches

Sliced lungs were similarly rinsed and treated with SDS with different concentrations at 24 which is shown in Table 1. Lastly, sliced lungs were rinsed and treated with 1M sodium chloride for 1 h at room temperature for single detergent. Sliced lungs were flushed with PBS before they were used for experiments.

Table 1: 24h decellularization approaches for rat lung decellularization

Approach	Methods	Decellularization approaches
24 h	1	SDC 0.5% 23h, NaCl 1 M 1h
	2	SDC 1% 23h, NaCl 1 M 1h
	3	SDC 1.5% 23h, NaCl 1 M 1h
	4	SDC 2% 23h, NaCl 1 M 1h

Forty-eight-hour decellularization approaches

In this time, sliced lungs were rinsed and treated by mixed detergents and single detergent of SDC. These detergents were used at 48 h which is shown in Table 2. Sliced lungs were flushed with PBS before they were used for experiments.

Table 2: 48h decellularization approaches for rat lung decellularization

Approach	Methods	Decellularization approaches		
		24 h		24 h
	5	SDC 0. 5% 47h, NaCl 1 M 1h		
	6	SDC 1% 47h, NaCl 1 M 1h		
	7	SDC 1.5% 47h, NaCl 1 M 1h		
	8	SDC 2% 47h, NaCl 1 M 1h		
	9	SDC 0. 5%	+	SDS 0.05%
	10	SDC 1%	+	SDS 0.1%
	11	SDC 1.5%	+	SDS 0.15%
	12	SDC 2%	+	SDS 0.2%
	13	SDC 1%	+	SDS 0.05%
	14	SDC 1.5%	+	SDS 0.1%
48 h	15	SDC 0. 5%	+	Triton X-100 0.05%
	16	SDC 1%	+	Triton X-100 0.1%
	17	SDC 1.5%	+	Triton X-100 0.15%
	18	SDC 2%	+	Triton X-100 0.2%
	19	SDC 1%	+	Triton X-100 0.05%
	20	SDC 1.5%	+	Triton X-100 0.1%
	21	SDC 0. 5%	+	CHAPS 2mM
	22	SDC 1%	+	CHAPS 4mM
	23	SDC 1.5%	+	CHAPS 6mM
	24	SDC 2%	+	CHAPS 8mM
	25	SDC 1%	+	CHAPS 2mM
	26	SDC 1.5%	+	CHAPS 4mM

Ninety-six-hour decellularization approaches

In this time, sliced lungs were rinsed and treated by mixed detergents and single detergent of SDC. These detergents were used at 96 h which is shown in Table 3. Sliced lungs were flushed with PBS before they were used for experiments.

Table 3: 96h decellularization approaches for rat lung decellularization

Approach	Methods	Decellularization approaches		
		48 h		48 h
	27	SDC 0. 5%	+	SDS 0.05%
	28	SDC 1%	+	SDS 0.1%
	29	SDC 1.5%	+	SDS 0.15%
	30	SDC 2%	+	SDS 0.2%
	31	SDC 1%	+	SDS 0.05%
	32	SDC 1.5%	+	SDS 0.1%
	33	SDC 0. 5%	+	Triton X-100 0.05%
	34	SDC 1%	+	Triton X-100 0.1%
96 h	35	SDC 1.5%	+	Triton X-100 0.15%
	36	SDC 2%	+	Triton X-100 0.2%
	37	SDC 1%	+	Triton X-100 0.05%
	38	SDC 1.5%	+	Triton X-100 0.1%
	39	SDC 0. 5%	+	CHAPS 2mM
	40	SDC 1%	+	CHAPS 4mM
	41	SDC 1.5%	+	CHAPS 6mM
	42	SDC 2%	+	CHAPS 8mM
	43	SDC 1%	+	CHAPS 2mM
	44	SDC 1.5%	+	CHAPS 4mM

Lung histology

H&E staining protocol was utilized to tissue assay. Sliced lungs tissue (before and after decellularization) were fixed in paraformaldehyde 10% after fixation transferred to tissue processor device to dehydration and clearing. Finally, they were embedded in paraffin and sectioned in 5-micron thickness. Sections were stained with H&E, Trichrome- Masson staining and Elastin staining and then, they were observed under a light microscope [22-26]. H&E staining was utilized to visually quantify the nuclei loss and three-dimensional lung architecture by comparing nuclei counts in paraffin sections of normal and decellularized sliced lungs. Nuclei were counted from three random images at 100 × magnification using a counting tool. Trichrome-Masson staining and Elastin staining were done for evaluating the level of collagen and elastin, respectively.

Statistical analysis

All studies were conducted at least in biological triplicate. Data were statistically analyzed by t-test. P values less than 0.05 were considered significant.

Results

Analysis of a 24-h decellularized rat lung by Hematoxylin and Eosin, Trichrome- Masson and Elastin staining

This 24-h decellularization process could not completely preserve the architecture of the lung and also, could not completely eliminate the nuclei utilizing SDC detergent. But, method 1 was better than other methods in 24 h decellularization which this method maintained the three-dimensional structure at very low levels and also. This method could not preserve the proteins ECM composition such as Collagen and Elastin (Figure 2).

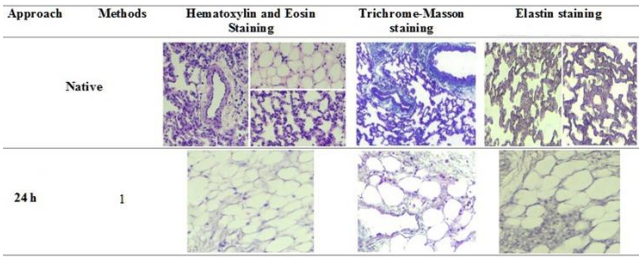


Figure 2: Hematoxylin and Eosin Staining showed structures and nuclei present in native and decellularized lungs. Trichrome-Masson staining showed collagen levels in native and decellularized lungs (Blue colour). Elastin staining showed elastin levels in native and decellularized lungs (Blue colour to black colour). Representative images for all conditions are shown (100 × magnification)

Analysis of a 48-h decellularized rat lung by Hematoxylin and Eosin, Trichrome-Masson and Elastin staining

SDC detergent

In this approach, SDC detergent was not able to maintain the three-dimensional, Collagen and Elastin. The architecture of the lung was destroyed by

increasing the concentration of SDC from 0.5% to 2%. In addition, a significant reduction was seen in Collagen and Elastin. Method 5 was better than other methods in single SDC detergent in 48 h decellularization (Figure 3) and it could relatively preserve the three-dimensional but could not maintain Collagen and Elastin.

Mixed detergents of SDC with SDS, Triton X-100 or CHAPS

Results showed that methods 9, 15, 21, 22 and 25 were considered as the best methods in maintaining of 3D and protein ECM composition (Collagen and Elastin) (Figure 3). Methods 9 and 15 could relatively preserve the three-dimensional and remove nuclei from lung tissue but Collagen and elastin were not seen in SDC+SDS and SDC+Triton X-100 detergent, respectively.

Methods 21, 22 and 25 relatively maintained proteins ECM (Collagen and Elastin) and also, a significant reduction in the nuclei was seen in 48 h decellularization of SDC+ CHAPS. In addition, Method 22 maintained the 3D, Collagen and elastin better than methods 21 and 25 (Figure 3).

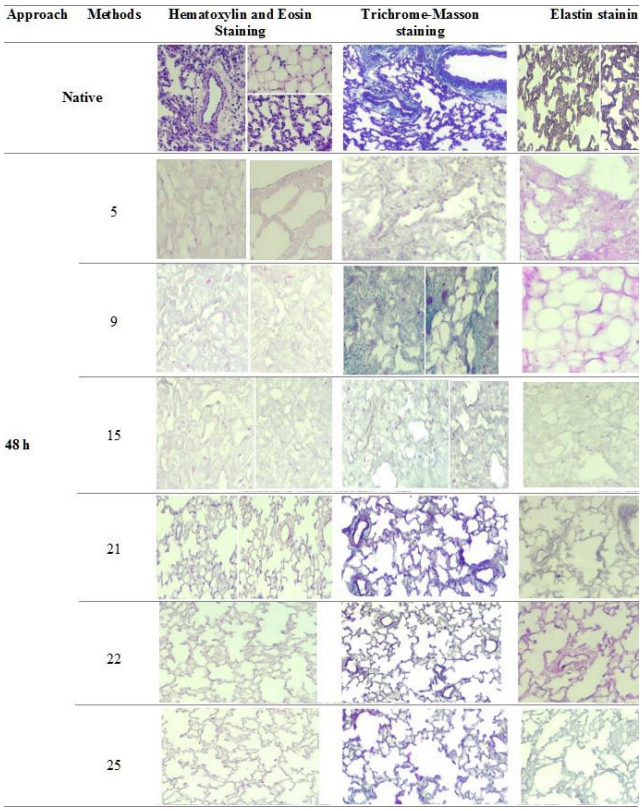


Figure 3: Hematoxylin and Eosin Staining showed structures and nuclei present in native and decellularized lungs. Trichrome-Masson staining showed collagen levels in native and decellularized lungs (Blue colour). Elastin staining showed elastin levels in native and decellularized lungs (Blue colour to black colour). Representative images for all conditions are shown (100 × magnification)

Analysis of a 96-h decellularized rat lung by Hematoxylin and Eosin, Trichrome - Masson and Elastin staining

In Ninety-six hour decellularization, methods 27, 33, 39 and 43 were the best decellularization approaches which methods 27 and 33 maintained the three-dimensional structure at very low levels and a significant reduction were not seen in the nuclei in SDC+SDS and SDC+Triton X-100 approaches, respectively. Methods 39 and 43 preserved the architecture of the lung while eliminating the nuclei in SDC+CHAPS approaches and also, the portions ECM composition (Collagen and Elastin) were decreased with increasing the time from 48 h to 96 h decellularization.

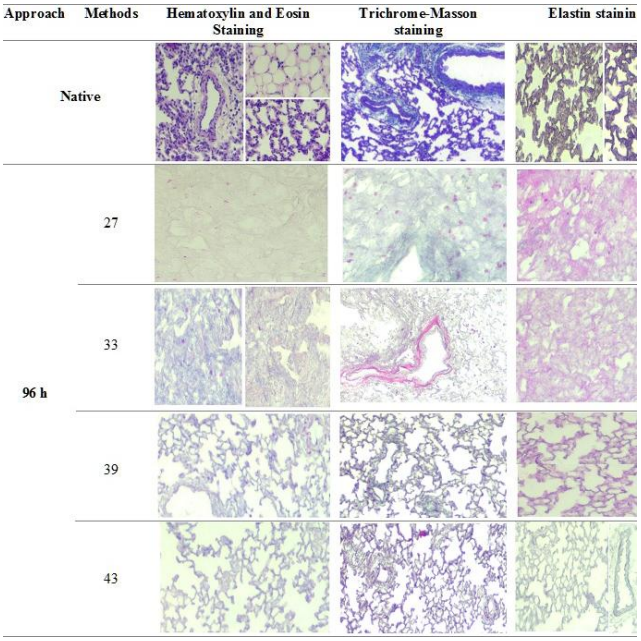


Figure 3: Hematoxylin and Eosin Staining showed structures and nuclei present in native and decellularized lungs. Trichrome-Masson staining showed collagen levels in native and decellularized lungs (Blue colour). Elastin staining showed elastin levels in native and decellularized lungs (Blue colour to black colour). Representative images for all conditions are shown (100 × magnification)

Discussion

In the percent century, pulmonary diseases are becoming world healthcare problems [27]. Chronic obstructive pulmonary diseases (COPD) such as emphysema and chronic obstructive bronchitis have caused more than 120,000 deaths in adults in the US in one year [28]. The last stage of pulmonary diseases is lung transplantation which has many challenges such as transplant rejection, immunosuppression, and

a shortage of donor organ. Waitlist of lung transplantation is increasing and many patients on the waiting list for lung transplant pass out before suitable donor organ get found because of the critical shortage of transplantable donor lungs [2, 4]. Lung tissue has a complex bio-structure which has various structural, functional compositions [11] and proteins ECM composition including collagen, laminin, elastin, fibronectin and so on [4]. Many types of research have been done for producing a lung scaffold from biomaterial but it has a lot of limitations. Recently, decellularization is appearing as decellularized biomimetic scaffold to support differentiated stem cells which it can be a significant treatment for lung diseases [13, 22]. In this regard, lung organ is decellularized and lung ECM's own cells are removed from the extracellular matrix and also, proteins ECM composition and growth factor are maintained which they are necessary for proliferation, differentiation and migration of stem cells but decreasing them during decellularization can be a major problem [4]. Native extracellular matrix has the optimum substrate for cell attachment, spreading, growth and differentiation.

The goal of the decellularization approach is to remove cellular and nuclear material while maintaining three dimensional and ECM protein compositions and also, reducing any damage to lung ECM. There are several types of decellularization methods such as physical, chemical and enzymatic methods [16, 17]. Physical approaches can destruct the complex lung ECM which this method includes freezing, pressure, sonication and agitation, while enzymatic approaches cannot be cost-effective due to large volumes of liquid would be needed for decellularization of a whole organ such as proteases, nucleases, and calcium chelators [21].

Trypsin has been used in the enzymatic method which it can remove fibronectin, laminin, and elastin. Because of the importance of these compositions, enzymatic approaches have not been mostly used in decellularization. But, chemical approaches are cost-effective and have several of techniques such as alkaline or acidic solutions, detergents (SDS, SDC, CHAPS and …) hypotonic or hypertonic solutions, and chelators [16, 17, 21]. Jensen et al (2012) studied on the evaluation of 24h decellularization process (Triton-X100 0.1% and sodium deoxycholate 2%) which they showed lung scaffolds with preservation of key ECM proteins collagen I, collagen IV, fibronectin, and laminin, but with the loss of nuclear proteins [13]. Price et al (2015) used pig lung for decellularization by an automated method and compared to manual method and they found that an automated method offered more consistent matrix and reduced the decellularization procedure required time [29]. Neill et al (2013) used CHAPS 8 mM and SDS 1.8 mM for human and porcine decellularization and they found that Porcine lungs could be decellularized using CHAPS to produce lung ECM scaffolds with properties resembling those of human lungs, for pulmonary tissue engineering and they proposed that porcine lung ECM can be an excellent screening platform for the envisioned human tissue engineering applications of decellularized lungs [30].

In this study, three-time approaches were used for decellularization (24, 48 and 96 h). In 24 h decellularization, method 1 could relatively maintain 3D and remove nuclei but all methods could not preserve Collagen and Elastin and also, there was significant of destruction with increasing the concentration of SDC from 0.5% to 2%. In 48 h decellularization, methods 5, 9, 15 could not preserve the integrity of the 3D and ECM proteins but they were better than other approaches in SDC, SDC+SDS and SDC+Triton X-100, respectively. On the other hand, methods 21, 22 and 25 were the best decellularization approaches which preserved the architecture of the lung while eliminating the nuclei and maintained a considerable of Collagen and Elastin.

In addition, among these approaches, method 22 was remarkable in maintaining of 3D and ECM proteins (Collagen and Elastin). In 96 h decellularization, methods 27 and 33 could not maintain the 3D, Collagen and elastin but they were better than other approaches in SDC+SDS and SDC+Triton X-100, respectively. Methods 39 and 43 preserved the architecture of the lung while eliminating the nuclei and relatively maintained Collagen and Elastin. Maghsoudlou et al (2013) used rat lung for decellularization using the intermittent intra-tracheal flow of detergent-enzymatic treatment and they presented a methodology which was applicable to rat and sheep lungs, preserved lung architecture and ECM and reduced the generating time [31]. Petersen et al (2012), two different techniques were examined using either the detergent 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) or sodium dodecyl sulphate (SDS). CHAPS-based decellularization resulted in the elimination of the majority of cellular components (including DNA) and maintenance of collagen and elastin, whereas SDS-based decellularization caused collagen depletion and less mechanical stability [16].

Cortiella et al (2010) demonstrated that the first attempt to produce and use whole decellularized lung and they compared the influence of decellularized lung by SDS 0.1%, Gelfoam, Matrigel, and a collagen I hydrogel matrix on the mESC attachment, differentiation and subsequent formation of complex tissue and they found that decellularized lung allowed for better retention of cells with more differentiation of mESCs into epithelial and endothelial lineages [32]. In this research, we used single and mixed detergents for decellularization and results showed that destruction was increased with increasing the concentration of detergent. Single detergent showed a significant reduction in maintaining of three-dimensional of lung and ECM

proteins (Collagen and Elastin). But, the best methods were mixed detergents of SDC and CHAPS in low concentration in 48 and 96 h decellularization. On the other hand, there was a considerable loss of Collagen and elastin and also, damage in 3D with increasing time. We conclude that the best method for decellularization was approach 22 which related to SDC 1%+CHAPS 4mM in 48 h decellularization. Finally, engineered scaffold of lung tissue can be used in the laboratory to study a wide variety of important aspects of pulmonary biology and physiology.

Acknowledgements

This study is extracted from PhD thesis which has been approved by Baqiyatallah University of Medical Sciences, Tehran, Iran. This work was supported by the Iran National Science Foundation (INSF).

References

1. ALA. Data American Lung Association: Lung Disease Data, 2008:1-188.

2. Tebyanian H, Karami A, Motavallian E, Aslani J, Samadikuchaksaraei A, Arjmand B, and Nourani MR. Histologic analyses of different concentrations of TritonX-100 and Sodium dodecyl sulfate detergent in lung decellularization. Cellular and Molecular Biology. 2017; 63(7):46-51. https://doi.org/10.14715/cmb/2017.63.7.8 PMid:28838339

3. Mulligan MS, Shearon TH, Weill D, Pagani FD, Moore J, and Murray S. Heart and lung transplantation in the United States. American Journal of Transplantation. 2008; 8(4p2):977-987.

4. Prakash YS, Tschumperlin DJ, and Stenmark KR. Coming to terms with tissue engineering and regenerative medicine in the lung. American Journal of Physiology - Lung Cellular and Molecular Physiology. 2015; 309(7):L625-L638. https://doi.org/10.1152/ajplung.00204.2015 PMid:26254424 PMCid:PMC4593835

5. Nichols JE, Niles JA, and Cortiella J. Design and development of tissue engineered lung: Progress and challenges. Organogenesis. 2009; 5(2):57-61. https://doi.org/10.4161/org.5.2.8564 PMid:19794900 PMCid:PMC2710526

6. Reichenspurner H. Overview of tacrolimus-based immunosuppression after heart or lung transplantation. The Journal of heart and lung transplantation. 2005; 24(2):119-130. https://doi.org/10.1016/j.healun.2004.02.022 PMid:15701425

7. Ott HC, Matthiesen TS, Goh S-K, Black LD, Kren SM, Netoff TI, and Taylor DA. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med. 2008; 14(2):213-221. https://doi.org/10.1038/nm1684 PMid:18193059

8. Schmidt D, Mol A, Odermatt B, Neuenschwander S, Breymann C, Gössi M, Genoni M, Zund G, and Hoerstrup SP. Engineering of Biologically Active Living Heart Valve Leaflets Using Human Umbilical Cord–Derived Progenitor Cells. Tissue engineering. 2006; 12(11):3223-3232. https://doi.org/10.1089/ten.2006.12.3223 PMid:17518636

9. Niklason LE, Gao J, Abbott WM, Hirschi KK, Houser S, Marini R, and Langer R. Functional arteries grown in vitro. Science. 1999; 284(5413):489-493. https://doi.org/10.1126/science.284.5413.489 PMid:10205057

10. Atala A, Bauer SB, Soker S, Yoo JJ, and Retik AB. Tissue-engineered autologous bladders for patients needing cystoplasty. The Lancet. 2006; 367(9518):1241-1246. https://doi.org/10.1016/S0140-6736(06)68438-9

11. Badylak SF, Taylor D, and Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. Annu Rev Biomed Eng. 2011; 13:27-53. https://doi.org/10.1146/annurev-bioeng-071910-124743 PMid:21417722

12. Vertrees RA, Zwischenberger JB, Boor PJ, Popov V, McCarthy M, Goodwin T, and Solley T. Cellular differentiation in three-dimensional lung cell cultures. Cancer biology & therapy. 2008; 7(3):404-411. https://doi.org/10.4161/cbt.7.3.5368

13. Jensen T, Roszell B, Zang F, Girard E, Matson A, Thrall R, Jaworski DM, Hatton C, Weiss DJ, and Finck C. A Rapid Lung De-cellularization Protocol Supports Embryonic Stem Cell Differentiation In Vitro and Following Implantation. Tissue Engineering. Part C, Methods. 2012; 18(8):632-646. https://doi.org/10.1089/ten.tec.2011.0584 PMid:22404373 PMCid:PMC3401389

14. Petersen TH, Calle EA, Zhao L, Lee EJ, Gui L, Raredon MB, Gavrilov K, Yi T, Zhuang ZW, Breuer C, Herzog E, and Niklason LE. Tissue-Engineered Lungs for in Vivo Implantation. Science (New York, N.Y.). 2010; 329 (5991):538-541. https://doi.org/10.1126/science.1189345 PMid:20576850 PMCid:PMC3640463

15. Mirsadraee S, Wilcox HE, Watterson KG, Kearney JN, Hunt J, Fisher J, and Ingham E. Biocompatibility of acellular human pericardium. Journal of Surgical Research. 2007; 143(2):407-414. https://doi.org/10.1016/j.jss.2007.01.026 PMid:17574597

16. Petersen TH, Calle EA, Colehour MB, and Niklason LE. Matrix Composition and Mechanics of Decellularized Lung Scaffolds. Cells, Tissues, Organs. 2012; 195 (3): 222-231. https://doi.org/10.1159/000324896 PMid:21502745 PMCid:PMC3696368

17. Petersen TH. In vitro development of engineered lung tissue, 2009.

18. Lin YM, Boccaccini AR, Polak JM, Bishop AE, and Maquet V. Biocompatibility of Poly-DL-lactic acid (PDLLA) for Lung Tissue Engineering. Journal of Biomaterials Applications. 2006; 21(2):109-118. https://doi.org/10.1177/0885328206057952 PMid:16443629

19. Conconi MT, Coppi PD, Liddo RD, Vigolo S, Zanon GF, Parnigotto PP, and Nussdorfer GG. Tracheal matrices, obtained by a detergent-enzymatic method, support in vitro the adhesion of chondrocytes and tracheal epithelial cells. Transplant international. 2005; 18(6):727-734. https://doi.org/10.1111/j.1432-2277.2005.00082.x PMid:15910302

20. Bernard MP, Chu ML, Myers JC, Ramirez F, Eikenberry EF, and Prockop DJ. Nucleotide sequences of complementary deoxyribonucleic acids for the pro alpha 1 chain of human type I procollagen. Statistical evaluation of structures that are conserved during evolution. Biochemistry. 1983; 22(22):5213-5223. https://doi.org/10.1021/bi00291a023 PMid:6689127

21. Gilbert TW, Sellaro TL, and Badylak SF. Decellularization of tissues and organs. Biomaterials. 2006; 27(19):3675-3683. https://doi.org/10.1016/j.biomaterials.2006.02.014

22. Daly AB, Wallis JM, Borg ZD, Bonvillain RW, Deng B, Ballif BA, Jaworski DM, Allen GB, and Weiss DJ. Initial binding and re-cellularization of de-cellularized mouse lung scaffolds with bone marrow-derived mesenchymal stromal cells. Tissue Eng Part A. 2011; 18(1-2):1-16. https://doi.org/10.1089/ten.tea.2011.0301 PMid:21756220 PMCid:PMC4066256

23. Babavalian H, Latifi AM, Shokrgozar MA, Bonakdar S, Shakeri F, and Tebyanian H. Healing Effects of Synthetic and Commercial Alginate Hydrogel Dressings on Wounds: A Comparative Study.

Trauma Mon. 2016 Aug (In press). https://doi.org/10.5812/traumamon.38941

24. Babavalian H, latifi AM, Shokrgozar MA, Bonakdar S, Tebyanian H, and Shakeri F. Cloning and expression of recombinant human platelet-derived growth factor-BB in Pichia Pink. Cellular and Molecular Biology. 2016; 62(8):45-51. PMid:27545214

25. Karami A, Tebyanian H, Goodarzi V, and Shiri S. Planarians: an in vivo model for regenerative medicine. International journal of stem cells. 2015; 8(2):128. https://doi.org/10.15283/ijsc.2015.8.2.128 PMid:26634061 PMCid:PMC4651277

26. Shakeri F, Tebyanian H, Karami A, Babavalian H, and Tahmasbi MH. Effect of Topical Phenytoin on Wound Healing. Trauma Mon. 2017; 22(5).

27. Lal MK, Manktelow BN, Draper ES, and Field DJ. Chronic lung disease of prematurity and intrauterine growth retardation: a population-based study. Pediatrics. 2003; 111(3):483-487. https://doi.org/10.1542/peds.111.3.483 PMid:12612225

28. Mini-o AM, Murphy SL, Xu J, and Kochanek KD. Deaths: final data for 2008. National vital statistics reports: from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System. 2011; 59(10):1-126.

29. Price AP, Godin LM, Domek A, Cotter T, D'Cunha J, Taylor DA, and Panoskaltsis-Mortari A. Automated Decellularization of Intact, Human-Sized Lungs for Tissue Engineering. Tissue Engineering. Part C, Methods. 2015; 21(1):94-103. https://doi.org/10.1089/ten.tec.2013.0756 PMid:24826875 PMCid:PMC4290793

30. O'Neill JD, Anfang R, Anandappa A, Costa J, Javidfar JJ, Wobma HM, Singh G, Freytes DO, Bacchetta MD, Sonett JR, and Vunjak-Novakovic G. Decellularization of Human and Porcine Lung Tissues for Pulmonary Tissue Engineering. The Annals of thoracic surgery. 2013; 96(3):1046-1056. https://doi.org/10.1016/j.athoracsur.2013.04.022 PMid:23870827 PMCid:PMC4033908

31. Maghsoudlou P, Georgiades F, Tyraskis A, Totonelli G, Loukogeorgakis SP, Orlando G, Shangaris P, Lange P, Delalande J-M, Burns AJ, Cenedese A, Sebire NJ, Turmaine M, Guest BN, Alcorn JF, Atala A, Birchall MA, Elliott MJ, Eaton S, Pierro A, Gilbert TW, and De Coppi P. Preservation of micro-architecture and angiogenic potential in a pulmonary acellular matrix obtained using intermittent intra-tracheal flow of detergent enzymatic treatment. Biomaterials. 2013; 34(28):6638-6648. https://doi.org/10.1016/j.biomaterials.2013.05.015 PMid:23727263 PMCid:PMC3988964

32. Cortiella J, Niles J, Cantu A, Brettler A, Pham A, Vargas G, Winston S, Wang J, Walls S, and Nichols JE. Influence of Acellular Natural Lung Matrix on Murine Embryonic Stem Cell Differentiation and Tissue Formation. Tissue Engineering Part A. 2010; 16(8):2565-2580. https://doi.org/10.1089/ten.tea.2009.0730 PMid:20408765

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):866-874.
https://doi.org/10.3889/oamjms.2017.210
eISSN: 1857-9655
Basic Science

Antigenotoxic and Antioxidant Activity of Methanol Stem Bark Extract of *Napoleona Vogelii Hook & Planch* (Lecythidaceae) In Cyclophosphamide-Induced Genotoxicity

Victor Ikumawoyi*, Esther Agbaje, Olufunsho Awodele

Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi araba, Lagos, Nigeria

Citation: Ikumawoyi V, Agbaje E, Awodele O. Antigenotoxic and Antioxidant Activity of Methanol Stem Bark Extract of Napoleona Vogelii Hook & Planch (Lecythidaceae) In Cyclophosphamide-Induced Genotoxicity. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):866-874. https://doi.org/10.3889/oamjms.2017.210

Keywords: antigenotoxic; antioxidant; cyclophosphamide; micronucleus; phytochemicals

***Correspondence:** Victor Olabowale Ikumawoyi. Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Idi araba, Lagos, Nigeria. +2348039706148. E-mail: walevt@gmail.com

Received: 20-Mar-2017; **Revised:** 19-Apr-2017; **Accepted:** 19-May-2017; **Online first:** 09-Dec-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: *Napoleona vogelii* is used in traditional medicine for cancer management.

AIM: The study was conducted to evaluate the antigenotoxic and antioxidant activities of methanol stem bark extract of *N. vogelii* in male Sprague Dawley rats.

MATERIALS AND METHOD: Thirty male Sprague Dawley rats were randomly divided into group 1 (control) administered 10 mL/kg distilled water, groups 2 and 3 were co-administered 100 mg/kg, 200 mg/kg of *N. vogelli* and 5 mg/kg cyclophosphamide (CPA) respectively for 7 days p.o. Groups 4 and 5 were administered only 5 mg/kg CPA and 200 mg/kg NV respectively.

RESULTS: The LD50 oral was greater than 4 g/kg. There were significant ($p < 0.0001$) increases in plasma enzymatic and non-enzymatic antioxidant enzymes and significant ($p < 0.0001$) decrease in percentage micronuclei in bone marrow of extract treated rats compared to rats administered 5 mg/kg CPA alone. There was steatosis pointing to cytotoxic injury in the liver of rats co-administered 200 mg/kg NV and 5 mg/kg CPA. Gas chromatography-mass spectrometry analysis of the extract showed the presence of phytol and unsaturated fatty acids.

CONCLUSION: *N. vogelii* possesses antigenotoxic and antioxidant activities associated with the presence of phytochemicals, phytol and unsaturated fatty acids.

Introduction

Oxidative stress occurs when the generation of free radicals and reactive intermediates in a system exceeds the system's ability to neutralise and eliminate them [1]. It reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage [2]. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA [2]. Oxidative stress is thought to be involved in the development of some disease conditions such as cancer [3], Alzheimer's disease [4], heart failure [5], myocardial infarction [6], fragile X syndrome [7], and sickle cell disease [8] among others.

Cyclophosphamide (CPA) is used for several types of cancers including leukaemia, breast and ovarian cancers. It is cytotoxic with a low margin of safety, and it's a cell cycle non-specific drug which acts on both resting as well as dividing cells. The use of CPA may result in cardiac dysfunction, pulmonary toxicity and incidence of its genotoxicity have been reported [9]. The cellular mechanism of CPA toxicity is mediated by an increase in free radicals through the generation of intracellular phosphoramide mustard and acrolein, the principal alkylating metabolite of CPA [10]. Acrolein interferes with the tissue antioxidant defence system producing highly reactive oxygen free radicals and is mutagenic to mammalian cells [10], and an increase in free-radical production mediated by CPA metabolites stimulates lipid peroxidation leading to an increase in

malondialdehyde production [11]. Genotoxins include both radiation and chemical genotoxins, and there are three primary effects that genotoxins can have on organisms by affecting their genetic information [12]. Genotoxins can be carcinogens (cancer-causing agents), mutagens, or mutation-causing agents, or teratogens, birth defect-causing agents. In most cases, genotoxicity leads to mutations in various cells and other bodily systems and can result in a host of other pathologies, from cancer to a wide variety of different diseases [12].

The mammalian in vivo micronucleus assay is recommended by the International Conference on Harmonization (ICH) guidelines as part of the genotoxicity testing battery required during the development of new drugs [13]. Mechanistic studies [14] have shown that micronucleus formation may be due to free radical generation from an agent leading to lipid peroxidation of membranes causing the breakages of the deoxyribonucleic acid (DNA) and covalent binding between the products of lipid peroxidation and DNA. The World Health Organization estimates that up to 80% of the African population relies on the traditional medicinal system for some aspects of their primary health care [15]. Plants play a significant role in maintaining human health and improving the quality of human life serving as valuable components of food, as well as in cosmetics, dyes, and medicines [16].

Napoleona vogelii is found mostly in rain forests and along sea shores extending from Sierra Leone to Nigeria [17]. About 20 genera and 450 species have been identified in the tropical regions of Africa, Asia, Australia and it's distributed mostly in African countries of Nigeria, Ghana, Guinea, Togo and Benin [18]. The methanolic leaf extract of *N. vogelii* is used in the treatment of peptic ulcer disease [19]; furthermore, the leaves are also used in the treatment of a cough, asthma and hypoglycaemia [20]. The stem bark decoction is used topically against dermatosis and drunk to treat sexual asthenia [21] and its used in traditional medicine used in the treatment of cough, asthma, inflammatory conditions [22, 23] and cancer [24]; thus, this study was designed to investigate the antigenotoxic and antioxidant activity of the methanol stem bark extract of *N. vogelii* in cyclophosphamide-treated rats and identifying the constituent bioactive principles by Gas chromatography-mass spectrometry (GCMS) analysis.

Materials and Methods

Drugs and chemicals

Cyclophosphamide (Kwality Pharmaceuticals Pvt. Ltd. Amritsar – India), phosphate buffered saline (PBS), Methanol (Sigma-Aldrich Chemie GmbH, Germany), Normal saline (Unique pharmaceuticals Ltd, Ogun State, Nigeria), Giemsa stain (Sigma-Aldrich Chemie GmbH, Germany).

Plant extraction

The stem bark of *N. vogelii* was collected from a secondary forest in Abatadu village, Ikire township of Osun state in the South Western part of Nigeria and duly authenticated by Mr. O. Oyebanji and Professor J. D. Olowokudejo at the herbarium of the Department of Botany and Microbiology, University of Lagos, Nigeria where a voucher specimen (LUH 6524) was deposited. It was washed, chopped into small pieces and air-dried to a constant weight. The dried stem bark was then pulverised into a fine powder using a grinding mill, and 500 g of the powder was macerated in 2.5 L of methanol. The mixture was stirred and left to stand for seven days at room temperature. It was then filtered using a muslin cloth and a 125 mm Whatman filter paper (GE Healthcare UK Limited, United Kingdom). The filtrate was concentrated using a rotary evaporator (Buchi R – 215 Rotavapor (pump-) V – 700 Switzerland) at 45°C, to yield a solid brown extract. The yield of the extract derived using the formula: weight of extract/weight of starting plant material ×100 was 2.22%. The extract was stored in an air-tight container until required.

Phytochemical screening

The qualitative phytochemical screening was done according to the methods of Trease and Evans [25].

Determination of total flavonoids

Total soluble flavonoid of the extract was determined with aluminium chloride using quercetin as standard [26]. A 1 ml of sample solution (100 µg/ ml) was mixed with 3 ml of methanol, 0.2 ml of 10% Aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. The resulting mixture was incubated at room temperature for 30 minutes, and the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol.

Determination of total phenolic content

Total phenolic content in the extract was estimated using the modified Folin-Ciocalteu method of Wolfe et al., [27]. An aliquot of the extract was mixed with 2.5 ml of Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 2 ml of 75 g/l of sodium carbonate. The tubes were vigorously shaken for 15 s and allowed to stand for 30 min at

40˚C for colour development. Absorbance was measured at 765 nm using T80 UV/VIS spectrophotometer. The total phenolic content in mg/ 100 g was calculated as gallic acid equivalent from the calibration curve.

Determination of total tannin content

Total tannin content was estimated by adopting the procedure of Sun et al. [28]. To 1 ml of the extract solution, 3 ml of 4 % vanillin-methanol solution and 1.5 ml hydrochloric acid was added. The mixture was allowed to stand for 15 min. The absorbance was then measured at 500 nm using T80 UV/VIS spectrophotometer, and the result expressed as catechin equivalent in mg/100 g.

Determination of total saponin content

The method used was that of Obadoni and Ochuko [29]. The sample was ground, and 0.02 mg was put in a conical flask, and 100 cm3 of 20 % aqueous ethanol was added. The sample \was heated over a hot water bath for four h with continuous stirring at about 55˚C. The mixture was filtered and the residue re-extracted with another 200 ml or 20 % ethanol. The extract was reduced to 40 ml over a water bath at about 90˚C. The concentrate was transferred to a 250 ml separating funnel, and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered, while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The n-butanol extract was washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample was dried in the oven to a constant weight; the saponin content was calculated as a percentage.

Experimental animals

Male rats of 4 months old weighing between 100 – 150 g and mice of about 3.5 – 4 months weighing between 20 – 30 g used were obtained from the Laboratory Animal Centre of the College of Medicine of the University of Lagos, Lagos, Nigeria, where they were also kept. The animals were maintained under standard environmental conditions (23-25˚C, 12h/12h light/dark cycle) and they were sustained on standard rodent feed (Livestock Feed Plc, Lagos, Nigeria) and clean drinking water. The animals were acclimatised for seven days before commencement of the experiment and the procedures were in conformity with The Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health [30] for studies involving experimental animals [30].

Acute toxicity

Five (5) groups of 5 mice each were fasted for 12 h and were then treated with 100 mg/ml of the extract at doses of 0.5 g/kg, 1 g/kg, 2 g/kg, 3 g/kg and 4 g/kg which ranges between 0.1 ml to 1 ml in divided doses p.o. Animals were observed for two h after extract administration for behavioural symptoms of toxicity and mortality and then after 24 h for mortality. They were further observed for 14 days for signs of delayed toxicity.

Collection of samples for micronucleus and antioxidant enzymes assay

The micronucleus assay was carried out by a modification of the method of Heddle and Salamone, [31]; Timwell and Ashley, [32] and blood levels of antioxidant enzymes; catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) and MDA was done according to the protocol of Sun and Zigma [33].

Male rats were divided into 5 groups (n = 6); control rats treated with 10 mL/kg distilled water, while the remaining four groups received 5 mg/kg CPA, 100 mg/kg *N. vogelii* and 5 mg/kg CPA, 200 mg/kg *N. vogelii* and 5 mg/kg CPA and 200 mg/kg *N. vogelii* respectively for 7 days. The dosing schedule of cyclophosphamide was chosen according to a modification of the method of Doherty et al. [34] and the extract was administered 30 mins post cyclophosphamide administration.

On the day after termination of administration, normal and treated animals were sacrificed by cervical dislocation and blood samples collected through the retro-orbital plexus vein of the eye for antioxidant enzymes assay. For the micronucleus test, the femur of the animals was harvested and washed in phosphate buffered saline (PBS), and the two edges were cut off. A 2 ml syringe containing PBS was used to wash out the bone marrow on a slide and then smeared and left to dry. The slides were then fixed in absolute methanol and then stained for 15 min using 10 % Giemsa and at least 2000 cells per rat were scored at × 100 for MN in polychromatic erythrocytes (MNPCE).

Determination of Superoxide dismutase activity

Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by Sun and Zigma [33]. The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of the blood sample and 0.03 ml of epinephrine in 0.005 N HCl was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate

(epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min.

Determination of Catalase activity

Serum catalase activity was determined according to the method of Beers and Sizer as described by Usoh et al. [35] by measuring the decrease in absorbance at 240 nm due to the decomposition of H2O2 in a UV recording spectrophotometer. The reaction mixture (3 ml) contained 0.1 ml of serum in phosphate buffer (50 mM, pH 7.0) and 2.9 ml of 30 mM H_2O_2 in phosphate buffer of pH 7.0. The specific activity of catalase was expressed as moles of H_2O_2 reduced per minute per mg protein.

Determination of Reduced glutathione

The reduced glutathione (GSH) content was estimated according to the method described by Sedlak and Lindsay [36]. To the homogenate 10% TCA was added and centrifuged. 1.0 ml of the supernatant was treated with 0.5ml of Ellman's reagent (19.8mg of 5,5-dithiobisnitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412 nm.

Determination of Malondialdehyde activity

Malondialdehyde (MDA), an index of lipid peroxidation was determined using the method of Buege and Aust [37]. 1.0 ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA) tricarboxylic acid- thiobarbituric acid-hydrochloric acid reagent boiled at 100^{o}C for 15 min and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed, and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA- a complex of 1.56 × 105 M-1CM-1.

Determination of Glutathione-S-transferase activity

Glutathione –S- transferase activity was determined according to Habig et al. [38]. This is based on the fact that all known glutathione –S-transferase demonstrate a relatively high activity with 1-Chloro-2, 4-dinitrobenzene (CDNB) as the second substrate. Consequently, the conventional assay for glutathione –S- transferase activity utilises 1-Chloro-2, 4-dinitrobenzene as substrate. When this substrate is conjugated with reduced glutathione (GSH) its maximum absorption shifts to a longer wavelength. The absorption increases at the new wavelength of 340 nm which provides a direct measurement of the enzymatic reaction.

Gas chromatography-mass spectrometry

GC-MS analysis of the methanol extract of *N. vogelii* was performed using a Shimadzu GP-2010 gas chromatograph equipped with Rtx-5MS (30m X 0.25mm, 0.25µm) column. Helium was used as the carrier gas at a flow rate of 1ml/min. Using an injection volume of 1.0 µL. The sample was injected in a split mode of 10:1. Mass spectral scan range was set at 35 - 550 (m/z). The injector temperature was kept at 250^{o}C, and ion source temperature was 200^{o}C. The oven temperature was maintained at 40 oC, and the interface temperature was at 250°C. Relative quantity of the chemical compounds present in the extract was expressed as a percentage based on peak area produced in the chromatogram.

Histopathological assessment

Tissues fixed in 10% formol-saline were dehydrated in graded alcohol, embedded in paraffin, and cut into 4- to 5- µm-thick sections. The sections were stained with hematoxylin-eosin for photomicroscopic assessment using a Model N-400ME photomicroscope (CEL-TECH Diagnostics, Hamburg, Germany).

Statistical analysis

Statistical analysis was done using One-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc multiple comparison tests using GraphPad Prism 6.0 (GraphPad Software, CA, USA). Results were considered significant at $p < 0.05$.

Results

Acute toxicity

There was no mortality recorded on the administration of methanol stem bark extract of *N. vogelii* up to 4000 mg/kg p.o. The LD50 is greater than 4 g/kg.

Qualitative phytochemical analysis

The methanol stem bark extract of *N. vogelii* was found to contain flavonoids, phenols, saponins, tannins, phlorotannin and cardiac glycoside.

Quantitative phytochemical screening of

methanol stem bark extract of *N. vogelii.* The Table below shows the phytochemical constituents of *N. vogelii* quantitatively in mg/100 g (Table 1).

Table 1: Quantitative phytochemical screening of methanol stem bark extract of *N. vogelii*

Constituents	Quantity mg/100g
Flavonoid	87.88 ± 0.32
Phenol	24.88 ± 0.47
Saponin	35.55 ± 0.19
Tannin	13.01 ± 0.84

Effect of N. vogelii on mean percentage micronuclei (%MNPCE) and polychromatic erythrocytes (%PCE) in cyclophosphamide-treated rats

There was a significant ($p < 0.0001$) increase in percentage micronuclei at 5 mg/kg CPA alone (4.90 ± 0.11) compared to control (0.12 ± 0.04). Co-administration of 200 mg/kg NV and 5 mg/kg CPA resulted in a significant ($p < 0.0001$) decrease (2.40 ± 0.05) in percentage micronuclei compared to 5 mg/kg CPA alone (4.90 ± 0.11).

There was also a significant ($p < 0.0001$) decrease in percentage micronuclei on co-treatment with 100 mg/kg NV and 5 mg/kg CPA (3.20 ± 0.02) compared to 5 mg/kg CPA alone (4.90 ± 0.11).

A significant ($p < 0.0001$) decrease in percentage micronuclei at 200 mg/kg NV alone (0.66 ± 0.02) compared to 5 mg/kg CPA alone (4.90 ± 0.11) was also observed (Table 2).

Table 2: Effect of *N. vogelii* on mean percentage micronuclei and polychromatic erythrocytes in cyclophosphamide-treated rats

	% MNPCE	Mean MNPCE	% PCE
CONTROL	0.12 ± 0.04	1.20 ± 0.37	100.00 ± 0.04
5 mg/kg CPA	4.90 ± 0.11a	49.00 ± 1.10	95.00 ± 0.11a
100 mg/kg NV + 5 mg/kg CPA	3.20 ± 0.02a, b	32.00 ± 0.24	97.00 ± 0.03a, b
200 mg/kg NV + 5 mg/kg CPA	2.40 ± 0.05a, b	24.00 ± 0.51	98.00 ± 0.05a, b
200 mg/kg NV	0.66 ± 0.02a, b	6.60 ± 0.24	99.00 ± 0.02a, b

MNPCE; micronucleated polychromatic erythrocytes, PCE; polychromatic erythrocytes. Results are mean ± SEM. a $p < 0.0001$ vs control, b $p < 0.0001$ vs CPA. One Way ANOVA followed by Tukey's post hoc multiple comparison tests.

Effect of methanol stem bark extract of on *N. vogelii* on antioxidant parameters in cyclophosphamide-treated rats

Treatment with 5 mg/kg CPA resulted in a significant reduction in GSH, SOD, CAT and GST with corresponding significant increases in MDA compared to control.

Treatment with *N. vogelii* at all doses resulted in significant increases in these antioxidant enzymes compared to 5 mg/kg CPA alone. There was significant reduction in MDA levels at 200 mg/kg NV alone compared with rats co-administered 100 mg/kg + 5 mg/kg CPA and 200 mg/kg NV + 5 mg/kg CPA. (Fig. 1A-E).

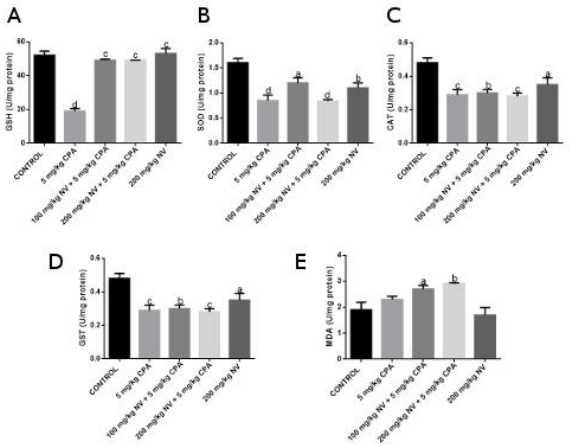


Figure 1: A) Effect of N. vogelii on plasma GSH level in cyclophosphamide-treated rats; B) Effect of N. vogelii on SOD level in cyclophosphamide-treated rats;C) Effect of N. vogelii on CAT level in cyclophosphamide-treated rats; D) Effect of N. vogelii on GST level in cyclophosphamide-treated rats; E: Effect of N. vogelii on MDA level in cyclophosphamide-treated rats. Results are mean ± SEM. a $p < 0.05$, b $p < 0.01$ vs 200 mg/kg NV. One Way ANOVA followed by Tukey's post hoc multiple comparison tests

Histology

Histological photomicrographs showed that the liver presented with steatosis around the portal tracts pointing to cytotoxic injury on co-administration of 200 mg/kg *N. vogelii* and 5 mg/kg CPA with mild portal tract inflammation at 200 mg/kg NV alone. <Fig. 2A-D>

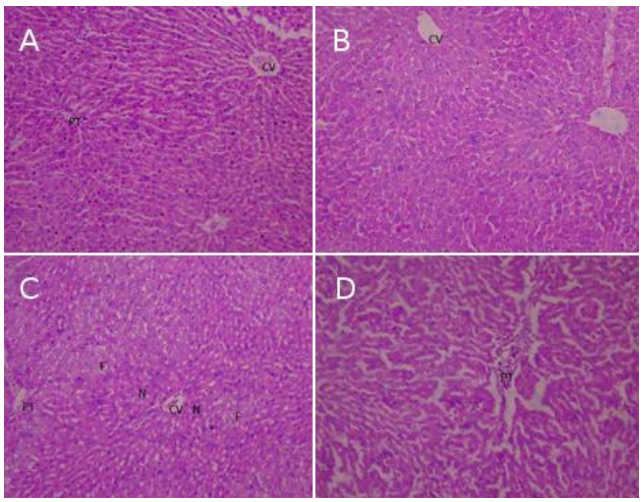


Figure 2: Photomicrographs of the liver extract and CPA treated rats.A – CONTROL: Normal appearing hepatocytes are radiating from the central veins (CV) to the portal tracts (PT). No abnormalities were seen; B - 100 mg/kg NV + 5 mg/kg CPA: Normal appearing hepatocytes radiating from the central veins (CV) to the portal tracts. No abnormalities were seen; C - 200 mg/kg NV + 5 mg/kg CPA: Abnormal appearing fat containing hepatocytes (F: fatty change, steatosis) disposed around the portal tracts pointing to cytotoxic injury. D - 200 mg/kg NV: Normal appearing hepatocytes radiating from the central veins (CV) to the portal tracts with mild portal tract (PT) inflammation

The kidney and heart showed no abnormalities (Fig not shown).

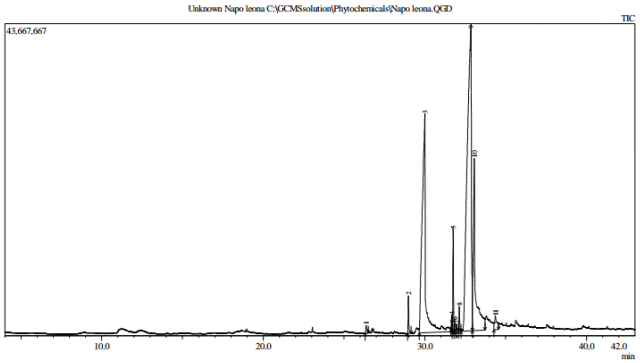
Compounds identified in stem bark extract of N. vogelii by GC-MS

GC-MS chromatogram of methanol stem bark extract of *N. vogelii* along with their retention time (RT) and peak area is shown in (Fig. 3 and Table 3).

Table 3: Compounds identified in stem bark extract of *N. vogelii* by GC-MS

No	Retention time	Name of compound	Peak area %
1	26.37	Tridecanoic acid	0.38
2	28.98	Pentadecanoic acid	0.97
3	30.00	n-Hexadecanoic acid	29.81
4	31.66	9,12-Octadecadienoic acid	0.23
5	31.75	9-Octadecenoic acid	2.64
6	31.85	7-Hexadecenoic acid	0.41
7	31.97	Phytol	0.34
8	32.14	Methyl stearate	0.67
9	32.85	Oleic acid	48.57
10	33.06	Octadecanoic acid	14.66
11	34.38	Cis-10-Nonadecenoic acid	1.33

Eleven compounds were identified and are as follows; the diterpene alcohol; phytol (0.34%) and unsaturated fatty acids; 9 – Octadecenoic (2.64%), 9, 12 – Octadecadienoic (0.23%), Tridecanoic (0.38%), Pentadecanoic (0.97%), n- Hexadecanoic (29.81%), 7- Hexadecenoic (0.41%), Oleic (48.57%), Octadecanoic (14.66%) and cis–10–Nonadecenoic acids (1.33%) including methyl stearate (0.67%).



Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	26.367	5914756	0.38	1112850	0.89	5.31	Tridecanoic acid
2	28.981	14943118	0.97	5292570	4.22	2.82	Pentadecanoic acid, methyl ester
3	30.003	461141135	29.81	30375720	24.25	14.92	n-Hexadecanoic acid
4	31.661	3560040	0.23	1469844	1.17	2.42	9,12-Octadecadienoic acid (Z,Z)-
5	31.754	40771474	2.64	14463535	11.55	2.82	9-Octadecenoic acid (Z)-, methyl ester
6	31.845	6293412	0.41	1476062	1.18	4.26	7-Hexadecenoic acid, methyl ester, (Z)-
7	31.969	5289998	0.34	1045123	0.83	5.06	Phytol
8	32.135	10350743	0.67	3468350	2.77	2.98	Methyl stearate
9	32.845	751293709	48.57	41722976	33.31	18.01	Oleic Acid
10	33.064	226840386	14.66	22937622	18.31	9.89	Octadecanoic acid
11	34.376	20504445	1.33	1904508	1.52	10.77	cis-10-Nonadecenoic acid
		1546903216	100.00	125269160	100.00		

Figure 3: GC-MS chromatogram of the methanol stem bark extract of N. vogelii

Discussion

Chemoprotective agents in everyday life have been documented as effective in preventing the increase of cancer frequency in human populations [39]. Experimental evidence suggests that free radicals and reactive oxygen species can be involved in a high number of diseases, including diabetes, hypertension, cancers, stroke [40], and many dietary antioxidants have been shown to be potentially beneficial agents by reducing oxidative stress involved in the development of different chronic diseases, including cancer [41].

The present study was conducted to evaluate the in-vivo antioxidant activity of methanol stem bark extract of *N. vogelii* in cyclophosphamide (CPA) induced genotoxicity in rats. There was no mortality recorded on oral administration of *N. vogelii* up to 4 g/kg in mice. This showed that the oral LD50 is greater than 4 g /kg and the extract is safe on acute exposure.

For the antioxidant enzymes assay, administration of the extract to CPA-treated rats significantly altered the level of blood antioxidant enzymes. Treatment with 5 mg/kg CPA significantly decreased the levels of reduced glutathione, catalase, and superoxide dismutase with a corresponding increase in the level of malondialdehyde compared to the control. Several studies indicate that CPA has a pro-oxidant character and generation of oxidative stress after administration leads to decrease in the activities of antioxidant enzymes and increase in lipid peroxidation in liver, lungs and serum of treated animals [42-44]. Treatment with the methanol stem bark extract of *N. vogelii* at different doses significantly increased the levels of these antioxidant enzymes and correspondingly decreased the level of MDA compared to animals treated 5 mg/kg CPA only. The antioxidant activities of putative antioxidants have been attributed to various mechanisms, such as the prevention of chain initiation, transition metal ion catalyst binding, peroxides decomposition, prevention of continued proton abstraction, and radical scavenging [45].

The increased level of these antioxidant enzymes demonstrates the free radical scavenging activity of the extract and thus could be beneficial in conditions requiring CPA therapy [46]. Phytochemical screening showed that the extract contains some pharmacologically active substances such as flavonoids, tannins, saponins, phenolic compounds, phlorotannin and cardiac glycoside. Phenolic acids and flavonoids have been the object of a great number of studies for their anti-oxidative activity which is mainly because of their capacity to act as free radical scavengers and/or metal chelators [47, 48]. Both compounds have attracted considerable interest in the past few years due to their many potential health benefits. As polyphenols, phenolic acids and flavonoids are powerful antioxidants and have been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasodilatory actions [49, 50]. Saponin also decreases blood lipids, lower cancer risks as well as possess antioxidant activity [51].

A positive result from the in vitro micronucleus test indicates that the test substance induces chromosome damage or damage to the cell division apparatus [52, 53]. In this study, cyclophosphamide resulted in significant increase in micronucleated polychromatic erythrocytes. Curtis et al. [14] have shown that micronucleus formation may be due to free radical generation from an agent leading to lipid peroxidation of membrane causing the breakages of the deoxyribonucleic acid (DNA) and covalent binding between the product of lipid peroxidation and DNA. Treatment with the extract significantly reduced the percentage of micronucleated polychromatic erythrocytes which can be correlated with the significantly increased level of GSH, CAT and SOD in CPA-treated rats with the highest antioxidant and hence anti-genotoxic effect observed in animals co-administered 200 mg/kg of the extract and 5 mg/kg CPA. This suggests that the extract can combat the genotoxic effect of CPA by enhancing the antioxidant system. The presence of phytoconstituents like flavonoids, tannins and saponins in the extract may be responsible for the significant antioxidant effects which may be the mechanism of antigenotoxicity elicited by the extract. Anti-genotoxic activity may be ascribed to flavonoids [54] and tannins [55, 56].

GCMS analysis of the extract showed the presence of the diterpene alcohol; phytol and unsaturated fatty acids such as; 9 – Octadecenoic, 9, 12 – Octadecadienoic, Tridecanoic, Pentadecanoic, n-Hexadecanoic, 7- Hexadecenoic, Oleic, Octadecanoic and cis – 10 – Nonadecenoic acids including methyl stearate. Presence of these metabolites in the extract may contribute to its antigenotoxic activity due to the cytotoxic nature of phytol and the fatty acids present. Lee et al. [57] had reported the antigenotoxic and anticancer activities of phytol. 9, 12-octadecadienoic acid has been documented to possess cancer preventive effects [58] possibly via an antigenotoxic mechanism as depicted by this study. n-Hexadecanoic acid has antioxidant [59, 60] and anti-tumour activity against human leukemic cells and murine cells [61, 62] and octadecanoic acid had been documented to possess significant cytotoxicity [63]. Parthipan et al. [64] have described the antitumor effects of oleic acid also possibly mediated via an antigenotoxic mechanism according to our study.

Histological presentation of the kidney, liver and heart tissues showed normal architecture in control and treated rats. There was fatty infiltration of hepatocytes; fats being disposed around the portal tracts which portend cytotoxic injury in animals co-administered 200 mg/kg *N. vogelii* and 5 mg/kg CPA. This may be as a result of the toxic effect of CPA as certain drugs and toxins commonly show forms of steatosis [65].

In conclusion, the methanol stem bark extract of *N. vogelii* possesses antigenotoxic and antioxidant activity which may be associated with the presence of flavonoids, phytol, oleic acid and other unsaturated fatty acids.

References

1. Sies H. Oxidative stress. Academic Press: San Diego, 1985; 1-8.

2. Chandra K, Salman AS, Mohd A, Sweety R, Ali KN. Protection against FCA induced oxidative stress-induced DNA damage as a model of arthritis and in vitro anti-arthritic potential of Costus speciosus rhizome extract. IJPPR. 2015; 7 (2): 383–389.

3. Halliwell B. Free radicals in biology and medicine. 4th ed. - Oxford: Oxford University Press, 2007:851.

4. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007; 39 (1): 44 - 84. https://doi.org/10.1016/j.biocel.2006.07.001 PMid:16978905

5. Singh, N., Dhalla, A.K., Seneviratne, C, Singal PK. Mol Cell Biochem. 1995; 147:77. https://doi.org/10.1007/BF00944786 PMid:7494558

6. Ramond A, Godin-Ribuot D, Ribuot C, Totoson P, Koritchneva I, Cachot S, Levy P, Joyeux-Faure M. Oxidative stress mediates cardiac infarction aggravation induced by intermittent hypoxia. Fundam Clin Pharmacol. 2011; 27 (3): 252–261. https://doi.org/10.1111/j.1472-8206.2011.01015.x PMid:22145601

7. Dean OM, van den Buuse M, Berk M, Copolov DL, Mavros C, Bush AI. N-acetyl cysteine restores brain glutathione loss in combined 2-cyclohexene-1-one and D-amphetamine-treated rats: relevance to schizophrenia and bipolar disorder. Neurosci Lett. 2011; 499 (3): 149–53. https://doi.org/10.1016/j.neulet.2011.05.027 PMid:21621586

8. de Diego-Otero Y, Romero-Zerbo Y, El Bekay R, Decara J, Sanchez L, Rodriguez-de Fonseca F, del Arco-Herrera I. Alpha-tocopherol protects against oxidative stress in the fragile X knockout mouse: an experimental therapeutic approach for the Fmr1 deficiency. Neuropsychopharmacology. 2009; 34 (4): 1011–26. https://doi.org/10.1038/npp.2008.152 PMid:18843266

9. Česen M, Eleršek T, Novak M, Žegura B, Kosjek T, Filipič M, Heath E. Ecotoxicity and genotoxicity of cyclophosphamide, ifosfamide, their metabolites/transformation products and their mixtures. Environmental Pollution. 2016; 210: 192 – 201. https://doi.org/10.1016/j.envpol.2015.12.017 PMid:26735164

10. Senthilkumar S, Yogeeta SK, Subashini R, Devaki T. Attenuation of cyclophosphamide induced toxicity by squalene in experimental rats. Chem Biol Interact. 2006; 160: 252 - 260. https://doi.org/10.1016/j.cbi.2006.02.004 PMid:16554041

11. Oboh, G., Rocha JBT. Polyphenols in red pepper [Capsicum annuum var. aviculare (Tepin)] and their protective effect on some Pro-oxidants induced lipid peroxidation in Brain and Liver. Eur Food Res Technol. 2007; 225:239-247. https://doi.org/10.1007/s00217-006-0410-1

12. Shah SU Importance of Genotoxicity & S2A guidelines for genotoxicity testing for pharmaceuticals. IOSR Journal of Pharmacy and Biological Sciences. 2012; 1(2): 43-54. https://doi.org/10.9790/3008-0124354

13. International Conference on Harmonization Guidelines, Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals, Step 4, 1999.

14. Curtis JF, Hughes MF, Mason RP, Eling TE. Peroxidase catalase oxidation of (bi) sulphite: reaction of free radical metabolites of (bi) sulfite with 7, 8-dihydroxy-7, 8- dihydrobenzo (α)-pyrene. Carcinogenesis. 1988; 9:2015-2021. https://doi.org/10.1093/carcin/9.11.2015 PMid:3141075

15. WHO media centre, 2003. http://www.who.int/mediacentre/factsheets/2003/fs134/en/

16. Chaabane F, Boubaker J, Loussaif A, Neffati A, Kilani-Jaziri S, Ghedira K, Chekir-Ghedira L. Antioxidant, genotoxic and antigenotoxic activities of daphne gnidium leaf extracts. BMC Complementary and Alternative Medicine. 2012; 12:153. https://doi.org/10.1186/1472-6882-12-153 PMid:22974481 PMCid:PMC3462690

17. Keay RWJ, Onochie CFA, Standfield DP. Nigerian trees. Department of Forest Research. 1964; 1:139-140.

18. Enye JC, Chineka HN, Onubeze DPM, Nweke I. Wound Healing Effect of Methanol Leaf Extract of Napoleona vogelii (Lecythidaceae). Journal of Dental and Medical Sciences. 2013; 8 (6):31-35. https://doi.org/10.9790/0853-0863134

19. Akah PA, Nnaeto O, Nworu S, Ezike AC. Medicinal Plants Used in the Traditional Treatment of Peptic Ulcer Diseases: A Case Study of Napoleona vogelii. Hook & Planch Lecythidaceae. Respiratory Journal of Pharmacology. 2007; 1: 67-74.

20. Jhansi Rani M. Mohana lakshmi S, Saravana Kumar A. Review on herbal drugs for anti-ulcer property. International Journal of Biological & Pharmaceutical Research. 2010;1(1).

21. Muganzaa DM, Fruth BI, Lamia JN, Mesiaa GK, Kambua OK, Tonaa GL, Kanyangaa RC, Cosc P, Maesc L, Apersd S, Pieters L. In vitro antiprotozoal and cytotoxic activity of 33 ethonopharmacologically selected medicinal plants from Democratic Republic of Congo. Journal of Ethnopharmacology. 2012; 141:301–308. https://doi.org/10.1016/j.jep.2012.02.035 PMid:22394563

22. Odugbemi T. Outlines and pictures of medicinal plants from Nigeria. University of Lagos Press, 2008; 138.

23. Iwu M. Hand book of African medicinal plants CRC Press, 40. 1993.

24. Soladoye MO, Amusa NA, Raji-Esan SO, Chukwuma EC, Taiwo AA. Ethnobotanical Survey of Anti-Cancer Plants in Ogun State, Nigeria. Annals of Biological Research. 2010; 1(4):261–273.

25. Trease GE, Evans WC. Pharmacognosy. 13th Ed. Bailliere Tindall Books Publishers. By Cas Sell and Collines Macmillan Publishers, Ltd. London, 1989: pp 1-8.

26. Chang C, Yang M, Wen H. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis. 2002; 10:178−182.

27. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. Journal of Agriculture and Food Chemistry. 2003; 51:609-614. https://doi.org/10.1021/jf020782a PMid:12537430

28. Sun JS, Tsuang YW, Chen IJ, Huang WC, Hang YS, Lu FJ. An ultraweak chemiluminescene study on oxidative stress in rabbit following acute thermal injury. Burns. 1998; 24:225-231. https://doi.org/10.1016/S0305-4179(97)00115-0

29. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta states of Nigeria. Global J. Pure Appl. Sci. 2001; 8:203-208.

30. National Institute of Health, USA: Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002.

31. Heddle JA, Salamone MF. Chromosomal aberrations and bone marrow toxicity. Environ Health Perspect. 1981; 39:23-27. https://doi.org/10.1289/ehp.813923

32. Tinwell H, Ashby J. Comparison of Acridine Orange and Giemsa stains in several mouse bone marrow micronucleus assays—including a triple dose study. Mutagenesis. 1989; 4(6): 476-481. https://doi.org/10.1093/mutage/4.6.476 PMid:2695763

33. Sun M, Zigma S. An improved spectrophotometer assay of superoxide dismutase based on epinephrine antioxidation. Anal Biochem. 1978; 90:81 – 9. https://doi.org/10.1016/0003-2697(78)90010-6

34. Doherty AT, Hayes J, Holme P, O'Donovan M. Chromosome aberration frequency in rat peripheral lymphocytes increases with repeated dosing with hexamethylphosphoramide or cyclophosphamide. Mutagenesis. 2012. https://doi.org/10.1093/mutage/ges016 PMid:22492203

35. Usoh IF, Akpan EJ, Etim EO, Farombi EO. Antioxidant actions of dried flower extract of Hibiscus Sabdariffa L. on sodium, arsenite-induced oxidative stress in rats. Pakistan J Nutri. 2005; 4: 135-141. https://doi.org/10.3923/pjn.2005.135.141

36. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analyt Biochem.1968; 25: 1192–1205. https://doi.org/10.1016/0003-2697(68)90092-4

37. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978; 52: 302-310. https://doi.org/10.1016/S0076-6879(78)52032-6

38. Habig WA, Pabst MJ, Jacoby WB. Glutathione transferases. The first step in mercapturic acid formation. Journal of Biological Chemistry. 1974; 249:7130-7139. PMid:4436300

39. Rodeiro I, Olguín S, Santes R, Herrera JA, Pérez CL, Mangas R, Hernández Y, Fernández G, Hernández I, Hernández-Ojeda S, Camacho-Carranza R, Valencia-Olvera A, Espinosa-Aguirre JJ. Gas Chromatography-Mass Spectrometry Analysis of Ulva fasciata (Green Seaweed) Extract and Evaluation of Its Cytoprotective and Antigenotoxic Effects. Evid Based Complement Alternat Med. 2015; 2015:520598. https://doi.org/10.1155/2015/520598 PMid:26612994 PMCid:PMC4647032

40. Sies H. Oxidative stress: Oxidants and antioxidants. Experimental Physiology. 1997; 82(2):291-295. https://doi.org/10.1113/expphysiol.1997.sp004024 PMid:9129943

41. Khan N, Mukhtar H. Dietary agents for prevention and treatment of lung cancer. Cancer Letters. 2015; 359(2):155–164. https://doi.org/10.1016/j.canlet.2015.01.038 PMid:25644088 PMCid:PMC4409137

42. Patel JM, Block ER. Cyclophosphamide-induced depression of the antioxidant defense mechanisms of the lungs. Exp Lung Res. 1985; 8:153-165. https://doi.org/10.3109/01902148509057519

43. Barja de Quiroga G, Perez-Campo R, Lopez-Torres M. Antioxidant defences and peroxidation in liver and brain of aged rats. Biochem J. 1990; 272:247-250. https://doi.org/10.1042/bj2720247 PMid:2176082 PMCid:PMC1149684

44. Perez R, Lopez M, Barja de Quiroga G. (1991). Aging and lung antioxidant enzymes, glutathione and lipid peroxidation in rats. Free Rad Biol Med. 1991; 10:35-39. https://doi.org/10.1016/0891-5849(91)90019-Y

45. Diplock AT. Will the good fairies please prove us that vitamin E lessens human degenerative disease? Free Radic Res. 1997; 27:511-532. https://doi.org/10.3109/10715769709065791 PMid:9518068

46. Ikumawoyi VO, Awodele O, Rotimi K, Fashina AY. Evaluation of the effects of the hydro-ethanolic root extract of Zanthoxylum zanthoxyloides on hematological parameters and oxidative stress in cyclophosphamide treated rats. Afr J Tradit Complement Altern Med. 2016; 13(5):153-159.

47. Anderson KJ, Teuber SS, Gobeille A, Cremin P, Waterhouse AL, Steinberg FM. Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. Journal of Nutrition. 2001; 131: 2837-2842. PMid:11694605

48. Hameed ES. Total phenolic contents and free radical scavenging activity of certain Egyptian Ficus species leaf samples. Food Chemistry. 2009; 114:127.

49. Mattila P, Hellstrom J. Phenolic acids in potatoes, vegetables, and some of their products. Journal of Food Composition Analysis. 2007; 20:152-160. https://doi.org/10.1016/j.jfca.2006.05.007

50. Galeotti F, Barile E, Curir P, Dolci M, Lanzotti V. Flavonoids from carnation (Dianthuscaryophyllus) and their antifungal activity. Phytochemical Letters. 2008; 1:44-48. https://doi.org/10.1016/j.phytol.2007.10.001

51. Eze SO, Amajoh CV. Phytochemicals, vitamins,macro and micro elements and antimicrobial analysis of the stem bark of napoleona vogelii (akpaesu). 2015; 11 (9): 3930-3939.

52. Kirsch-Volders M, Elhajouji A, Cundari E, Van Hummelen P.

The in vitro micronucleus test: a multi-endpoint assay to detect simultaneously mitotic delay, apoptosis, chromosomal breakage, chromosome loss and non-disjunction. Mutation Research, 1997; 392: 19–30. https://doi.org/10.1016/S0165-1218(97)00042-6

53. Fenech M. The in vitro micronucleus technique. Mutat. Res. 2000; 455 81–95. https://doi.org/10.1016/S0027-5107(00)00065-8

54. Calomme M, Pieters L, Vlietink A, Berghe DV. Inhibition of bacterial mutagenesis flavonoids. Planta Med. 1996; 92:222–226. https://doi.org/10.1055/s-2006-957864 PMid:8693033

55. Lee KT, Sohn IC, Park HJ, Kim DW, Jung GO, Park KY. Essential moiety of antimutagenic and cytotoxic activity of hederagenin monodesmosides and bidesmosides isolated from the stem bark of Kalapanox pictus. Planta Med. 2000;66:329–332. https://doi.org/10.1055/s-2000-8539 PMid:10865448

56. Baratto MC, Tattini M, Galardi C, Pinelli P, Romani A, Visioli F, Basosi R, Pongi R. Antioxidant activity of galloyl quinic derivatives isolated from P.lentiscus leaves. Free Radic Res. 2003; 37:405–412. https://doi.org/10.1080/1071576031000068618 PMid:12747734

57. Lee KL, Lee SH, Park KY. Anticancer activity of phytol and eicosatrienoic acid identified from Perilla leaves. Journal of the Korean Society of Food Science and Nutrition. 1999; 28:1107–1112.

58. Mangunwidjaja DS, Kardono SR, Iswantini LBSD, Gas chromatography and Gas Chromatography-Mass Spectrometry analysis of Indonesian Croton tiglium seeds. J.Applied Sci. 2006; 6:1576-1580. https://doi.org/10.3923/jas.2006.1576.1580

59. Jegadeeswari P, Nishanthini A, Muthukumaraswamy S, Mohan VR. GC-MS analysis of bioactive components of Aristolochia krysagathra (Aristolochiaceae) J Curr Chem Pharm Sci. 2012; 2:226-236.

60. Upgade A, Anusha B. Characterization and medicinal importance of phytoconstiuents of Carica papaya from down south Indian region using gas chromatography and mass spectroscopy Asian J Pharm Clinical Res. 2013; 6(4):101-106.

61. Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J, Kamei Y: Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. Anticancer Res. 2002; 22:2587-2590. PMid:12529968

62. Semary NAE, Ghazy SM, Naby MMAE: Investigating the taxonomy and bioactivity of an Egyptian Chlorococcum isolate. Aust J Basic Appl Sci. 2009;3:1540−1551.

63. Keawsa-ard S, Liawruangrath B, Liawruangrath S, Teerawutgulrag A, Pyne SG: Chemical constituents and antioxidant and biological activities of the essential oil from leaves of Solanum spirale. Nat Prod Commun. 2012;7:955-958. PMid:22908592

64. Parthipan B, Suky MGT, Mohan VR. GC-MS Analysis of Phytocomponents in Pleiospermium alatum (Wall. ex Wight & Arn) Swingle, (Rutaceae). Journal of Pharmacognosy and Phytochemistry. 2015; 4(1): 216-222.

65. Brunt EM. Pathology of fatty liver disease. Modern Pathology. 2007; 20:S40 – S48. https://doi.org/10.1038/modpathol.3800680

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):875-879.
https://doi.org/10.3889/oamjms.2017.206
eISSN: 1857-9655
Basic Science

Hepatitis B virus Genotypes in West Azarbayjan Province, Northwest Iran

Mohammad Hasan Khadem Ansari[1*], Yousef Rasmi[1,2], Laya Abbasi[3]

[1]Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran; [2]Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran; [3]Department of Microbiology, Azad Islamic University-Urmia Branch, Urmia, Iran

Citation: Khadem Ansari MH, Rasmi Y, Abbasi L. Hepatitis B virus Genotypes in West Azarbayjan Province, Northwest Iran. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):875-879. https://doi.org/10.3889/oamjms.2017.206

Keywords: Hepatitis B Genotype; HBsAg; Nested PCR; Sequencing.

***Correspondence:** Mohammad Hasan Khadem Ansari, Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran. E-mail: mhansari1@gmail.com

Received: 10-Apr-2017; **Revised:** 02-Oct-2017; **Accepted:** 03-Oct-2017; **Online first:** 11-Dec-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Infections caused by Hepatitis B are one of the world health's most serious problems. According to assessments, nearly 500,000 to 1.2 million people die each year due to chronic hepatitis, cirrhosis of the liver and hepatocellular carcinoma. Hepatitis B is one of the diseases which can be transferred through blood and its products. Clinical importance of genotypes of hepatitis B virus and their relations with mutations are well known.

AIM: Since epidemiological data resulting from determining genotypes and sub-genotypes of hepatitis B can help a lot in defining a vaccination plan, antiretroviral therapy, detection and prevention of diseases, genotypes of this virus in hepatitis B patients were evaluated in West Azarbaijan province.

MATERIALS AND METHODS: In this cross-sectional study, serum samples of 100 hepatitis B patients (70 male/30 female) were taken randomly from Urmia University of Medical Sciences (UMSU) referrals, Urmia, Iran; and were tested positive for the presence of surface antigens of hepatitis B virus (HBsAg) using ELISA method. In the first method, after extracting the DNA of the virus, sequencing of S genes was carried out using Sanger method, and the sequences were aligned and edited using Bioedit software. In the next step, phylogenic analysis of the sequences was done in comparison with the reference sequences which were extracted from a gene bank, utilising Neighbour-joining assay method with CLUSTRAL W software. To ensure genotyping accuracy, the samples were tested once more, using Nested PCR method.

RESULTS: The results were consistent with the sequence method and the dominant genotype in patients suffering hepatitis was type D. In other words, Iranian's HBV genotypic types are homogeneous and in close coordination with each other.

CONCLUSIONS: The results reveal that D genotype is the main genotype of HBV in West Azarbayjan province, northwest Iran. Presence of this genotype was in conformity conformed withto the low rate of acute liver diseases caused by hepatitis B chronic infection, cirrhosis of the liver and hepatocellular carcinoma.

Introduction

Hepatitis B is one of the global health problems. According to the statistics of world World health Health organizationOrganization, it's it is the third most prevailing infectious and contagious disease after Tuberculosis and Malaria and is the fifth cause of death from infectious disease in the world. There are 2 billion people infected with hepatitis B and more than 350 million people (5% of total population of the world) are reported to be chronic carriers of hepatitis B [1].

There are four million new clinical hepatitis B patients each year and one million of hepatitis B carriers die from active chronic hepatitis and cirrhosis of liver annually. Hepatitis B virus (HBV) is one the causes of acute and chronic hepatitis and liver cancer in Asia and especially in China, Taiwan, Africa and South America [2]. Hepatitis B genome is a double-stranded DNA which is not completely circular and has has 3200 base pairs. Hepatitis B genome have

four open reading frames called S, C, P and X. Using advanced evolutionary analysis and based on HBV similarity of similarity HBV between full genome of virus, 8 genotypes are identified which are based on more than 8% complete A-H genome sequence variation [3].

DNA of the virus consists of 4 groups of genes: *S gene* (surface shield of antigen or s protein codes HBsAg), *C gene* (Hepatitis B Nucleocapsid proteins, viral capsid or nuclear antigen or HbcAg and codes e antigen), *P gene* (DNA polymerase) and *X gene* (which codes transactivating protein) which increases hepatitis B virus duplication. *P gene* covers almost 180 percent of genome of virus, fully covers *S gene* and a part of it overlaps with *C* and *X genes*. In addition, pre-core and core promotore shares are a significant area of genome sequence with *X gene* [3, 4].

Studies show that clinical outbreak, prognosis and response to treatment of the disease depends on genotype of virus. Hepatitis B genotype has a certain type of geographical distribution. In North America, Northern Europe, India and Africa, A genotype, in Asia, B and C genotypes, in Middle East, Mediterranean area and India, D genotype, in west and south Africa, E genotype, in Southern and central America, F genotype and in America and Europe, G and H genotype are dominant [5-8].

All genotypes of HBV will cause liver disease, but their clinical signs are different. C genotype is prevalent in cirrhotic patients. A genotype often lead to chronicity of illness and D genotype is typically observed in patients who are intravenous drug users. But some researches demonstrate that B genotype was more frequently observed in Taiwan youngsters who didn't have cirrhosis of liver rather than D genotype. D genotype was also less connected to acute liver diseases and cancer compared to A genotype [5, 9].

Studies conducted on a limited number of patients show that chronicity ratio in individuals with A and D genotypes is higher than B and C genotypes. The ratio of development of persistent HBV infection after severing hepatitis B infection is higher in A patients rather than B and C genotype patients. They also reported a higher chronicity after HBV infection in D genotype patients [10].

The only host to HBV is a human being, and chronic carriers are the only source of virus in nature. It is estimated that more than 35% of Iranians had contact with HBV and 3% are chronic carriers which 1.7% of them are in Fars province and more than 5% are in Sistan-Va-Balouchestan province. In a study carried out on 250,000 of healthy volunteers of blood donation, 3.6% of men and 1.6% of women were carriers of HBsAg [11, 12].

Anti-HBV antibody was identified in 37% of this population. Thus, it seems that 8% of Iranians which are suffering hepatitis B will be chronic carriers. Studies conducted in the last decade demonstrate that in patients with cirrhosis of the liver, 70% to 84% have evidence of contacting HBV and 51% to 56% are vectors. Also, in Iranian patients with hepatocellular carcinoma, HBsAg was positive in 72% of virus encounters, and carriage rate is reported to be 46% [2, 11, 12].

Since epidemiologic data resulting from determining genotypes and sub-genotypes of hepatitis B virus help a lot in defining a vaccination program, antiretroviral therapy, diagnosing and prevention of the disease, this research was conducted to study hepatitis B virus genotypes in West Azarbaijan, northwest Iran; province which was not studied previously.

Material and Methods

Cross sectional study were carried out on 100 patients (70 male/30 female) in 2014-2015 year. hepatitis B patients were chosen randomly from UMSU hospitals. Five milliliters of blood were collected from each patient. After 15 minutes incubation at room temperature samples were centrifuged at 1000 g. Serum were separated and kept at -40 degree centigrade until the experiments were preformed. Sample wereSamples were assayed for presence of HBsAg by using the ELISA method (PishTaz Teb, Tehran, Iran).

In order to determine hepatitis B genotypeing, positive 100 serum samples which are positive in the ELISA method, DNA purification carried out by using Nucleic acid extract kit(QIA amp DNA miniKit, Germany) which was based on affinity chromatography utilizing silica columns, and DNA extracted samples divided in two parts.

HBV genotyping by sequencing

The first part of the extracted DNA from hepatitis B virus was sent to South Korea with the aid of Iran Pasteur Institute. Sequencing of *S genes* were carried out using Sanger method, and the sequences were aligned and edited using Bioedit software. In the next step, phylogenic analysis of the sequences were done in comparison with the reference sequences which were extracted from a gene bank, utilizing Neighbor joining assay method with CLUSTRAL W software.

NESTED PCR method

Also nested Nested PCR method was carried

out by hepatitis B virus kit (Genekam Biotechnology AG, Germany) by second part of extracted DNA, extracted materials as described before.

Primers were used a are shown in table 1, [13]. Electrophoresis were carried out by PCR products in 60 volts in TAE buffer (2 M Tris-Acetate, 0.05 M EDTA-K2) and 2% agarose gel containing syber Syber green dye. DNA bands in compare with molecular size marker with maximum 500 bp in visible zero UV rays to the gel.

Table 1: Designed primers for identification of hepatitis B genes using nested PCR method

Primer	Sequence a (position, specificity, and polarity)
first PCR	
P1b	5'-TCA CCA TAT TCT TGG GAA CAA GA-3' (nt 2823-2845,
S1-2	universal, sense)
	5'-CGA ACC ACT GAA CAA ATG GC-3' (nt 685-704, universal, antisense)
2nd PCR	
Mix A	
B2	5'-GGC TCM AGT TCM GGA ACA GT-3' (nt 67-86 types A to E
BA1R	specific, sense)
BC1R	5'-CTC GCG GAG ATT GAC GAG ATG T-3' (nt 113-134, type A specific, antisense)
	5'-CAG CCT AGG AAT CCT GAT GTT G-3' (nt 165-186, type C specific, antisense)
Mix B	
BD1	5'-GCC AAC AAG GTA GGA GCT-3' (not 2979-2996, type D
BE1	specific, sense)
BF1	5'-CAC CAG AAA TCC AGA TTG GGA CCA –3' (nt 2955-2978,
B2R	type E specific, sense)
	5'-GYT ACG GTC CAG GGT TAC CA-3' (nt 3032-3051, type F specific, sense)
	5'-GGA GGC GGA TYT GCT GGC AA-3' (nt 3078-3097, types D to F specific, antisense)

Results

After genotyping HBV with S gene sequence utilizing Sanger sequencing method, all samples shows hepatitis B virus D genotypes.

Also carrying out Nested PCR by nested method genotyping and compare with DNA molecular size with maximum of 500 bp in agarose gel all samples were revealed in 119 bp witch which is the sign of type D genotype.

There are no other genotypes were observed in our experiments, figure Figure 1.

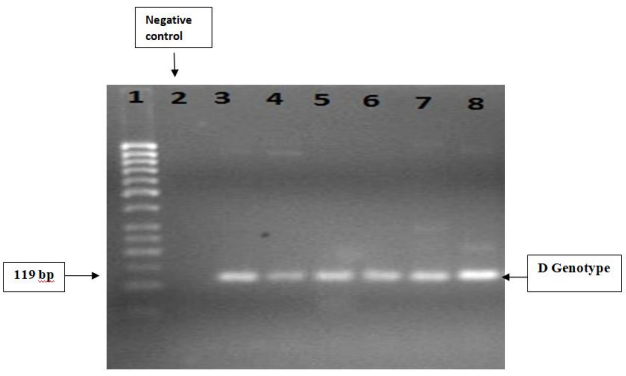


Figure 1: According to the marker, all bands belong to 119 bp and identified D genotype which for type A in 68 bp, for type B in 281 bp, for type C in 122 bp, for type E in 167 bp and for type F in 97 bp no bands were visible

Discussion

Hepatitis B chronic infection is a widespread disease which infected more than 5% of the world's population and is one of the biggest health problems of Iran and the world. The prevalence of hepatitis B is endemic in the world. 90% of the world's population are living in areas with high prevalence of 8% of positive HBsAg or with medium prevalence of 82-28% of HBsAg [11]. Iran is located in the middle eastMiddle East and according to Center for Disease Control, havehas medium prevalence of the infection. Prevalence of positive HBsAg in 1357 1982 in Iran was reported to be 2.5%-7.2% [12, 14]. Even though hepatitis B has low prevalence in Bahrain and Kuwait, medium in Iran, USA and United Arab Emirates and high in Oman, Palestine, Yemen and Sweden, positive HBsAg prevalence in ordinary people was reportedly 1.7% and in blood donors was reported to be 0.5% [12, 15, 16].

This study was first carried out in West Azarbayjan province, north west Iran; in order to determine hepatitis B genotypes among hepatitis B patients utilizing sequencing method and Nested PCR method on polymerase gene of hepatitis B virus. In this method, blood samples of one hundred patients which were randomly chosen and tested positive for the presence of surface antigens of Hepatitis hepatitis B virus (HBsAg) using ELISA method and HBV with PCR method, were taken and the experiment were carried out on these samples.

Studies show that there is a meaningful relation between HBV genotypes with illness severity, Cirrhosis cirrhosis of the liver and Hepatocellular hepatocellular carcinoma and response to treatment [17]. Recent data suggests that patients with C genotype would probably have a greater chance of being subject to severe liver diseases while those who have B genotypes have a greater chance of hepatocellular carcinoma [18, 19]. According to the latest studies, 8%-20% of individuals with hepatitis B are its carriers and prevalence of hepatitis B in Middle East and Iran is reported to be 2%-7% [12, 20]

One of the discussions made in hepatitis B researches, is the geographical distribution of this virus which has different patterns in different regions of the world. D genotype is dominant in Middle East and Mediterranean countries which are confirmed through multiple studies [21].

In a research carried out by Eftekhari et al., in 2010 on positive HbsAg patients, GAP-PCR method was utilized in order to determine the genotype. Results showed that in 60% of the individuals, HBc-Ab antibody was tested positive and the dominant genotype in these patients was type D [22].

In a similar study by Goodarzi et al. in 2007 [23] which was conducted to determine the genotype

of HBV in Iran, *S* and *C genes* of the virus were studied using sequencing and phylogenic analysis method in preS area. The results of their investigation demonstrated that in HCC patients all extracted genotypes were typed D. Albeit in positive HBsAg patients, utilising reverse hybridisation method demonstrated that in 86% of chronic hepatitis patients, 10% of Cirrhosis patients and 2.7% of carriers, the only identified genotype is type D [24].

In researches done in different areas of Iran, the dominant genotype is D genotype, but some studies reported B and C genotypes [11]. The research was conducted by Karimi et al. in 2013 [25] on serums of 116 patients with the positive surface antigen of HBV. The results showed that 19.8% were positive concerning hepatitis B nucleic acid and the dominant genotype in these individuals was D genotype in 73.9% of the cases. 26.1% of patients were found to have C genotype.

In a similar study in India, A and D genotypes were dominant. According to this study, D, A and C genotypes were observed in 57.3%, 18% and 11.5% of patients with chronic HBV infection respectively, respectively. D genotype was more prevalent in chronic liver patients in New Delhi [24-26]. In north east areas genotype C is dominant in high risk individuals and is more connected with liver disease progression [25-27]. Other researches of the same kind performed in middle easternMiddle Eastern countries like Saudi Arabia, lead to similar results which shows D genotype in more than 80% of hepatitis B (HBV) patients [2628].

In other areas such as Mediterranean area, Middle East and South Eastern Asia, D genotype is prevalent and similar results in Turkey demonstrated that all 44 patients which were tested, had D genotypes [2729].

In this study, D genotype was found to be dominant in infected patients. In other words, HBV genotypic types are in close connection with each other and are homogeneous and there is no connection between the genotype of virus and the investigated population (epidemiologically). This fully complies with the results previously obtained in Iran, Middle East, Afghanistan, Central Asia, Turkey, Egypt, New Delhi and British Colombia [2830].

Despite all the investigations carried out in Iran, data on HBV genotypes are very limited because of the fact that in some cases, differences in genotypes can be found within a country and thus the necessity of regional studies in order to determine HBV genotypes in West Azarbayjan province is justified [2931].

The results of this study demonstrate that the dominant genotype in West Azarbayjan is similar to other regions of Iran. D genotype is the dominant genotype in Iran and other Middle Eastern countries. In a research conducted by Alavian et al., the only genotype found in HBV patients in Iran was type D [9]. Utilizing different methods in the studies lead to similar results. Therefore it can be conducted that the dominant genotype in Iran was type D. Analogous results despite different methods utilized, are an evidence of gene dominancy [3032].

In a study performed by Yalchin et al., liver enzymes level were investigated and were found to be normal. In this research, ALT and AST liver enzymes were measured and were found to be normal. It seems like enzyme variation doesn't have a meaningful connection with HBV virus and does not change significantly [2729] and was in conformity with the present research.

In conclusion, the results show that D genotype is the main genotype of HBV in West Azarbayjan province. This study opens the door to perform further studies such as understanding the effects of antiviral drugs on HBV infected patients. Researches on subgenotypes of HBV and its serotypes can be conducted, and the original and effective anti-viral drugs may be used to treat infected patients.

These results can be utilised in the immunological and genetic diagnosis of HBV to make hepatitis B diagnostic kits and quality control panels for evaluation of diagnostic methods of the virus. Genotyping can be done in bigger cities of West Azarbayjan and may be compared to the present results.

References

1. Kuhns MC, Busch MP. New strategies for blood donor screening for hepatitis B virus: nucleic acid testing versus immunoassay methods. Mol Diagn Ther. 2006; 10(2):77-91. https://doi.org/10.1007/BF03256447

2. Mojiri A, Behzad-Behbahani A, Saberifirozi M, Ardabili M, Beheshti M, et al. Hepatitis B virus genotypes in southwest Iran: Molecular, serological and clinical outcomes. World J Gastroenterol. 2008; 14(10): 1510-1513. https://doi.org/10.3748/wjg.14.1510 PMid:18330939 PMCid:PMC2693743

3. Chu CJ, Keeffe EB, Han SH, Perrillo RP, Min AD, et al. Hepatitis B virus genotypes in the United States: results of a nationwide study. Gastroenterology. 2003; 125(2): 444-451. https://doi.org/10.1016/S0016-5085(03)00895-3

4. Arauze-Ruiz P, Norder H, BH. R, Genotpye H. A new Amerindian genotype of hepatitis B virus revealed in Central America. Journal of General Virology. 2002; 83: 2059-2073. https://doi.org/10.1099/0022-1317-83-8-2059 PMid:12124470

5. Sanchez-Tapias JM, Costa J. Influence of hepatitis B virus genotype on the long term out come of chronic hepatitis B in western patients. Gastroenterology. 2002; 123: 1848-1856. https://doi.org/10.1053/gast.2002.37041 PMid:12454842

6. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B virus genotypes and spontaneous hepatitis Be antigen seroconversion in Taiwanese hepatitis B carriers. J Med Virol. 2004; 72: 363-369. https://doi.org/10.1002/jmv.10534 PMid:14748059

7. Kidd-Ljunggren K, Miyakawa Y, Kidd AH. Genetic variability in

hepatitis B viruses. J General Virol. 2002; 83(6): 1267-1280. https://doi.org/10.1099/0022-1317-83-6-1267 PMid:12029141

8. Stuyver L, De Gendt S, Van Geyt C, Zoulim F, Fried M, et al. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. J Gen Virol. 2000; 81 67-74. https://doi.org/10.1099/0022-1317-81-1-67 PMid:10640543

9. Alavian SM, Keyvani H, Rezai M, Ashayeri N, Sadeghi HM. Preliminary report of hepatitis B virus genotype prevalence in Iran. World J Gastroenterol. 2006; 12: 5211-5213. PMid:16937535 PMCid:PMC4088022

10. Liu CJ, Kao JH. Global perspective on the natural history of chronic hepatitis B: role of hepatitis B virus genotypes A to J. Semin Liver Dis. 2013; 33: 97-102. https://doi.org/10.1055/s-0033-1345716 PMid:23749665

11. Haghshenas MR, Arabi M, Mousavi T. Hepatitis B genotypes in iran. Mater Sociomed. 2014; 26: 129-133. https://doi.org/10.5455/msm.2014.26.129-133 PMid:24944540 PMCid:PMC4035145

12. Merat S, Malekzadeh R, Rezvan H, Khatibian M. Hepatitis B in Iran. Arch Iran Med. 2000;3(4):192-201.

13. Geramizadeh B, Kaboli R, Behzad-Behbahani A, Rahsaz M, Azarpira N, et al. A nested PCR method for the identification of hepatitis B virus genotype in paraffin blocks of formalin-fixed liver biopsies. Arch Iran Med. 2008; 11: 455-458. PMid:18588380

14. Farzadegan H, Harbour C, Ala F. The prevalence of hepatitis B surface antigen and its antibody in blood donors and high risk groups in Iran. Vox Sang. 1979; 37: 182-186. https://doi.org/10.1111/j.1423-0410.1979.tb02289.x PMid:494588

15. Aghakhani A, Hamkar R, Zamani N, Eslamifar A, Banifazl M, et al. Hepatitis B virus genotype in Iranian patients with hepatocellular carcinoma. 2009; 13(6): 685—689.

16. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat. 2004; 11: 97-107. https://doi.org/10.1046/j.1365-2893.2003.00487.x PMid:14996343

17. Kidd-Ljunggren K, Miyakawa Y, Kidd AH. Genetic variability in hepatitis B viruses. J General Virol. 2002; 83(6): 1267-1280. https://doi.org/10.1099/0022-1317-83-6-1267 PMid:12029141

18. Mumtaz K, Hamid S, Ahmed S, Moatter T, Mushtaq S, et al. A study of genotypes, mutants and nucleotide sequence of hepatitis B virus in Pakistan: HBV genotypes in pakistan. Hepat Mon. 2001; 11(1): 14-18.

19. Kao JH, Chen PJ, Lai MY, Chen DS. Occult hepatitis B virus infection and clinical outcomes of patients with chronic hepatitis C. J Clin Microbiol. 2002; 40(11): 4069-4071. https://doi.org/10.1128/JCM.40.11.4068-4071.2002 PMCid:PMC139665

20. Arababadi MK, Nasiri Ahmadabadi B, Yousefi Daredor H, Kennedy D. Epidemiology of occult hepatitis B infection among thalassemic, hemophilia, and hemodialysis patients. Hepat Mon. 2012; 12(5): 315-319. https://doi.org/10.5812/hepatmon.5934 PMid:22783342 PMCid:PMC3389356

21. Mayerat C, Mantegani A, Frei C. Does hepatitis B virus (HBV) genotype influence the clinical outcome of HBV infection? J Viral Hepat. 1999; 6: 299-304. https://doi.org/10.1046/j.1365-2893.1999.00174.x PMid:10607244

22. Eftekhari Y, Arababadi MK, Hakimi H, Zarandi ER. Common HBV Genotype in Southeastern Iranian Patients. Arch Iran Med. 2010; 13 (2): 147-149. PMid:20187670

23. Goodarzi Z, Malekzadeh R, Montazeri G, Alavian SM, Qurbanalizadgan M, Daram M, et al. Phylogenetic Analysis of HBV Based on PreS Region in Iranian Hepatocellular Carcinoma Patients. Hepatitis Monthly. 2007; 7(4): 201-215.24.

24. Alavian SM, Asari S, Manzori-Joybari H. Prevalence and risk factors of HDV infection in HBV infected cases. Govaresh. 2004; 9: 217-221.

25. Karimi A, Salimzadeh L, Bagheri N, Heidari K, Hossein SM. Genotyping of Hepatitis B Virus in HBsAg Positive Individuals Referred to the Health Centers of Shahrekord, Iran. Armaghane Danesh. 2012; 17(5): 407-415.

26. Kar P, Polipalli SK, Chattopadhyay S, Hussain Z, Malik A, et al. Prevalence of hepatitis B virus genotype D in precore mutants among chronic liver disease patients from New Delhi, India. Dig Dis Sci. 2007; 52(2): 565-569. https://doi.org/10.1007/s10620-006-9625-z PMid:17211692

27. Saha MK, Chakrabarti S, Panda S, Naik TN, Manna B, et al. Prevalence of HCV & HBV infection amongst HIV seropositive intravenous drug users & their non-injecting wives in Manipur, India. Indian J Med Res. 2000; 111: 37-39. PMid:10824464

28. Abdo AA, Al-Jarallah BM, Sanai FM, Hersi AS, Al-Swat K, et al. (Hepatitis B genotypes: relation to clinical outcome in patients with chronic hepatitis B in Saudi Arabia). World J Gastroenterol. 2006; 12(43): 7019-7024. https://doi.org/10.3748/wjg.v12.i43.7019 PMid:17109498 PMCid:PMC4087347

29. Yalcin K, Degertekin H, Bahcecioglu IH, Demir A, Aladag M, et al. Hepatitis B virus genotype D prevails in patients with persistently elevated or normal ALT levels in Turkey. Infection. 2004; 32(1): 24-29. https://doi.org/10.1007/s15010-004-3010-7 PMid:15007739

30. Vaezjalali M, Alavian SM, Jazayeri SM, Nategh R, Mahmoodi M, et al. Genotype of Hepatitis B Virus Isolates from Iranian Chronic Carriers of the Virus. Hepatitis Monthly. 2008; 8(2): 97-100.

31. Kramvis A, Restorp K, Norder H, Botha JF, Magnius LO, et al. Full genome analysis of hepatitis B virus genotype E strains from South-Western Africa and Madagascar reveals low genetic variability. J Med Virol. 2005; 47-52: 77.

32. Alavian SM, Tabatabaei SV. Meta-analysis of factors associated with sustained viral response in patients on hemodialysis treated with standard or pegylated interferon for hepatitis C infection. Iran J Kidney Dis. 2010; 4: 181-194. PMid:20622305

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):880-892.
https://doi.org/10.3889/oamjms.2017.173
eISSN: 1857-9655
Basic Science

Protective Effects of the Third Generation Vasodilatory Beta - Blocker Nebivolol against D-Galactosamine - Induced Hepatorenal Syndrome in Rats

Ahmed Atwa[1*], Rehab Hegazy[2], Rania Mohsen[3], Neamat Yassin[2], Sanaa Kenawy[3]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Egyptian Russian University, Badr City, Egypt; [2]Department of Pharmacology, Medical Division, National Institution Research, Giza, Egypt; [3]Departement of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Citation: Atwa A, Hegazy R, Mohsen R, Yassin N, Kenawy S. Protective Effects of the Third Generation Vasodilatory Beta - Blocker Nebivolol against D-Galactosamine - Induced Hepatorenal Syndrome in Rats. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):880-892. https://doi.org/10.3889/oamjms.2017.173

Keywords: Hepatorenal syndrome; Nebivolol; Nitric oxide; Sprague-Dawley rats; Galactosamine

***Correspondence:** Ahmed Atwa. Department of Pharmacology and Toxicology, Faculty of Pharmacy, Egyptian Russian University, Badr City, Egypt. E-mail: dr.atwa_911@hotmail.com

Received: 03-Aug-2017; **Revised:** 27-Aug-2017; **Accepted:** 25-Nov-2017; **Online first:** 13-Dec-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Renal dysfunction is very common in patients with advanced liver cirrhosis and portal hypertension. The development of renal failure in the absence of clinical, anatomical or pathological causes renal of failure is termed hepatorenal syndrome (HRS).

AIM: The present study was constructed to investigate the possible protective effects of nebivolol (Nebi) against D-galactosamine (Gal)-induced HRS in rats.

MATERIAL AND METHODS: Rats were treated with Nebi for ten successive days. On the 8th day of the experiment, they received a single dose of Gal. Serum levels of Cr, BUN, Na+ and K+ as well as AST, ALT, total bilirubin (TB), NH3 and endothelin-1 (ET-1) were determined following Gal administration. Moreover, renal and liver contents of MDA, GSH, F2-isoprostanes (F2-IPs), tumor necrosis factor-alpha (TNF-α), nuclear factor kappa-B (NF-κB), total nitric oxide (NO), in addition to activities of caspase-3 (Cas-3), heme oxygenase-1 (HO-1), inducible and endothelial NO synthase (iNOS and eNOS) enzymes were also assessed. Finally, histopathological examination was performed.

RESULTS: Nebi attenuated Gal-induced renal and hepatic dysfunction. It also decreased the Gal-induced oxidative stress and inflammatory recruitment.

CONCLUSION: Results demonstrated both nephroprotective and hepatoprotective effects of Nebi against HRS and suggested a role of its antioxidant, anti-inflammatory, anti-apoptotic and NO-releasing properties.

Introduction

Renal failure occurs in 40-80% of patients with end-stage liver disease and is associated with an unfavourable prognosis. The development of renal failure in the absence of clinical, anatomical, or pathological causes of renal failure is termed the HRS [1].

Typical features of HRS include oliguria, hyponatremia, azotemia and hyponatremia. Although the pathophysiological mechanism underlying HRS is still incompletely understood, marked renal vasoconstriction in the presence of splanchnic and systemic vasodilation may play an important role, and may thus reduce the renal arterial blood flow and the glomerular filtration rate, resulting in renal impairment [2-4]. One of the hallmarks of HRS is that there are relatively few histological changes in the kidneys, and that renal failure is secondary to haemodynamic and functional changes in the kidney. So far, no effective strategies are available for the treatment or prevention of HRS. Instead, patients are usually managed by maintaining their adequate hemodynamic status and intravascular volume. A better understanding of the pathophysiological mechanism underlying the transition from liver damage to renal failure helps to

guide its treatment [2, 4, 5].

Galactosamine is a potent hepatotoxic substance, which can cause hepatocyte death both by necrosis and apoptosis secondary to inhibition of hepatic RNA synthesis [6]. Studies also found that animals rapidly developed functional acute renal failure in addition to acute damage and liver failure, following intoxication with Gal [7]. Following a single injection of high dose Gal, rats develop acute liver failure with development of a hyperdynamic circulation. It was reported that Gal- induced liver injury is associated with the development of renal failure [8].

Many factors may contribute to Gal-induced HRS. Patients who develop HRS, particularly in the context of acute liver failure or alcoholic hepatitis, have increased circulating concentrations of the potent vasoconstrictor peptide endothelin-1 (ET-1) [9].

Additionally, NO is elevated in patients with cirrhosis; the imbalance between it and vasoconstrictors such as ET-1 in the renal microcirculation has been proposed to be responsible for the deterioration of kidney function in these patients. Moreover, a progressive rise in levels of NO had been proposed during progressive renal dysfunction in cirrhosis [10]. NO produced by iNOS is reported to have aggravated liver and kidney injury, while eNOS expression preserved physiological functions [11].

Moreover, oxidative stress is markedly elevated in chronic liver disease and has gain attention as a potentially important factor in altered hemodynamics and renal dysfunction in cirrhosis. It induces renal vasoconstriction not only by quenching NO, but also by increasing production of F2-IPs (F2-isoprostanes; formed as a result of free radical-mediated non-enzymatic peroxidation of membrane-bound arachidonic acid which can be used to evaluate local or systemic lipid peroxidation in vivo) and ET-1 in addition to damaging DNA and provoking apoptosis [12]. Markedly increased levels of both factors in patients with HRS in conjunction with increased systemic oxidative stress in cirrhosis raises the possibility of a pathogenetic role of oxidative stress in HRS [13]. Excessive oxidative stress has been suggested as a reason for HO-1 up-regulation, and this enzyme is known to play a role in the inflammatory process and oxidative tissue damage in Gal-induced acute liver injury. On the other hand, previous studies denoted that decreased renal HO-1 expression plays an important role in the pathogenesis of experimental HRS [14].

It has been well recognized that an unregulated inflammatory response is a key mechanism of Gal-induced acute hepatotoxicity. TNF-α is a pro-inflammatory cytokine secreted by liver kupffer cells as an inflammatory response [15]. It modulates the necrotic, apoptotic and inflammatory pathways in Gal-induced hepatotoxicity by activating transcription factors as NF-κB. In respect of apoptosis, TNF-α combines with TNF-α receptor on the hepatocyte membrane activates caspase-3 and eventually induces apoptosis at an early stage through a series of signal transmission [16]. It has been reported that the transcription factor NFκB plays an important role in the induction of iNOS because an NFκB binding site has been identified in the promoter region of the iNOS gene. Inducible NOS-induced NO production is believed to play an important role in hepatocellular injury following endotoxemia and TNF-α stimulation [17].

Portal hypertension is an almost unavoidable complication of cirrhosis and provides the driving force for most of its complications, such as oesophagal and gastric varices, variceal bleeding, ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, as well as portal-systemic encephalopathy. For medical treatment of portal hypertension, beta-blockers are used to decrease splanchnic inflow and may be combined with nitrates to reduce intrahepatic resistance [18].

Nebivolol is a third generation selective β1-adrenergic receptor blocker with vasodilator properties mediated by a direct stimulatory effect on the eNOS-L-arginine-NO pathway [19]. Treatment with Nebi has been shown to decrease renal fibrosis and glomerular injury as well as improving endothelial dysfunction. These effects have been attributed to vasodilatation, reduction in oxidative stress in addition to the enhancement of NO bioavailability [20]. Nebi may have beneficial effects on portal pressure, by decreasing splanchnic blood flow and decreasing intrahepatic resistance. Indeed, Nebi has been shown to be effective in a small case series of portal hypertensive patients with and without ascites [21].

Taken into consideration, these pharmacological properties of Nebi, with its renoprotective and hepatoprotective effects, could be of potential interest to patients with HRS. For that, the present study was performed to investigate the possible protective effects of Nebi against Gal-induced HRS in rats.

Material and Methods

Animals

Adult male Sprague Dawley rats, weighing 250-300 gram, were used in the present study. Standard food pellets and tap water were supplied ad libitum. Rats were kept under controlled conditions, with a 12 h light/dark cycles, at an ambient temperature of 22 ± 2^{o}C and a humidity of 65–70%. This study was carried out in strict accordance with the recommendations in the guide for the care and

use of laboratory animals of the National Institutes of Health. The study protocol was approved by the guidelines of the Research Ethical Committee of the Faculty of Pharmacy, Cairo University, Cairo, Egypt (Permit Number: PT 734).

Drugs

Nebi was obtained from Sigma-Aldrich (USA). It was available as a powder and used in the current study at two dose levels of 10 and 20 mg/kg/day, p.o. [22]. Nebi was freshly prepared at the beginning of each experiment by being suspended in distilled water and volumes were adjusted so that each rat received 1 ml suspension/100 g body weight [23]. All other used chemicals were of the highest purity available.

Experimental Design

Hepatorenal syndrome was induced in rats using a single dose of Gal solution in sterile saline (1.1 g/kg, i.p.) [7]. Animals were randomly allocated into four groups; each group consisted of 12 rats. The first and second groups received saline and served as normal and control groups, respectively. Rats of all groups except the first received a single dose of Gal solution in sterile saline g/kg, i.p. on the 8th day of the experiment. Groups 3 & 4 received Nebi (10 & 20 mg/kg/day, respectively, p.o.). Administration of Nebi was carried out for ten successive days. Animals were allowed free access to food and tap water during the experiment.

Serum biochemical analysis

Blood samples were withdrawn via the retro-orbital plexus under ether anaesthesia from all rats on day 10, after two h of the last drug administration. Sera were separated for assessment of renal functions by measuring blood urea nitrogen (BUN), serum creatinine (SCr), potassium (K+) and sodium (Na+) levels, using specific commercial kits, (Stanbio, USA, catalogue No. 2050), (Quimica Clinica Aplicada S.A., Spain, catalogue No. 998891), (Quimica Clinica Aplicada S.A., Spain, catalogue No. 99111), and (Teco Diagnostics, USA, catalogue No. S600-50), respectively. Additionally, liver function tests were also assessed by measuring aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TB) in addition to ammonia (NH3) levels using specific commercial kits, (Quimica Clinica Aplicada S.A., Spain, catalogue No. 998720), (Quimica Clinica Aplicada S.A., Spain, catalogue No. 997610), (Quimica Clinica Aplicada S.A., Spain, catalogue No. 992714) and (MyBioSource Co., Inc., California, USA, catalogue No. MBS841579), respectively. Moreover, endothelin-1 (ET-1) level was estimated to judge the severity of vasoconstriction, using specific commercial ELISA kit (Immuno Biological Laboratories Co., Ltd, Gunma, Japan, catalogue No. 27165).

Renal and liver tissue biochemical and histopathological analysis

Directly after collecting the blood samples, rats were sacrificed by cervical dislocation under ether anaesthesia, and both kidneys and liver tissues were isolated. The right kidneys and part of the liver tissues were rinsed in chilled 0.9 % NaCl (pH 7.4) then homogenised using a homogeniser (MPW- 120, Med instruments, Poland) to yield a 20% (w/v) tissue homogenate. The homogenates were used for estimation of kidney and liver contents of lipid peroxides in term of malondialdehyde (MDA) [24], reduced glutathione (GSH) [25], F2-isoprostanes (F2-IPs) using commercial ELISA kit (OXIS Health Products Co., Inc., Portland, catalogue No. 21049), tumor necrosis factor-alpha (TNF-α) using commercial ELISA kit (RayBiotech Co., Norcross GA, USA, catalogue No. ELR-TNF-α-001c), nuclear factor kappa-B (NF-κB) using commercial reagent kit (Wuhan Eiaab Science Co., Wuhan, China, catalogue No. E1824r), nitric oxide (NO) measured as NO_3-/NO_2- (nitrite and nitrate, stable metabolites of NO) using commercial reagent kit (Cayman chemical company, Germany, catalogue No. 780001). Moreover, kidney and liver activities of caspase-3 (Cas-3), inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS) as well as heme-oxygenase-1 (HO-1) enzymes were also assessed, using specific commercial ELISA kits, (Uscn Life Science Co., Wuhan, China, catalogue No. E90626Mu), (Bioassay Technology Laboratory Co., Shanghai, China, catalogue No. E0704ra), (Wuhan Eiaab Science Co., Wuhan, China, catalogue No. E0868r) and (Uscn Life Science Co., Wuhan, China, catalogue No. E90584ra), respectively.

The left kidneys and the remaining parts of liver tissues from all groups were removed and fixed in 10% neutral buffered formal saline for at least 72 h. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in soft paraffin. Paraffin sections of 5 μm thick were stained with haematoxylin and eosin (H&E) [26], for histopathological examination. Images were captured and processed using Adobe Photoshop version 8.0.

Statistical Analysis

All the values are presented as means a ± standard error of the means (SEM). Comparisons between different groups were carried out using one-way analysis of variance (ANOVA) followed by Tukey HSD test for multiple comparisons [27]. GraphPad Prism software, version 5 was used to carry out these statistical tests. The difference was considered significant when $p < 0.05$.

Results

Serum biochemical analysis

Induction of HRS in rats by a single dose of Gal markedly increased SCr and BUN levels on day ten by 217% and 372%, respectively. A marked decrease in Na+ level by 10% and increase in K+ by 54% level was also observed in Gal treated rats as compared with normal rats. Pretreatment of rats with Nebi (10 & 20 mg/kg) led to a significant reduction in SCr by 33% and 51% as well as BUN by 41% and 64%, respectively, compared to Gal group. Pretreatment of rats with Nebi (10 and 20 mg/kg) led to a significant elevation in Na+ by 8% and 10%, respectively, and to a significant reduction in K+ by 17% and 28%, respectively, compared to Gal group (Table 1).

Table 1: Effects of Nebi on serum levels of creatinine, blood urea nitrogen, sodium and potassium

Parameters	SCr (mg/dl)	BUN (mg/dl)	Na^+ (mEq/L)	K^+ (mmol/L)
Groups				
Normal (Saline)	b 0.64 ± 0.04	b 16.71 ± 0.57	b 137.86 ± 0.34	b 3.81 ± 0.09
Control Gal (1.1 g/kg)	a 2.03 ± 0.06	a 78.80 ± 0.59	a 123.57 ± 0.57	a 5.87 ± 0.07
Nebi (10 mg/kg) + Gal	a b 1.36 ± 0.02	a b 46.57 ± 0.65	a b 132.86 ± 0.34	a b 4.90 ± 0.03
Nebi (20 mg/kg) + Gal	a b 1.00 ± 0.03	a b 28.29 ± 0.87	a b 136.00 ± 0.31	a b 4.20 ± 0.08

Saline, rats treated with saline and considered as normal rats; Gal, rats treated with galactosamine and served as control; Nebi + Gal, rats treated with galactosamine and nebivolol; SCr, serum creatinine; BUN, blood urea nitrogen; Na+, serum sodium; K+, serum potassium. Data are presented as mean ± SE, n=12; a Significantly different from Saline; $p < 0.05$; b Significantly different from Gal; $p < 0.05$.

Saline, rats treated with saline and considered as normal rats; Gal, rats treated with galactosamine and served as control; Nebi + Gal, rats treated with galactosamine and nebivolol; SCr, serum creatinine; BUN, blood urea nitrogen; Na+, serum sodium; K+, serum potassium. Induction of HRS in rats by a single dose of Gal markedly increased AST, ALT, NH_3 and TB levels on day ten by 339%, 432%, 1493% and 406%, respectively. Pretreatment of rats with Nebi (10 & 20 mg/kg) notably declined levels of AST by 50% and 71%, ALT by 51% and 73%, NH_3 by 68% and 84% and TB by 45% and 62%, respectively, compared to Gal group (Table 2).

Table 2: Effect of Nebi on serum levels of aspartate aminotransferase, alanine aminotransferase, ammonia and total bilirubin

Parameters	AST	ALT	NH_3 (µg/ml)	TB
Groups	(U/ml)	(U/ml)		(mg/dl)
Normal (Saline)	b 23.00 ± 0.62	b 22.86 ± 0.55	b 0.58 ± 0.01	b 0.35 ± 0.01
Control Gal (1.1 g/kg)	a 100.86 ± 0.91	a 121.57 ± 0.95	a 9.24 ± 0.24	a 1.77 ± 0.04
Nebi (10 mg/kg) + Gal	a b 50.14 ± 0.51	a b 59.43 ± 0.20	a b 2.99 ± 0.07	a b 0.97 ± 0.01
Nebi (20 mg/kg) + Gal	a b 28.86 ± 0.34	a b 33.29 ± 1.13	a b 1.52 ± 0.04	a b 0.67 ± 0.01

Saline, rats treated with saline and considered as normal rats; Gal, rats treated with galactosamine and served as control; Nebi + Gal, rats treated with galactosamine and nebivolol; AST, serum aspartate aminotransferase; ALT, alanine aminotransferase; NH_3, serum ammonia; TB, total bilirubin. Data are presented as mean ± SE, n=12. A Significantly different from Saline; $p < 0.05$. B Significantly different from Gal; $p < 0.05$.

Induction of HRS in rats by a single dose of Gal markedly increased ET-1 levels on day ten by 759%. Pretreatment of rats with Nebi (10 and 20 mg/kg) resulted in a significant cutback in ET-1 levels by 57% and 73%, respectively, compared to Gal group (Table 3).

Table 3: Effect of Nebi on serum levels of endothelin-1

Parameters	ET-1 (pg/ml)
Groups	
Normal (Saline)	b 10.34 ± 0.33
Control Gal (1.1 g/kg)	a 88.87 ± 0.55
Nebi (10 mg/kg) + Gal	a b 38.00 ± 0.82
Nebi (20 mg/kg) + Gal	a b 24.29 ± 0.53

Saline, rats treated with saline and considered as normal rats; Gal, rats treated with galactosamine and served as control; Nebi + Gal, rats treated with galactosamine and nebivolol; ET-1, serum endothelin-1. Data are presented as mean ± SE, n=12. A Significantly different from Saline; $p < 0.05$. B Significantly different from Gal; $p < 0.05$.

Renal and liver tissue biochemical analysis

Induction of HRS in rats using Gal obviously augmented the normal renal and hepatic MDA contents by 493% and 508%, respectively and diminished GSH contents by 76% and 78%, respectively. Pretreatment of rats with Nebi (10 and 20 mg/kg) led to a significant dwindle in renal MDA contents by 59% and 76% and hepatic MDA contents by 57% and 72%, respectively, compared to Gal group (Fig. 1a). On the other hand, a recognisable rise in renal GSH contents by 71% and 123% and hepatic GSH contents by 87% and 136%, respectively, were seen as compared to Gal group (Fig. 1b).

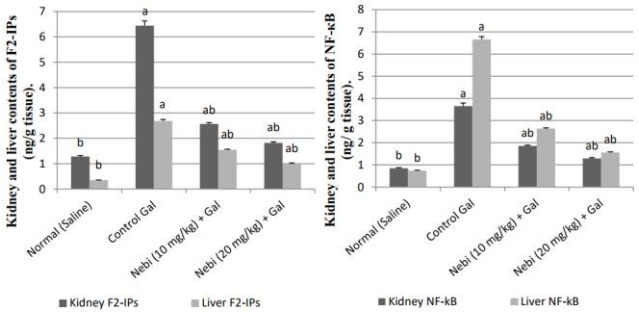


Figure 1: Effect of Nebi on Kidney and liver contents of MDA (left) and Kidney and liver contents of GSH (right). Saline, rats treated with saline and considered as normal rats; Gal, rats treated with galactosamine and served as control; Nebi + Gal, rats treated with galactosamine and nebivolol; MDA, malondialdehyde; GSH, reduced glutathione. Data are presented as mean ± SE, n=12. A Significantly different from Saline; p < 0.05. b Significantly different from Gal; p < 0.05

Induction of HRS in rats using Gal strikingly increased the normal renal and hepatic F2-IPs contents by 403% and 666%, respectively and increased NF-κB contents by 329% and 799%, respectively. Pretreatment of rats with Nebi (10 and 20 mg/kg) led to a significant decline in renal F2-IPs contents by 60% and 72% and hepatic F2-IPs contents by 42% and 63%, respectively, compared to

Gal group (Fig. 2a). Moreover, Pretreatment of rats with Nebi (10 and 20 mg/kg) led to a significant drop in renal NF-κB contents by 49% and 65% as well as hepatic NF-κB contents by 60% and 77%, respectively, compared to Gal group (Fig. 2b).

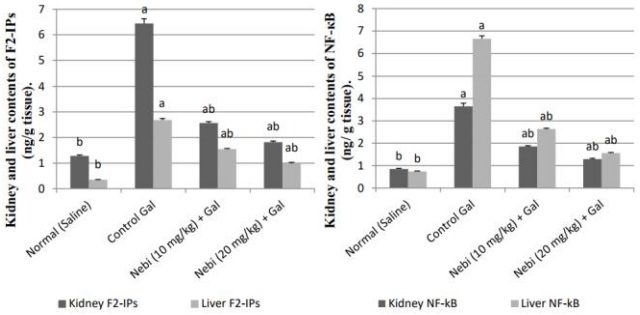


Figure 2: Effect of Nebi on Kidney and liver contents of F2-IPs (left) and Kidney and liver contents of NF-κB (right). Saline, rats treated with saline and considered as normal rats; Gal, rats treated with galactosamine and served as control; Nebi + Gal, rats treated with galactosamine and nebivolol; F2-IPs, F2-isoprostanes; NF-κB, nuclear factor-κB. Data are presented as mean ± SE, n=12. A Significantly different from Saline; p < 0.05. b Significantly different from Gal; p < 0.05

Induction of HRS in rats using Gal evidently amplified the normal renal and hepatic TNF-α content by 950% and 666%, respectively and augmented Cas-3 activity by 699% and 629%, respectively. Pretreatment of rats with Nebi (10 and 20 mg/kg) led to a significant reduction in renal TNF-α contents by 57% and 73% and hepatic TNF-α contents by 60% and 76%, respectively, compared to Gal group (Fig. 3a). Additionally, Nebi (10 & 20 mg/kg) achieved a marked fall in renal Cas-3 activity by 51% and 74% and hepatic Cas-3 activity by 57% and 76%, respectively, compared to Gal group (Fig. 3b).

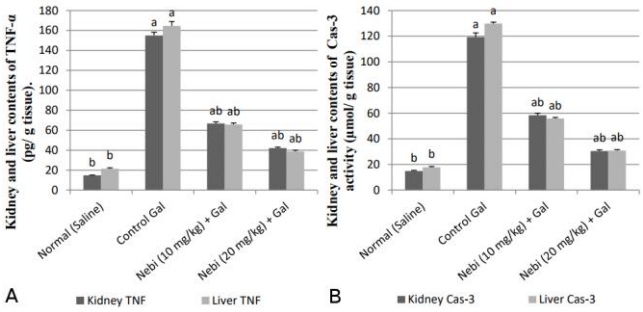


Figure 3: Effect of Nebi on (A) Kidney and liver contents of TNF-α and (B) Kidney and liver Cas-3 activity. Saline, rats treated with saline and considered as normal rats; Gal, rats treated with galactosamine and served as control; Nebi + Gal, rats treated with galactosamine and nebivolol; TNF-α, tumour necrosis factor-alpha; Cas-3, caspase-3. Data are presented as mean ± SE, n=12. a Significantly different from Saline; p < 0.05. b Significantly different from Gal; p < 0.05

Induction of HRS in rats using Gal markedly intensified the normal renal and hepatic NOx contents by 533% and 563%, respectively and raised renal & hepatic iNOS activity by 592% and 311%, respectively. Pretreatment of rats with Nebi (10 and 20 mg/kg) led to a significant decline in renal NOx contents by 58% and 76% and hepatic NOx contents by 60% and 78%, respectively, compared to Gal group (Fig. 4a). In the same line, pretreatment of rats with Nebi (10 and 20 mg/kg) led to a significant regression in renal iNOS activity by 63% and 77% and hepatic iNOS activity by 56% and 68%, respectively, compared to Gal group (Fig. 4b).

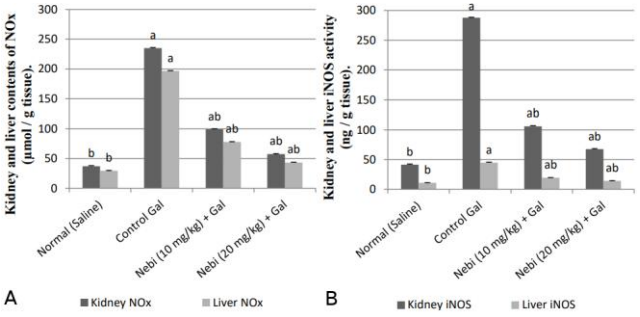


Figure 4: Effect of Nebi on (A) Kidney and liver contents of NOx and (B) Kidney and liver iNOS activity. Saline, rats treated with saline and considered as normal rats; Gal, rats treated with galactosamine and served as control; Nebi + Gal, rats treated with galactosamine and nebivolol; NOx, nitrite and nitrate, stable metabolites of NO; iNOS, inducible nitric oxide synthase. Data are presented as mean ± SE, n=12. a Significantly different from Saline; p < 0.05. b Significantly different from Gal; p < 0.05

Induction of HRS in rats using Gal prominently decreased the normal renal HO-1 activity by 76% and increased hepatic HO-1 activity by 717%. On the other hand, normal renal eNOS activity decreased by 52% and normal hepatic eNOS activity increased by 903%. Pretreatment of rats with Nebi (10 and 20 mg/kg) led to a significant surge in renal HO-1 activity by 86% and 210% and renal eNOS activity by 53% and 96%, respectively, compared to Gal group. On the contrary, pretreatment of rats with Nebi (10 and 20 mg/kg) led to a significant reduction in hepatic HO-1 activity by 56% and 69% in addition to hepatic eNOS activity by 67% and 84%, respectively, compared to Gal group (Fig. 5 a & b).

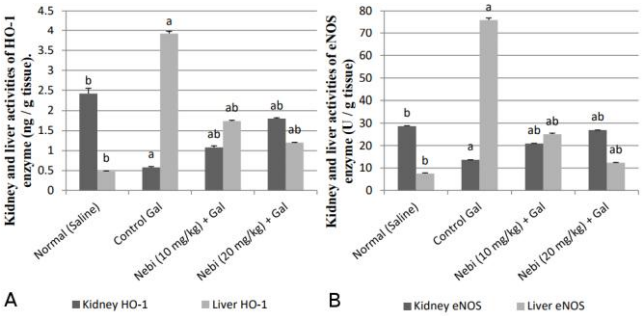


Figure 5: Effect of Nebi on (A) Kidney and liver HO-1 activity and (B) Kidney and liver eNOS activity. Saline, rats treated with saline and considered as normal rats; Gal, rats treated with galactosamine and served as control; Nebi + Gal, rats treated with galactosamine and nebivolol; HO-1, heme-oxygenase-1; eNOS, endothelial nitric oxide synthase. Data are presented as mean ± SE, n=12. a Significantly different from Saline; p < 0.05. b Significantly different from Gal; p < 0.05

Histopathological features of the renal and liver tissues

The renal tissue of the normal rats showed the normal histological structure of the glomeruli (g) and tubules (t) at the cortex besides the normal histological structure of the tubules at the corticomedullary portion (cm) (Fig. 6 A & B).

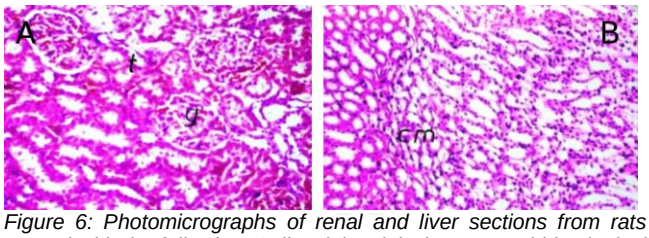


Figure 6: Photomicrographs of renal and liver sections from rats treated with the following: Saline (A) & (B) show normal histological structure of the glomeruli (g) and tubules (t) at the cortex besides the normal histological structure of the tubules at the corticomedullary portion (cm)

The liver tissue of the normal rats showed the normal histological structure of the portal area (Pa) and surrounding hepatocytes (h) besides the normal histological structure of the of the central vein (cv) (Fig. 7C & D).

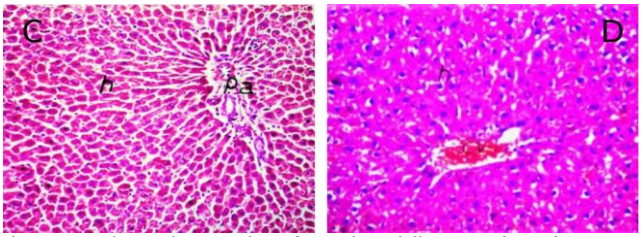


Figure 7: Photomicrographs of renal and liver sections from rats treated with the following: Saline (C) & (D) show the normal histological structure of the portal area (Pa) and surrounding hepatocytes (h) besides the normal histological structure of the of the central vein (cv)

In rats treated with Gal (1.1 g/kg) and sacrificed after 48 h from Gal administration, the renal tissues showed a marked congestion in the blood vessels (v) and glomeruli (g) associated with perivascular edema and inflammatory cells infiltration (m) and degeneration in the lining epithelium of the tubules (d) in addition to focal hemorrhage in the corticomedullary portion (h) (Fig. 8 E & F).

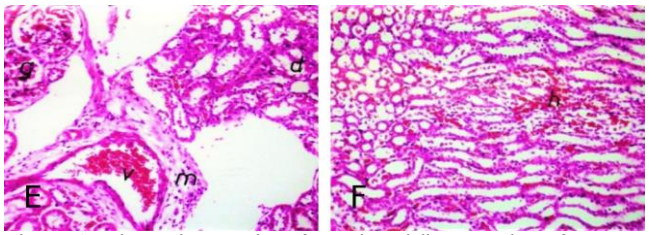


Figure 8: Photomicrographs of renal and liver sections from rats treated with the following: Gal (E & F) the renal tissues show a marked congestion in the blood vessels (v) and glomeruli (g) associated with perivascular edema and inflammatory cells infiltration (m) and degeneration in the lining epithelium of the tubules (d) in addition to focal hemorrhage in the corticomedullary portion (h) (H & E X 40)

In rats treated with Gal (1.1 g/kg) and sacrificed after 48 h from Gal administration, the liver tissues showed marked diffuse coagulative necrosis in the hepatocytes (hn) surrounding the central vein (cv) (Fig. 9 G).

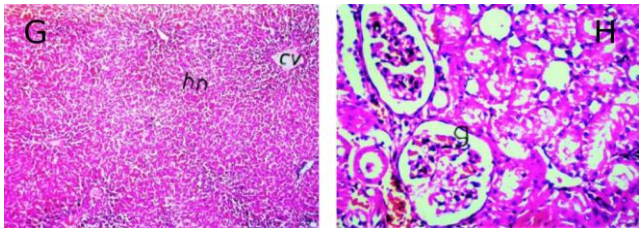


Figure 9: Photomicrographs of renal and liver sections from rats treated with the following: Gal (G) the renal tissues show a marked congestion in the blood vessels and glomeruli (g) associated with perivascular edema and inflammatory cells infiltration (m) and degeneration in the lining epithelium of the tubules (d) in addition to focal hemorrhage in the corticomedullary portion (h), while the liver tissues show marked diffuse coagulative necrosis in the hepatocytes (hn) surrounding the central vein (cv) (H & E X 16)

The renal tissues of rats with Gal-induced HRS that were pretreated with Nebi (10 mg/kg/day) showed glomerular congestion (g) in addition to tubular degeneration (d) with tubular cystic dilation (c) in corticomedullary portion (Fig. 9 H & Fig. 10 I). The liver tissues of rats with Gal-induced HRS that were pretreated with Nebi (10 mg/kg/day) showed inflammatory cells aggregation (m), congestion in portal vein as well bile duct hyperplasia (bd) in association with hepatocellular degeneration (arrow) (Fig. 10 J).

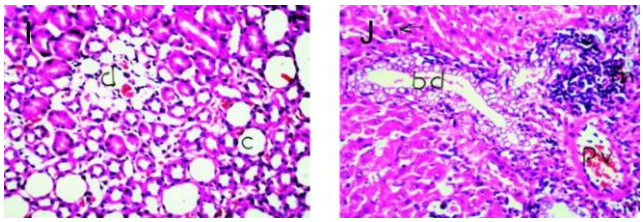


Figure 10: Photomicrographs of renal and liver sections from rats treated with the following: Nebi (10 mg) + Gal (I & J) the renal tissues show glomerular congestion (g) in addition to tubular degeneration (d) with tubular cystic dilation (c) in corticomedullary portion, while the liver tissues show inflammatory cells aggregation (m), congestion in portal vein as well bile duct hyperplasia (bd) in association with hepatocellular degeneration (arrow)

The renal tissues of rats with Gal-induced HRS that were pretreated with Nebi (20 mg/kg/day) showed inflammatory cells infiltration (m) in between the tubules of the cortex and congestion in blood vessels (v) with focal inflammatory cells infiltration in between the degenerated (d) and cystically dilated (c) tubules (Fig. 11 K & L**).**

The liver tissues of rats with Gal-induced HRS that were pretreated with Nebi (20 mg/kg/day) showed inflammatory cells infiltration (m) in between the degenerated hepatocytes (arrow) in addition to diffuse kupffer cells proliferation (arrow) in between the hepatocytes (Fig. 12 M & N).

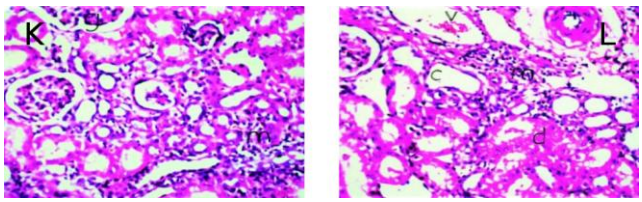


Figure 11: Photomicrographs of renal and liver sections from rats treated with the following: Nebi (20 mg) + Gal (K & L) the renal tissues show inflammatory cells infiltration (m) in between the tubules of the cortex and congestion in blood vessels (v) with focal inflammatory cells infiltration in between the degenerated (d) and cystically dilated (c) tubules. The liver tissues show inflammatory cells infiltration (m) in between the degenerated hepatocytes (arrow) (H & E X 40) in addition to diffuse kupffer cells proliferation (arrow) in between the hepatocytes (H & E X 80)

Discussion

A hepatorenal syndrome is a form of functional renal impairment due to debilitated renal blood flow, which happens typically in kidneys that are histologically normal, accompanied with severe complications of progressive liver disease and usually affects patients with cirrhosis and ascites [28].

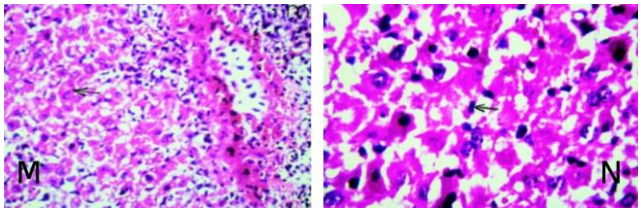


Figure 12: Photomicrographs of renal and liver sections from rats treated with the following: Nebi (20 mg) + Gal (M & N) the renal tissues show inflammatory cells infiltration (m) in between the tubules of the cortex and congestion in blood vessels (v) with focal inflammatory cells infiltration in between the degenerated (d) and cystically dilated (c) tubules. The liver tissues show inflammatory cells infiltration (m) in between the degenerated hepatocytes (arrow) (H & E X 40) in addition to diffuse kupffer cells proliferation (arrow) in between the hepatocytes (H & E X 80)

The current study revealed a significant increase in AST, ALT, NH3 and TB serum levels in control positive HRS group compared with the normal one, an effect that was documented in earlier studies [7, 28, 29].

A significant increase in SCr, BUN and K+, as well as significant decrease in serum Na+ levels, were observed in control positive HRS group compared to the normal one. These findings are corroborated by previous studies [7, 28, 29]. The pathogenesis of the development of renal failure in this model corresponds with the mechanisms observed in typical HRS. It progresses from damage to the liver parenchyma to the development of portal hypertension, enlargement of the splanchnic vascular bed, reduction of the effective volume of fluid in the systemic circulation, and subsequent vascular baroreceptor stimulation, followed by activation of numerous vasoconstriction factors, including the renin-angiotensin system, sympathetic nervous system, or arginine vasopressin system. These mechanisms lead to renal cortical vasoconstriction, renal hypoperfusion and renal failure [30].

In the present study, rats subjected to Gal provoked a significant elevation in serum ET-1 levels as compared with normal rats. This is by the outcomes of earlier studies [31-35]. Many factors may contribute to Gal-induced HRS. Patients who develop HRS, particularly in the context of acute liver failure or alcoholic hepatitis, have increased circulating concentrations of ET-1 [9].

In the present work, control positive HRS group depicted a significant increase in the renal and hepatic content of MDA, with a decrease in GSH contents. These results are in harmony with other studies [12, 36, 37]. Several mediators implicated in the pathogenesis of HRS are regulated through products of lipid peroxidation or redox changes in signalling pathways. Thus the development of oxidant stress may be important in the pathogenesis of HRS [38].

Moreover, Oxidative stress is markedly elevated in chronic liver disease and has gain attention as a potentially important factor in altered hemodynamics and renal dysfunction in cirrhosis. It induces renal vasoconstriction not only by quenching NO but also by increasing production of F2-IPs and ET-1 in addition to damaging DNA and provoking apoptosis [12]. Markedly increased levels of both factors in patients with HRS in conjunction with increased systemic oxidative stress in cirrhosis raises the possibility of a pathogenetic role of oxidative stress in HRS [13].

In the current work, F2-IPs contents in the liver and kidney were drastically boosted in the positive HRS group. This finding was by other studies [9, 39, 40]. The F2-IPs are formed by lipid peroxidation. One of the major F2-IPs formed in vivo, namely 8-iso-PGF2. F2-IPs synthesis is increased in patients with HRS and denotes increased lipid peroxidation [38]. Free-radical-generated F2-IPs also stimulates DNA synthesis and the ET-1 expression on endothelial cells. F2-IP is a highly potent renal vasoconstrictor that selectively increases pre-glomerular vascular resistance and leads to a reduction in the glomerular filtration rate [41].

Results of the current study illustrated that induction of HRS using Gal produced a substantial rise in the renal and hepatic contents of NF-κB, TNF-α as well as Cas-3 activity as compared with normal animals. The present data are in agreement with previous studies [12, 15-17, 35, 42]. It has been well recognized that an up regulated inflammatory response is a key mechanism of Gal-induced acute hepatotoxicity. TNF-α is a pro-inflammatory cytokine

secreted by liver kupffer cells as an inflammatory response [15]. TNF modulates the necrotic, apoptotic and inflammatory pathways in Gal-induced hepatotoxicity by activating transcription factors as NF-κB [43]. In respect of apoptosis, TNF-α combines with TNF-α receptor on the hepatocyte membrane activates caspase-3 and eventually induces apoptosis at an early stage through a series of signal transmission [16].

In the present study, rats subjected to Gal showed a significant elevation in the renal and hepatic contents of NOx besides iNOS activity. These results are consistent with prior studies [29, 42, 44-46]. Previous findings have shown that NO, a potent vasodilator, plays an important role in the development of hyperdynamic syndrome and peripheral vasodilation during cirrhosis [3]. Increased NO level and synthetase activity in patients with liver cirrhosis have adverse effects on the functions of renal tubules and glomeruli whereas inhibition of NO synthetase prevents the development of renal failure in an animal model of HRS [47, 48]. NO produced by iNOS is reported to have aggravated liver and kidney injury, while eNOS expression preserved physiological functions [11].

Furthermore, in patients with cirrhosis; the imbalance between NO and vasoconstrictors such as ET-1 in the renal microcirculation has been proposed to be responsible for the deterioration of kidney function in these patients [10]. Additionally, endotoxemia up-regulates inducible forms of HO-1 and iNOS. Inducible NOS is primarily found in Kupffer cells and hepatocytes. Once activated, it can produce up to 1000 times more NO than eNOS. Inducible NOS-induced NO production is believed to play an important role in hepatocellular injury following endotoxemia and TNF-α stimulation [17].

Previous studies also revealed that the transcription factor NFκB plays an important role in the induction of iNOS because an NFκB binding site has been identified in the promoter region of the iNOS gene and that blocking NFκB results in an attenuation of iNOS gene expression. Furthermore, ET-1 has been shown to activate NFκB in human myofibroblastic hepatic stellate cells [17].

In the present investigation, hepatic HO-1 in addition to eNOS activities were elevated, nevertheless all at once renal HO-1 besides eNOS activities were declined in Gal treated rats as compared with the normal group. A similar pattern coincided with previous studies [14, 49-53].

Excessive oxidative stress has been suggested as a reason for hepatic HO-1 up-regulation, and this enzyme is known to be readily inducible by stressors [54]. On the other hand, previous studies denoted that decreased renal HO-1 expression plays an important role in the pathogenesis of experimental HRS [14].

HO-1 has constitutive and inducible isoforms [55, 56]. HO-1, a 32-kDa inducible protein [57], catalyses the rate-limiting step in the oxidative degradation of heme to biliverdin, releasing equimolar amounts of CO and iron [55]. CO, a gaseous messenger similar to NO, mediates various physiological functions [58] including vasodilation [59]. HO-1 activity is the primary source of circulating CO [60], and HO-1 contributes to vasodilation mainly through HO-1-derived CO [61]. Thus, the declined HO-1 expression in the kidney may be responsible for a decrease in vasodilation. Also, oxidants can cause localised renal vasoconstriction [62]. Therefore, the antioxidant action of HO-1 and its products can preserve renal arterial blood flow. Decreased HO-1 expression in the kidney of Gal rats impairs their ability to buffer locally produced oxidants, thus leading to decreased renal arterial blood flow and deteriorated renal function. Additionally, eNOS expression follows a similar, tissue-specific pattern with HO-1 expression. Decreased eNOS and HO-1 expression in the kidneys, results in reduced amounts of NO and CO available resulting in renal vasoconstriction and reduced RBF occurring during cirrhosis [63]. Taken together, decreased HO-1 and eNOS expression in kidney plays an important role in the pathogenesis of experimental HRS [14].

Surprisingly, the HO-1 level was significantly higher in livers of Gal group, suggesting that there is more CO in the hepatic circulation [64]. It is possible that during cirrhosis the up-regulation of systemic CO resulting from increased HO-1 protein expression in the liver may also reduce HO-1 protein expression in the kidney due to a negative-feedback loop in an attempt to restore circulatory integrity [53].

Previous studies showed that over-expression of HO-1 could be harmful to the liver of rats with cirrhosis induced by bile duct ligation [14], which was also reported by [65]. In normal Sprague-Dawley (SD) rats, increased HO-1 activity as a pro-oxidant mechanism resulted in iron accumulation in the liver and increased portal pressure through hyperdynamic circulation and vasodilation; in contrast, decreased HO-1 activity reduced intracellular iron levels and oxidative stress besides reducing portal pressure and improving fibrosis [66].

HO-1 catalyses heme into iron and plays an important role in iron homeostasis. High levels of HO-1 could result in the accumulation of free divalent iron, thus increasing oxidative injury in fibroblast cell cultures [49]. Deposition of iron in the liver often triggers oxidative stress and inflammation and induces liver cell damage to membranes, proteins, and DNA [52].

Endothelial dysfunction is concomitant with changes in vascular structure associated with many forms of vascular diseases, such as portal hypertension, occurring in all forms of liver injury. This is associated with abnormal production of eNOS.

eNOS is up regulated by various mechanisms; including phosphorylation, subcellular localisation, and protein-protein interactions [67]. A large number of studies demonstrated that heat shock protein 90; a stress protein, interacting with eNOS plays a role in excessive NO production in the rat superior mesenteric artery [68].

Normal eNOS localisation is dramatically altered in endothelial cells of mesenteric arteries isolated from cirrhotic rats with portal hypertension. In those vessels, the Golgi localisation is lost, and eNOS diffuses within the cells and migrates more toward plasma membrane [69].

The severity of portal hypertension seems to be an important factor that influences eNOS activation in the splanchnic circulation. Initially, high portal pressure induces vasoconstriction in arterial splanchnic circulation due to a myogenic reflex caused by a sudden increase in portal pressure, which then causes phosphorylation and activation of eNOS through Akt/protein kinase B activation, ultimately leading to increase NO production and vasodilatation in the arteries of the splanchnic circulation [70].

In the current study, there was a significant histopathological alteration in the Gal- treated rats. The livers extensively displayed diffuse coagulative necrosis [35, 71]. In the same line, the kidneys revealed congestion in the blood vessels, glomeruli associated with perivascular oedema, inflammatory cells infiltration, degeneration in the lining epithelium of the tubules and focal haemorrhage in the corticomedullary portion due to the elevated level of ROS and the upshot of pro-inflammatory cytokines [42].

In the present study, animals treated with Nebi (10 & 20 mg/kg) exhibited a significant improvement in the liver above and renal function tests as compared with the diseased group. These findings are in agreement with previous studies using Nebi in different models of hepatotoxicity and nephrotoxicity [23, 72-74]. Nebi exerts NO-mediated vasodilation in the renal vasculature in addition to conventional beta-blocking effects. Published data indicate that higher doses of Nebi might increase β2 receptor blocking activity which could be beneficial regarding decreasing splanchnic blood flow and portal hypertension [75] which was also proved by a previous study [76].

Current treatment with Nebi showed a remarkable drop in ET-1 levels as compared with Gal treated rats. Nebi reduced ET-1 levels in human pulmonary endothelial cells from pulmonary arterial hypertensive lungs [77] and during oxidative stress in human umbilical vein endothelial cells [20]. Moreover, Nebi can reduce ET-1 secretion in human coronary endothelial and smooth muscle cells [78]. Moreover, an in-vivo study demonstrated that Nebi suppresses ET-1-mediated vasoconstrictor tone in adults with elevated blood pressure [79].

Obtained data in the current study showed that animals treated with Nebi revealed an obvious enhancement in the oxidative stress markers in both kidney and liver. This is manifested by a significant decreased in renal and hepatic MDA, F2-IPs contents in addition to a significant increase in the antioxidant pool of GSH contents. Recently, it was reported that Nebi showed marked amelioration of oxidative stress induced in different models of hepatic and renal injury [23, 72, 74, 80, 81]. Moreover, [82] et al. demonstrated that Nebi exerts systemic antioxidant effects through significantly decreasing the urinary excretion of the 8-iso-PGF2α (one of the major F2-IPs). Nebi's antioxidant activity is due to a reduction of ROS produced by an NADPH oxidase system that makes an important contribution to oxidative stress by uncoupling eNOS [83]. Increased tissue levels of ROS diminish the bioactivity of NO by conversion of locally released NO to peroxynitrite (ONOO−), which itself contributes to tissue injury and oxidative stress [84]. Nebi reduces the NO-scavenging radical superoxide anion, by redirecting deranged NOS activity, from superoxide to NO production, thereby reducing lipid peroxidation and oxidative stress [85]. Also, increase in GSH may be ascribed to the observed increase in HO-1 expression as HO-1 mediates an increase in GSH levels [86] and modulates iNOS [87]. By decreasing oxidative stress, Nebi inhibits NF-κB activation, which leads to the decrease of various pro-inflammatory cytokines [88].

In the present study, the effect of Nebi on renal and hepatic TNF-α contents is consistent with the results of [20] et al. who found that Nebi significantly reduced the oxidative stress-induced TNF-α gene expression in human umbilical vein endothelial cells. Furthermore, Nebi down-regulated TNF-α gene expression in human coronary artery smooth muscle cells [88]. On the other hand, Nebi decreased case-3 immuno-reactivity in cerebral ischemia/reperfusion in rats [89].

Surprisingly, the NO releaser, Nebi, in the present study reduced the hepatic and renal total NOx contents which increased with Gal administration. Likewise, hepatic and renal iNOS activities were significantly reduced in the presence of Nebi. This finding attracts attention that the source of such NOx increase occurred with Gal; it seems that the major spring of NOx is iNOS which was weakly expressed with Nebi treatment. Quantities of NO generated by eNOS is small while large amounts of NO are generated by iNOS [74]. It was reported that Nebi enhanced eNOS expression and reduced iNOS expression [90]. It is also known that sustained iNOS-mediated NO generation may mediate lipid peroxidation and pro-apoptotic effects [74].

Current treatment with Nebi markedly increased expression of renal eNOS and HO-1

activities as compared to HRS group which were weakly expressed with Gal toxicity. Decreased eNOS and HO-1 expression in the kidneys, resulted in reduced amounts of NO and CO available resulting in renal vasoconstriction and reduced RBF occurring during HRS which was counteracted by Nebi. On the other hand, Nebi noticeably diminished hepatic eNOS and HO-1 activities which were strongly conveyed in Gal toxicity. Previous studies showed that over-expression of HO-1 could be harmful to the liver of rats with cirrhosis induced by bile duct ligation [14], which was also reported by [65]. In normal Sprague Dawley (SD) rats, increased HO-1 activity as a pro-oxidant mechanism resulted in iron accumulation in the liver and increased portal pressure through vasodilation; in contrast, decreased HO-1 activity reduced intracellular iron levels and oxidative stress besides reducing portal pressure and improving fibrosis [66]. Endothelial dysfunction is associated with many forms of vascular diseases, such as portal hypertension, occurring in all forms of liver injury. During endothelial dysfunction, hyperactive endothelial cells are observed in patients with portal hypertension. This is associated with abnormal production of an endothelial cell-derived eNOS [67]. All this consequence was reversed with Nebi treatment.

In this experiment, there was a significant histopathological improvement in the liver and kidney of the Nebi (both doses) treated HRS group showing little glomerular congestion and minute tubular degeneration with tubular cystic dilation in a corticomedullary portion in kidney and congestion in portal vein as well as bile duct hyperplasia in the liver due to the decreased level of ROS and the down-regulation of pro-inflammatory cytokines [91].

Finally, the present study has highlighted for the first time, the possible mechanisms responsible for Nebi mediated HRS improvement and its antioxidant action. These findings support its useful effect in the prevention of HRS in patients with advanced liver diseases or as an add-on medication with known anti-HRS therapy.

In conclusion, the present study revealed that treatment of rats with Nebi (10 or 20 mg/kg/day, p.o.) protected against renal and hepatic damage involved in Gal-induced HRS. The findings demonstrated the involvement of the anti-oxidant, anti-inflammatory, anti-apoptotic and NO-releasing properties of this drug and suggested its involvement in the renoprotective and hepatoprotective effect in Gal-induced HRS model?

References

1. Anand R, Harry D, Holt S, Milner P, Dashwood M, Goodier D et al. Endothelin is an important determinant of renal function in a rat model of acute liver and renal failure. Gut. 2002;50(1):111-17. https://doi.org/10.1136/gut.50.1.111 PMid:11772977 PMCid:PMC1773076

2. Cardenas A. Hepatorenal syndrome: a dreaded complication of end-stage liver disease. The American journal of gastroenterology. 2005;100(2):460-7. https://doi.org/10.1111/j.1572-0241.2005.40952.x PMid:15667508

3. Martin P-Y, Ginès P, Schrier RW. Nitric oxide as a mediator of hemodynamic abnormalities and sodium and water retention in cirrhosis. New England Journal of Medicine. 1998;339(8):533-41. https://doi.org/10.1056/NEJM199808203390807 PMid:9709047

4. Wadei HM, Mai ML, Ahsan N, Gonwa TA. Hepatorenal syndrome: pathophysiology and management. Clinical journal of the American Society of Nephrology. CJASN. 2006;1(5):1066-79. https://doi.org/10.2215/CJN.01340406 PMid:17699328

5. Moreau R, Lebrec D. Acute renal failure in patients with cirrhosis: perspectives in the age of MELD. Hepatology. 2003;37(2):233-43. https://doi.org/10.1053/jhep.2003.50084 PMid:12540770

6. Keppler D, Lesch R, Reutter W, Decker K. Experimental hepatitis induced by D-galactosamine. Experimental and molecular pathology.1968;9(2):279-90. https://doi.org/10.1016/0014-4800(68)90042-7

7. Saracyn M, Patera J, Kocik J, Brytan M, Zdanowski R, Lubas A et al. Strain of experimental animals and modulation of nitric oxide pathway: their influence on development of renal failure in an experimental model of hepatorenal syndrome. Arch Med Sci. 2012;8(3):555-62. https://doi.org/10.5114/aoms.2012.29281 PMid:22852015 PMCid:PMC3400905

8. Javle P, Yates J, Kynaston H, Parsons K, Jenkins S. Hepatosplanchnic haemodynamics and renal blood flow and function in rats with liver failure. Gut. 1998;43(2):272-79. https://doi.org/10.1136/gut.43.2.272 PMid:10189857 PMCid:PMC1727229

9. Moore K, Wendon J, Frazer M, Karani J, Williams R, Badr K. Plasma endothelin immunoreactivity in liver disease and the hepatorenal syndrome. New England Journal of Medicine. 1992;327(25):1774-78. https://doi.org/10.1056/NEJM199212173272502 PMid:1435931

10. Kayali Z, Herring J, Baron P, Franco E, Ojogho O, Smith J et al. Increased plasma nitric oxide, L- arginine, and arginase-1 in cirrhotic patients with progressive renal dysfunction. J Gastroenterol Hepatol. 2009;24(6):1030-7. https://doi.org/10.1111/j.1440-1746.2008.05757.x PMid:19226382

11. Alva N, Cruz D, Sanchez S, Valentin JM, Bermudez J, Carbonell T. Nitric oxide as a mediator of fructose 1,6-bisphosphate protection in galactosamine-induced hepatotoxicity in rats. Nitric Oxide. 2013;28:17-23. https://doi.org/10.1016/j.niox.2012.09.004 PMid:23032643

12. Silveira KC, Viau CM, Colares JR, Saffi J, Marroni NP, Porawski M. Cirrhosis induces apoptosis in renal tissue through intracellular oxidative stress. Arquivos de gastroenterologia. 2015;52(1):65-71. https://doi.org/10.1590/S0004-28032015000100014 PMid:26017086

13. Assimakopoulos SF, Gogos C, Labropoulou-Karatza C. Could antioxidants be the "magic pill" for cirrhosis-related complications? A pathophysiological appraisal. Med Hypotheses. 2011;77(3):419-23. https://doi.org/10.1016/j.mehy.2011.05.034 PMid:21703771

14. Guo SB, Duan ZJ, Li Q, Sun XY. Effect of heme oxygenase-1 on renal function in rats with liver cirrhosis. World J Gastroenterol. 2011;17(3):322-8. https://doi.org/10.3748/wjg.v17.i3.322 PMid:21253390 PMCid:PMC3022291

15. Kang JW, Kim SJ, Kim HY, Cho SH, Kim KN, Lee SG et al. Protective effects of HV-P411 complex against D-galactosamine-induced hepatotoxicity in rats. The American journal of Chinese medicine. 2012;40(3):467-80.

https://doi.org/10.1142/S0192415X1250036X PMid:22745064

16. Aristatile B, Al-Assaf AH, Pugalendi KV. Carvacrol suppresses the expression of inflammatory marker genes in D-galactosamine-hepatotoxic rats. Asian Pac J Trop Med. 2013;6(3):205-11. https://doi.org/10.1016/S1995-7645(13)60024-3

17. Keller S, Karaa A, Paxian M, Clemens MG, Zhang JX. Inhibition of endothelin-1-mediated up-regulation of iNOS by bosentan ameliorates endotoxin-induced liver injury in cirrhosis. Shock. 2006;25(3):306-13. https://doi.org/10.1097/01.shk.0000196549.18258.6a PMid:16552365

18. Bosch J, Berzigotti A, Garcia-Pagan JC, Abraldes JG. The management of portal hypertension: rational basis, available treatments and future options. J Hepatol. 2008;48 (Suppl 1):S68-92. https://doi.org/10.1016/j.jhep.2008.01.021 PMid:18304681

19. Munzel T, Gori T. Nebivolol: the somewhat-different beta-adrenergic receptor blocker. J Am Coll Cardiol. 2009;54(16):1491-9. https://doi.org/10.1016/j.jacc.2009.05.066 PMid:19815121

20. Garbin U, Fratta Pasini A, Stranieri C, Manfro S, Mozzini C, Boccioletti V et al. Effects of nebivolol on endothelial gene expression during oxidative stress in human umbilical vein endothelial cells. Mediators of inflammation. 2008;2008:367590. https://doi.org/10.1155/2008/367590 PMid:18437228 PMCid:PMC2323596

21. Fedulenkova LV. [Correction of portal hypertension and renal dysfunction with help of nebivolol and lizinopril in patients with hepatic cirrhosis]. Eksp Klin Gastroenterol. 2009; (5):130-7. PMid:20201315

22. Reiberger T, Payer BA, Schwabl P, Hayden H, Horvatits T, Jager B et al. Nebivolol treatment increases splanchnic blood flow and portal pressure in cirrhotic rats via modulation of nitric oxide signalling. Liver Int. 2013;33(4):561-8. https://doi.org/10.1111/liv.12101 PMid:23331709

23. Atwa A, Hegazy R, Shaffie N, Yassin N, Kenawy S. Protective Effects of Vasodilatory Betaeta-Blockers Carvedilol and Nebivolol against Glycerol Model of Rhabdomyolysis-Induced Acute Renal Failure in Rats. Open Access Maced J Med Sci. 2016;4(3):329-36. https://doi.org/10.3889/oamjms.2016.082 PMid:27703551 PMCid:PMC5042611

24. Ruiz-Larrea MB, Leal AM, Liza M, Lacort M, de Groot H. Antioxidant effects of estradiol and 2- hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. Steroids.1994;59(6):383-8. https://doi.org/10.1016/0039-128X(94)90006-X

25. Baud L, Ardaillou R. Involvement of reactive oxygen species in kidney damage. Br Med Bull. 1993;49(3):621-9. https://doi.org/10.1093/oxfordjournals.bmb.a072635 PMid:8221027

26. Bancroft JD, Gamble M. Theory and practice of histological techniques: Elsevier Health Sciences, 2008.

27. Dunn OJ. Multiple Comparisons Using Rank Sums. Technometrics. 1964; 6(3):241-52. https://doi.org/10.1080/00401706.1964.10490181

28. Saracyn M, Zabkowski T, Zdanowski R, Brytan M, Patera J, Nowak Z et al. Effect of nitric oxide pathway regulation on water/sodium balance and renal function in a rodent model of acute liver and renal failure. Med Sci Monit. 2014;20:1735-44. https://doi.org/10.12659/MSM.890757 PMid:25270512 PMCid:PMC4186324

29. Saracyn M, Zdanowski R, Brytan M, Kade G, Nowak Z, Patera J et al. D-Galactosamine Intoxication in Experimental Animals: Is it Only an Experimental Model of Acute Liver Failure? Med Sci Monit. 2015;21:1469-77. https://doi.org/10.12659/MSM.893291 PMid:26009004 PMCid:PMC4451715

30. Javle P, Yates J, Kynaston HG, Parsons KF, Jenkins SA. Hepatosplanchnic haemodynamics and renal blood flow and function in rats with liver failure. Gut. 1998;43(2):272-9. https://doi.org/10.1136/gut.43.2.272 PMid:10189857 PMCid:PMC1727229

31. Anand R, Harry D, Holt S, Milner P, Dashwood M, Goodier D et al. Endothelin is an important determinant of renal function in a rat model of acute liver and renal failure. Gut. 2002;50(1):111-7. https://doi.org/10.1136/gut.50.1.111 PMid:11772977 PMCid:PMC1773076

32. Soper CP, Latif AB, Bending MR. Amelioration of hepatorenal syndrome with selective endothelin-A antagonist. Lancet. 1996; 347(9018):1842-3. https://doi.org/10.1016/S0140-6736(96)91667-0

33. Izzedine H, Kheder-Elfekih R, Deray G, Thabut D. Endothelin-receptor antagonist/N-acetylcysteine combination in type 1 hepatorenal syndrome. J Hepatol. 2009;50(5):1055-6. https://doi.org/10.1016/j.jhep.2009.02.002 PMid:19328583

34. Moore K. Endothelin and vascular function in liver disease. Gut. 2004;53(2):159-61. https://doi.org/10.1136/gut.2003.024703 PMid:14724140 PMCid:PMC1774918

35. Wang JB, Wang HT, Li LP, Yan YC, Wang W, Liu JY et al. Development of a rat model of D-galactosamine/lipopolysaccharide induced hepatorenal syndrome. World J Gastroenterol. 2015; 21(34):9927-35. https://doi.org/10.3748/wjg.v21.i34.9927 PMid:26379397 PMCid:PMC4566385

36. Wang Y, Li Y, Xie J, Zhang Y, Wang J, Sun X et al. Protective effects of probiotic Lactobacillus casei Zhang against endotoxin- and d-galactosamine-induced liver injury in rats via anti-oxidative and anti- inflammatory capacities. Int Immunopharmacol. 2013; 15(1):30-7. https://doi.org/10.1016/j.intimp.2012.10.026 PMid:23146349

37. Mahmoud MF, Zakaria S, Fahmy A. Can Chronic Nitric Oxide Inhibition Improve Liver and Renal Dysfunction in Bile Duct Ligated Rats? Advances in pharmacological sciences. 2015;2015:298792.

38. Morrow JD, Moore KP, Awad JA, Ravenscraft MD, Marini G, Badr KF et al. Marked overproduction of non-cyclooxygenase derived prostanoids (F2-isoprostanes) in the hepatorenal syndrome. Journal of lipid mediators. 1993;6(1-3):417-20. PMid:8358000

39. Moore K. Isoprostanes and the liver. Chemistry and physics of lipids. 2004;128(1-2):125-33. https://doi.org/10.1016/j.chemphyslip.2003.10.003 PMid:15037158

40. Fukunaga M, Yura T, Badr KF. Stimulatory effect of 8-Epi-PGF2 alpha, an F2-isoprostane, on endothelin- 1 release. Journal of cardiovascular pharmacology. 994;26:S51-2.

41. Takahashi K, Nammour TM, Fukunaga M, Ebert J, Morrow JD, Roberts LJ, 2nd et al. Glomerular actions of a free radical-generated novel prostaglandin, 8-epi-prostaglandin F2 alpha, in the rat. Evidence for interaction with thromboxane A2 receptors. J Clin Invest. 1992;90(1):136-41. https://doi.org/10.1172/JCI115826 PMid:1386085 PMCid:PMC443072

42. Ghosh M, Das J, Sil PC. D(+) galactosamine induced oxidative and nitrosative stress-mediated renal damage in rats via NF-kappaB and inducible nitric oxide synthase (iNOS) pathways is ameliorated by a polyphenol xanthone, mangiferin. Free Radic Res. 2012;46(2):116-32. https://doi.org/10.3109/10715762.2011.644240 PMid:22118634

43. Abe K, Ijiri M, Suzuki T, Taguchi K, Koyama Y, Isemura M. Green tea with a high catechin content suppresses inflammatory cytokine expression in the galactosamine-injured rat liver. Biomedical research. 2005;26(5):187-92. https://doi.org/10.2220/biomedres.26.187 PMid:16295694

44. Mahmoud WA, Abdelkader NA, Mansor A. Could serum

nitrate and nitrite levels possibly predict hepatorenal syndrome in hepatitis C virus-related liver cirrhosis? Indian J Gastroenterol. 2014;33(3):274-80. https://doi.org/10.1007/s12664-013-0427-x PMid:24287875

45. Gomez-Hurtado I, Zapater P, Bellot P, Pascual S, Perez-Mateo M, Such J et al. Interleukin-10-mediated heme oxygenase 1-induced underlying mechanism in inflammatory down-regulation by norfloxacin in cirrhosis. Hepatology. 2011; 53(3):935-44. https://doi.org/10.1002/hep.24102 PMid:21374664

46. Li TH, Lee PC, Lee KC, Hsieh YC, Tsai CY, Yang YY et al. Down-regulation of common NFkappaB- iNOS pathway by chronic Thalidomide treatment improves Hepatopulmonary Syndrome and Muscle Wasting in rats with Biliary Cirrhosis. Sci Rep. 2016; 6:39405.

47. Islas-Carbajal MC, Covarrubias A, Grijalva G, Alvarez-Rodriguez A, Armendariz-Borunda J, Rincon- Sanchez AR. Nitric oxide synthases inhibition results in renal failure improvement in cirrhotic rats. Liver international: official journal of the International Association for the Study of the Liver. 2005; 25(1):131-40. https://doi.org/10.1111/j.1478-3231.2005.01018.x PMid:15698410

48. Saracyn M, Wesolowski P, Nowak Z, Patera J, Kozlowski W, Wankowicz Z. [Role of nitric oxide system in pathogenesis of experimental model of hepatorenal syndrome]. Polski merkuriusz lekarski: organ Polskiego Towarzystwa Lekarskiego. 2008;24(142):293-7.

49. Wang QM, Du JL, Duan ZJ, Guo SB, Sun XY, Liu Z. Inhibiting heme oxygenase-1 attenuates rat liver fibrosis by removing iron accumulation. World J Gastroenterol. 2013;19(19):2921-34. https://doi.org/10.3748/wjg.v19.i19.2921 PMid:23704825 PMCid:PMC3660817

50. Guo SB, Li Q, Duan ZJ, Wang QM, Zhou Q, Sun XY. Octreotide attenuates liver fibrosis by inhibiting hepatic heme oxygenase-1 expression. Molecular medicine reports. 2015;11(1):83-90. https://doi.org/10.3892/mmr.2014.2735 PMid:25338529 PMCid:PMC4237075

51. Shafiei MS, Lui S, Rockey DC. Integrin-linked kinase regulates endothelial cell nitric oxide synthase expression in hepatic sinusoidal endothelial cells. Liver Int. 2015;35(4):1213-21. https://doi.org/10.1111/liv.12606 PMid:24906011 PMCid:PMC4258191

52. Wang QM, Duan ZJ, Du JL, Guo SB, Sun XY, Liu Z. Heme oxygenase/carbon monoxide pathway inhibition plays a role in ameliorating fibrosis following splenectomy. Int J Mol Med. 2013;31(5):1186-94. https://doi.org/10.3892/ijmm.2013.1309 PMid:23525258

53. Miyazono M, Garat C, Morris KG, Jr., Carter EP. Decreased renal heme oxygenase-1 expression contributes to decreased renal function during cirrhosis. Am J Physiol Renal Physiol. 2002;283(5):F1123-31. https://doi.org/10.1152/ajprenal.00363.2001 PMid:12372789

54. Yun N, Eum HA, Lee SM. Protective role of heme oxygenase-1 against liver damage caused by hepatic ischemia and reperfusion in rats. Antioxid Redox Signal. 2010;13(10):1503-12. https://doi.org/10.1089/ars.2009.2873 PMid:20446775

55. Christodoulides N, Durante W, Kroll MH, Schafer AI. Vascular smooth muscle cell heme oxygenases generate guanylyl cyclase–stimulatory carbon monoxide. Circulation. 1995;91(9):2306-09. https://doi.org/10.1161/01.CIR.91.9.2306 PMid:7729015

56. Morita T, Perrella MA, Lee M-E, Kourembanas S. Smooth muscle cell-derived carbon monoxide is a regulator of vascular cGMP. Proceedings of the National Academy of Sciences.1995;92(5):1475-79. https://doi.org/10.1073/pnas.92.5.1475

57. Verma A, Hirsch DJ, Glatt CE, Ronnett GV, Snyder SH. Carbon monoxide: a putative neural messenger. Science-New York Then Washington. 1993;259:381-81. https://doi.org/10.1126/science.7678352

58. Herve P, Lebrec D, Brenot F, Simonneau G, Humbert M, Sitbon O et al. Pulmonary vascular disorders in portal hypertension. European Respiratory Journal. 1998;11(5):1153-66. https://doi.org/10.1183/09031936.98.11051153 PMid:9648972

59. Chang S, Ohara N. Pulmonary circulatory dysfunction in rats with biliary cirrhosis. Am Rev Respir Dis.1992;148:798-805. https://doi.org/10.1164/ajrccm/145.4_Pt_1.798 PMid:1554205

60. Naik JS, O'Donaughy TL, Walker BR. Endogenous carbon monoxide is an endothelial-derived vasodilator factor in the mesenteric circulation. American Journal of Physiology-Heart and Circulatory Physiology. 2003;284(3):H838-H45. https://doi.org/10.1152/ajpheart.00747.2002 PMid:12446283

61. Carter EP, Hartsfield CL, Miyazono M, Jakkula M, Morris KG, McMurtry IF. Regulation of heme oxygenase-1 by nitric oxide during hepatopulmonary syndrome. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2002;283(2):L346-L53. https://doi.org/10.1152/ajplung.00385.2001 PMid:12114196

62. Bomzon A, Holt S, Moore K, editors. Bile acids, oxidative stress, and renal function in biliary obstruction. Seminars in nephrology, 1997.

63. Carter EP, Hartsfield CL, Miyazono M, Jakkula M, Morris KG, Jr., McMurtry IF. Regulation of heme oxygenase-1 by nitric oxide during hepatopulmonary syndrome. Am J Physiol Lung Cell Mol Physiol. 2002;283(2):L346-53. https://doi.org/10.1152/ajplung.00385.2001 PMid:12114196

64. Rodriguez F, Kemp R, Balazy M, Nasjletti A. Effects of Exogenous Heme on Renal Function. Hypertension. 2003;42(4):680-84. https://doi.org/10.1161/01.HYP.0000085785.40581.1A PMid:12900432

65. Froh M, Conzelmann L, Walbrun P, Netter S, Wiest R, Wheeler MD et al. Heme oxygenase-1 overexpression increases liver injury after bile duct ligation in rats. World J Gastroenterol. 2007; 13(25):3478-86. https://doi.org/10.3748/wjg.v13.i25.3478 PMid:17659695 PMCid:PMC4146784

66. Khan ZA, Barbin YP, Cukiernik M, Adams PC, Chakrabarti S. Heme-oxygenase-mediated iron accumulation in the liver. Can J Physiol Pharmacol. 2004;82(7):448-56. https://doi.org/10.1139/y04-052 PMid:15389291

67. Sessa WC. eNOS at a glance. J Cell Sci. 2004;117(Pt 2):2427-9. https://doi.org/10.1242/jcs.01165 PMid:15159447

68. Shah V, Wiest R, Garcia-Cardena G, Cadelina G, Groszmann RJ, Sessa WC. Hsp90 regulation of endothelial nitric oxide synthase contributes to vascular control in portal hypertension. Am J Physiol.1999;277(2 Pt 1):G463-8. PMid:10444461

69. Sowa G, Liu J, Papapetropoulos A, Rex-Haffner M, Hughes TE, Sessa WC. Trafficking of endothelial nitric-oxide synthase in living cells. Quantitative evidence supporting the role of palmitoylation as a kinetic trapping mechanism limiting membrane diffusion. J Biol Chem. 1999;274(32):22524-31. https://doi.org/10.1074/jbc.274.32.22524 PMid:10428829

70. Iwakiri Y, Tsai MH, McCabe TJ, Gratton JP, Fulton D, Groszmann RJ et al. Phosphorylation of eNOS initiates excessive NO production in early phases of portal hypertension. Am J Physiol Heart Circ Physiol. 2002;282(6):H2084-90. https://doi.org/10.1152/ajpheart.00675.2001 PMid:12003815

71. Wang JB, Wang DL, Wang HT, Wang ZH, Wen Y, Sun CM et al. Tumor necrosis factor-alpha-induced reduction of glomerular filtration rate in rats with fulminant hepatic failure. Lab Invest. 2014;94(7):740-51. https://doi.org/10.1038/labinvest.2014.71 PMid:24887412

72. Ulger BV, Erbis H, Turkcu G, Ekinci A, Turkoglu MA, Ekinci C et al. Nebivolol Ameliorates Hepatic Ischemia/Reperfusion Injury on Liver But Not on Distant Organs. Journal of investigative surgery : the official journal of the Academy of Surgical Research. 2015; 28(5):245-52. https://doi.org/10.3109/08941939.2015.1031923 PMid:26305470

73. Teixeira C, Franco E, Oliveira PA, Colaco B, Gama A, Carrola J et al. Effects of nebivolol on liver fibrosis induced by bile duct ligation in Wistar rats. In vivo. 2013;27(5):635-40. PMid:23988899

74. Morsy MA, Heeba GH. Nebivolol Ameliorates Cisplatin-Induced Nephrotoxicity in Rats. Basic & clinical pharmacology & toxicology. 2016;118(6):449-55. https://doi.org/10.1111/bcpt.12538 PMid:26617394

75. Bundkirchen A, Brixius K, Bolck B, Nguyen Q, Schwinger RH. Beta 1-adrenoceptor selectivity of nebivolol and bisoprolol. A comparison of [3H]CGP 12.177 and [125I]iodocyanopindolol binding studies. Eur J Pharmacol. 2003;460(1):19-26. https://doi.org/10.1016/S0014-2999(02)02875-3

76. Silkauskaite V, Kupcinskas J, Pranculis A, Jonaitis L, Petrenkiene V, Kupcinskas L. Acute and 14-day hepatic venous pressure gradient response to carvedilol and nebivolol in patients with liver cirrhosis. Medicina. 2013; 49(11):467-73.

77. Perros F, Ranchoux B, Izikki M, Bentebbal S, Happe C, Antigny F et al. Nebivolol for improving endothelial dysfunction, pulmonary vascular remodeling, and right heart function in pulmonary hypertension. J Am Coll Cardiol. 2015;65(7):668-80. https://doi.org/10.1016/j.jacc.2014.11.050 PMid:25677428

78. Brehm BR, Bertsch D, von Fallois J, Wolf SC. Beta-blockers of the third generation inhibit endothelin-1 liberation, mRNA production and proliferation of human coronary smooth muscle and endothelial cells. J Cardiovasc Pharmacol. 2000;36(5 Suppl 1):S401-3. https://doi.org/10.1097/00005344-200036051-00117 PMid:11078433

79. Diehl KJ, Stauffer BL, Dow CA, Bammert TD, Brunjes DL, Greiner JJ et al. Chronic Nebivolol Treatment Suppresses Endothelin-1-Mediated Vasoconstrictor Tone in Adults With Elevated Blood Pressure. Hypertension. 2016;67(6):1196-204. https://doi.org/10.1161/HYPERTENSIONAHA.115.06979 PMid:27113048 PMCid:PMC4871319

80. Akgullu C, Huyut MA, Boyacioglu M, Gules O, Eryilmaz U, Hekim T et al. Nebivolol to attenuate the effects of hyper-homocysteinaemia in rats. Atherosclerosis. 2015 ;240(1):33-9. https://doi.org/10.1016/j.atherosclerosis.2015.02.054 PMid:25746375

81. Rofaeil RR, Kamel MY, Abdelzaher WY. Different effects of selective beta1-adrenoceptor antagonists, nebivolol or atenolol in acetaminophen-induced hepatotoxicity of rats. Fundam Clin Pharmacol. 2017; 31(2):165-73. https://doi.org/10.1111/fcp.12253 PMid:27862262

82. Troost R, Schwedhelm E, Rojczyk S, Tsikas D, Frolich JC. Nebivolol decreases systemic oxidative stress in healthy volunteers. British journal of clinical pharmacology. 2000;50(4):377-9. https://doi.org/10.1046/j.1365-2125.2000.00258.x PMid:11012562 PMCid:PMC2014994

83. Cominacini L, Fratta Pasini A, Garbin U, Nava C, Davoli A, Criscuoli M et al. Nebivolol and its 4-keto derivative increase nitric oxide in endothelial cells by reducing its oxidative inactivation. J Am Coll Cardiol. 2003;42(10):1838-44. https://doi.org/10.1016/j.jacc.2003.06.011 PMid:14642697

84. Habibi J, Hayden MR, Sowers JR, Pulakat L, Tilmon RD, Manrique C et al. Nebivolol attenuates redox- sensitive glomerular and tubular mediated proteinuria in obese rats. Endocrinology. 2011;152(2):659-68. https://doi.org/10.1210/en.2010-1038 PMid:21177830 PMCid:PMC3037162

85. Ignarro LJ. Different pharmacological properties of two enantiomers in a unique beta-blocker, nebivolol. Cardiovasc Ther. 2008;26(2):115-34. https://doi.org/10.1111/j.1527-3466.2008.00044.x PMid:18485134

86. Li MH, Jang JH, Na HK, Cha YN, Surh YJ. Carbon monoxide produced by heme oxygenase-1 in response to nitrosative stress induces expression of glutamate-cysteine ligase in PC12 cells via activation of phosphatidylinositol 3-kinase and Nrf2 signaling. J Biol Chem. 2007;282(39):28577-86. https://doi.org/10.1074/jbc.M701916200 PMid:17681938

87. Stevenson TH, Gutierrez AF, Alderton WK, Lian L, Scrutton NS. Kinetics of CO binding to the haem domain of murine inducible nitric oxide synthase: differential effects of haem domain ligands. Biochem J. 2001;358(Pt 1):201-8. https://doi.org/10.1042/bj3580201 PMid:11485568 PMCid:PMC1222048

88. Wolf SC, Sauter G, Jobst J, Kempf VA, Risler T, Brehm BR. Major differences in gene expression in human coronary smooth muscle cells after nebivolol or metoprolol treatment. Int J Cardiol. 2008;125(1):4-10. https://doi.org/10.1016/j.ijcard.2007.02.045 PMid:17467819

89. Uzar E, Acar A, Evliyaoglu O, Firat U, Kamasak K, Gocmez C et al. The anti-oxidant and anti-apoptotic effects of nebivolol and zofenopril in a model of cerebral ischemia/reperfusion in rats. Prog Neuropsychopharmacol Biol Psychiatry. 2012;36(1):22-8. https://doi.org/10.1016/j.pnpbp.2011.08.011 PMid:21888941

90. Heeba GH, El-Hanafy AA. Nebivolol regulates eNOS and iNOS expressions and alleviates oxidative stress in cerebral ischemia/reperfusion injury in rats. Life Sci. 2012;90(11-12):388-95. https://doi.org/10.1016/j.lfs.2011.12.001 PMid:22226906

91. Moningka NC, Tsarova T, Sasser JM, Baylis C. Protective actions of nebivolol on chronic nitric oxide synthase inhibition-induced hypertension and chronic kidney disease in the rat: a comparison with angiotensin II receptor blockade. Nephrol Dial Transplant. 2012;27(3):913-20. https://doi.org/10.1093/ndt/gfr449 PMid:21856762 PMCid:PMC3289897

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):893-898.
https://doi.org/10.3889/oamjms.2017.198
eISSN: 1857-9655
Clinical Science

Effects of Pleuran (B–Glucan from *Pleurotus Ostreatus*) Supplementation on Incidence and Duration of COPD Exacerbations

Jordan Minov[1*], Jovanka Bislimovska-Karadzhinska[1], Tatjana Petrova[2], Kristin Vasilevska[3], Sasho Stoleski[1], Dragan Mijakoski[1]

[1]Institute for Occupational Health of Republic of Macedonia, Skopje; WHO Collaborating Center, Skopje, Republic of Macedonia; [2]Department of Pharmacy Practice, Chicago State University, Chicago, USA; [3]Institute for Epidemiology and Biostatistics, Faculty of Medicine, SS Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Minov J, Bislimovska-Karadzhinska J, Petrova T, Vasilevska K, Stoleski S, Mijakoski D. Effects of Pleuran (B–Glucan from Pleurotus Ostreatus) Supplementation on Incidence and Duration of COPD Exacerbations. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):893-898. https://doi.org/10.3889/oamjms.2017.198

Keywords: Chronic obstructive pulmonary disease; Exacerbations; Beta glucans; Pleuran; Supplement.

***Correspondence:** Jordan B. Minov. Institute for Occupational Health of R. Macedonia, Skopje – WHO Collaborating Center, Skopje, R. Macedonia. E-mail: minovj@hotmail.com

Received: 14-Sep-2017; **Revised:** 25-Sep-2017; **Accepted:** 26-Sep-2017; **Online first:** 16-Nov-2017

Funding: This research did not receive any financial support.

Competing Interests: The Publication Fee fro this article was covered by MEDIS DOO, Skopje, Republic of Macedonia.

Abstract

BACKGROUND: 1,3/1,6-β–glucans are recognised as immunomodulators in human and veterinary medicine for over 50 years.

AIM: To assess the effects of pleuran (1,3/1,6-β–glucan from *Pleurotus ostreatus*) on incidence and duration of bacterial exacerbations in patients with COPD.

METHODS: We performed an observational, non-randomized, open-label study including 32 COPD patients (Group D) in whom besides the recommended chronic treatment for the stable disease were administered supplement combination containing pleuran 100 mg, vitamin C 60 mg and zinc 5 mg once daily over a three month-period (Group 1). Also, an equal number of Group D COPD patients who besides the recommended treatment for stable disease received the supplement combination containing vitamin C 60 mg and zinc 5 mg once daily, matched to the study subjects of the Group 1 by sex and age served as control (Group 2).

RESULTS: Over the study period 57 exacerbations (24 in the Group 1 and 33 in the Group 2) were documented. A mean number of exacerbations over the study period was significantly lower in the Group1 (0.7 ± 0.4) as compared to their mean number in the Group 2 (1.0 ± 0.6) (P = 0.0218). Furthermore, a mean duration of exacerbations expressed in days needed for cure or clinical improvement (i.e. complete resolution of symptoms or return of the symptoms to their baseline severity) in the Group 1 (6.7 ± 0.8 days) was significantly shorter than the mean duration of exacerbations in the Group 2 (7.4 ± 1.3 days) (P = 0.0118). There was not reported any adverse effect during the study period by study subjects from both examined groups.

CONCLUSION: Our findings indicated that pleuran might impact the incidence and duration of bacterial exacerbations in patients with COPD. There is a need for further studies for more precise determination of the influence of pleuran on the course of COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) represents one of the principal demands of the public health at global level due to high morbidity, early mortality, high date rates and significant costs to health systems. The projection of the Global Burden of Disease Study indicates that COPD in 2020 will be the third leading cause of death worldwide (from sixth in 1990) and fifth leading cause of years lost (disability-adjusted life years - DALYs) through early mortality or handicap (12th in 1990) [1]. In addition, exacerbations of COPD, defined as acute events characterized by worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations leading to a change in medications, have substantial impact in the course of the disease because they negatively affect a patient's quality of life, accelerate the rate of decline of lung function and are associated with significant mortality, particularly in those requiring hospitalization. The most common cause of COPD exacerbations is believed to be bacterial respiratory infections [2, 3].

β–glucans comprise a diverse group of polysaccharides in which glucose molecules are

linked by β-linkages. β-1,3/1,6-glucans are branched chains of glucose molecules connected by β-1,3-glycosidic bonds in which the branching points are β-1,6 linkages. Some β-1,3/1,6-glucans are bioactive interacting with receptors on immune cells eliciting specific biological responses. The ability of β-1,3/1,6-glucans to activate innate immune cells depends on its branched structure, i.e. the chain length and the frequency of side chains are essential for immunomodulating properties of these β–glucans. Also, results of several studies indicated that insoluble β–glucans (i.e. non-absorbable and non-digestible) possessed higher immunomodulating activity than soluble ones [4-6].

β–glucans can be extracted from the cell walls of yeast, oat, barley, seaweeds, algae and bacteria, but the foremost source of medical glycans turns out to be fungal cell walls [7]. Pleuran is an insoluble 1,3/1,6-β–glucan from mushroom Pleurotus ostreatus. Some experimental studies on an animal model, as well as some experimental and clinical studies in humans, indicated immunomodulatory properties of pleuran that are based on its effects on immune cells of the Peyer's patches in the gut. After oral administration, pleuran comes in contact with immune cells of the Peyer's patches which expressed several receptors (e.g. Dectin-1, complement receptor-3, scavenger receptors, etc.) capable of recognising β–glucans in their various forms. Upon pleuran's binding to these receptors, a cascade of intracellular signaling that stimulates innate and subsequently adaptive immune responses is initiated, mainly through release of pro-inflammatory cytokines (complement components, interleukin-1α/β, interleukin-6, interleukin-8, interleukin-12, tumor necrosis factor-, eicosanoids, etc.) which improves the resistance to invading pathogens [8-11].

The aim of the present study was to assess the effects of pleuran on incidence and duration of bacterial exacerbations in patients with COPD.

Methods

Study design and setting

An observational, non-randomized, open study (a real life-study) was realized as a comparison of frequency and duration of bacterial exacerbations between a group D COPD patients who received the supplement combination containing pleuran 100 mg, vitamin C 60 mg and zinc 5 mg over a three month-period besides the chronic pharmacological treatment for stable disease recommended by actual GOLD and a group D COPD patients treated over a three month-period with the recommended chronic treatment for stable disease and the supplement combination containing vitamin C 60 mg and zinc 5 mg. It was performed in a period December 2016-April 2017 at the Institute for Occupational Health of Republic of Macedonia, Skopje.

Study subjects

The study population included 64 COPD patients, classified into group D according to the combined assessment of the disease, divided into two groups. The first group included 32 patients (18 males and 14 females, aged 48 to 71 years) who took the supplement combination containing pleuran 100 mg, vitamin C 60 mg and zinc 5 mg once daily in three months besides the recommended pharmacological treatment for the stable disease. The second group included an equal number of COPD patients (18 males and 14 females, aged 47 to 73 years) who received the supplement combination containing vitamin C 60 mg and zinc 5 mg once daily besides the recommended treatment for the stable disease, matched to the first group by sex, age and smoking status.

Patients with a history of asthma, lung cancer, or another significant respiratory disease, as well as those unable to complete diary cards, were excluded from the study. All study subjects were recruited in the stable phase of the disease, i.e. without any evidence of exacerbation for at least three weeks.

All study subjects were informed about the study, and their written consent was obtained.

Daily stable respiratory symptoms (baseline symptoms), medication use and history of exacerbations were noted in all subjects before entering the study. All study subjects underwent baseline and post-bronchodilator spirometry according to the actual recommendations of European Respiratory Society (ERS) and American Thoracic Society (ATS) [3, 12].

The Body Mass Index (BMI) as a measure of body fat based on height and weight that applies to adult population was determined in all study subjects by computed calculation using BMI calculator [13].

Classification of smoking status was done by the World Health Organization (WHO) recommendations [14]. Passive smoking or exposure to environmental tobacco smoke was defined as exposure to tobacco combustion products from smoking by others (at home, workplace, etc.), i.e. as a presence of at least one smoker in the household and the workplace [15, 16].

Diagnosis and assessment of COPD

According to the actual Global Initiative for Chronic Obstructive Lung Disease (GOLD) recommendations, COPD was considered by finding

of a post-bronchodilator ratio between forced expiratory volume in one second and forced vital capacity (FEV1/FVC ratio) less than 0.70 in symptomatic subjects (dyspnea, chronic cough or sputum production) with a history of exposure to risk factors for the diseases (noxious particles and gases).

Subjects with diagnosed COPD were classified according to the combined COPD assessment which included assessment of symptoms, the degree of airflow limitation and risk of exacerbations. COPD patients classified as a Group D were characterized by frequent symptoms (overall score of the COPD Assessment Test [CAT] equal or higher than 10), severe or very severe airflow limitation (FEV1 value ranging from 30 to 50% of its predicted value or less than 30% of its predicted value) and high risk of exacerbation (two or more exacerbations per year or one or more exacerbations requiring hospitalization per year) [2, 3].

Diagnosis and treatment of COPD exacerbation

According to the actual GOLD recommendations, COPD exacerbations were considered as acute events characterised by a worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations and led to a change in medication. The diagnosis of exacerbation was defined by the patient's symptoms, using the criteria described by Anthonisen et al. [17]. Probable bacterial aetiology was established when the exacerbation was Anthonisen type I (presence of three cardinal symptoms: increased dyspnea, sputum volume and purulence) or type II (presence of two cardinal symptoms) if increased purulence of sputum was one of the two symptoms.

The treatment of exacerbations with antibiotic was started empirically following the actual GOLD recommendations. In the cases with positive result of microbiological evaluation of sputum, the treatment was continued following the finding of the sensitivity of bacterias to a certain antibiotic. Oral corticosteroids were given as needed (a dose of 40 mg oral prednisone per day for five days). The course of exacerbation was evaluated as a function of the resolution of symptoms, and the treatment was considered to be successful if a cure or clinical improvement was achieved. The cure was defined as complete resolution of the cardinal symptoms, whereas the clinical improvement was defined as the return of the symptoms to their baseline severity [2, 3, 17].

Data collection (Daily diary card)

All study subjects maintained daily diary cards on which they noted any appearance of an increase in the intensity of major symptoms (dyspnea, sputum amount and sputum purulence) or minor symptoms (nasal discharge/congestion, sore throat, wheezing, cough, etc.) over their chronic (stable) symptoms. A member of the study team saw study subjects within 48 hours of the detection of deterioration in symptoms and diagnosis was confirmed of each case. Exacerbation and its resolution were defined as it is mentioned above. Exacerbation number and their duration were calculated for each study subjects based on data from diary cards for a three month-period of follow-up.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 for Windows. Continuous variables were expressed as mean values with standard deviation (SD), and the nominal variables as numbers and percentages. Analyses of the data included testing the differences in prevalence and comparison of the means by chi-square test (or Fisher's exact test where appropriate) and independent-samples T-test. A P-value less than 0.05 were considered as statistically significant.

Results

Demographic characteristics of the study subjects are shown in Table 1.

Table 1: Demographics of the study subjects

Variable	Group1 (n = 32)	Group 2 (n = 32)
M/F ratio	1.4	1.4
Mean age (years)	58.7 ± 6.4	59.4 ± 7.2
Mean BMI (kg/m^2)	25.9 ± 3.3	26.6 ± 2.8
Mean duration of COPD (years)	11.7 ± 4.2	10.9 ± 5.1
Mean values of spirometric parameters (% pred.)	67.6 ± 7.1	68.7 ± 6.8
FVC	42.3 ± 4.4	43.4 ± 4.1
FEV_1	0.63 ± 0.03	0.64 ± 0.02
FEV_1/FVC ratio		
Treatment of stable COPD	25 (78.1%)	26 (81.2%)
LA β_2-agonist + ICS	22 (68.8%)	23 (71.8%)
LA anticholinergic	6 (18.7%)	5 (15.6%)
Oral theophylline		
Number of exacerbations in the previous year	2.6 ± 0.3	2.7 ± 0.4
Smoking status		
Active smokers	11 (34.3%)	9 (28.1%)
Ex-smokers	17 (53.1%)	19 (59.4%)
Never smokers	4 (12.5%)	4 (12.5%)
Exposed to ETS	15 (46.8%)	18 (56.2%)
Comorbidities		
Arterial hypertension	9 (28.1%)	8 (25.0%)
Osteo-muscular disorders	5 (15.6%)	7 (21.8%)
Ischaemic heart disease	4 (12.5%)	5 (15.6%)
Diabetes mellitus type 2	3 (9.4%)	4 (12.5%)

Numerical data are expressed as a mean value with standard deviation; frequencies as number and percentage of study subjects with a certain

variable.

COPD: chronic obstructive pulmonary disease; M: male; F: female; BMI: body mass index; kg: kilogram; m: meter; % pred.: % of the predicted value; FVC: forced vital capacity; FEV1: forced expiratory volume in one second; LA: long-acting; ICS: inhaled corticosteroid; ETS: environmental tobacco smoke.

Over the study period, 57 exacerbations were documented (24 in the Group 1 and 33 in the Group 2) of which 12 required hospital treatment (five in the Group 1 [20.8%] and seven in the Group 2 [21.2%]). 43 of the 57 (75.4%) were treated only with oral antibiotics (19/24 [79.2%] in the Group1 and 24/33 [72.7%] in the Group 2) and 14 (24.6%) were treated with antibiotics and oral prednisolone (5/24 [20.8%] in the Group 1 and 9/33 [27.3%] in the Group 2).

A mean number of exacerbations over the study period was significantly lower in the Group1 (0.7 ± 0.4) as compared to their mean number in the Group 2 (1.0 ± 0.6) (P = 0.0218) (Figure 1).

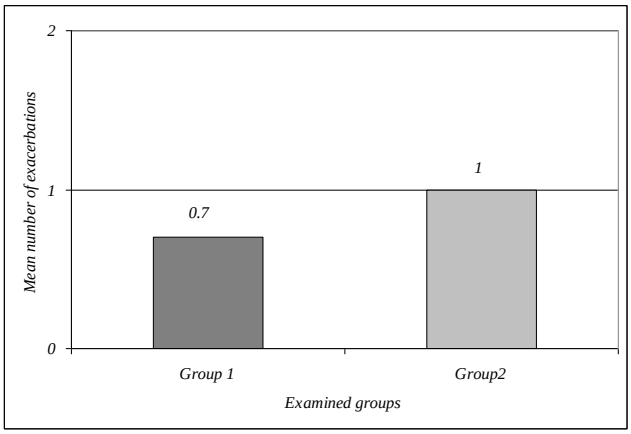


Figure 1: Mean number of exacerbations in the examined groups

A mean duration of exacerbations expressed in days needed for cure or clinical improvement (i.e. complete resolution of symptoms or return of the symptoms to their baseline severity) in the Group 1 (6.7 ± 0.8 days) was significantly shorter than the mean duration of exacerbations in the Group 2 (7.4 ± 1.3 days) (P = 0.0118) (Figure 2).

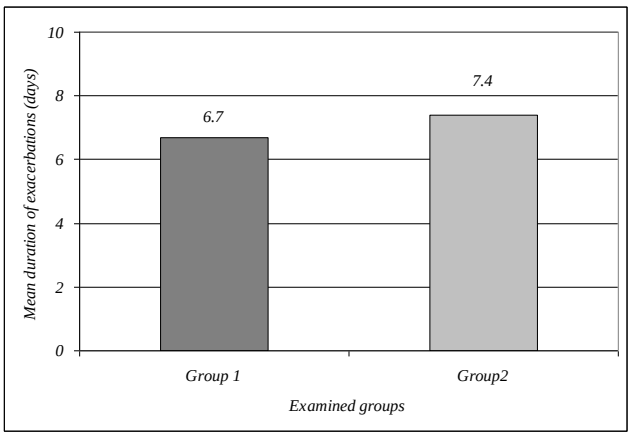


Figure 2: Mean duration of the exacerbations in the examined groups

Side effects of the used medicaments over the study period were not reported by any study subject from both examined groups.

Discussion

Pharmacological treatment of stable COPD is used to reduce symptoms, reduce frequency and severity of exacerbations, and improve health status and exercise tolerance. Existing medications for stable COPD have not been conclusively shown to modify the long-term decline in lung function that is the hallmark of the disease [2, 3]. As it is mentioned above, COPD is one of the most important public health problems in the last decades worldwide, so there are many investigations into new and more effective therapeutic options.

In the present study, we assessed the effects of pleuran (1,3/1,6-β–glucan from *Pleurotus ostreatus*) on the incidence and duration of exacerbations in patients with COPD. To our knowledge, the present study was the first one that investigated effects of pleuran on prevention of exacerbations in COPD patients. Examined groups included COPD patients classified into Group D, i.e. the COPD patients characterised by frequent symptoms, severe or very severe airflow limitation and high risk of exacerbation. Study subjects from both examined groups had similar demographic characteristics. Although tobacco smoke is recognised as the most important risk factor of COPD, in both groups, we found a large proportion of active and passive smokers which did not differ significantly from their prevalence in the general population in R. Macedonia documented in our previous studies [18, 19]. These findings suggested insufficient anti-smoking activities of healthcare workers among COPD patients besides smoking cessation is recommended as the first therapeutic option in the non-pharmacological treatment of the disease [2, 3].

The immunomodulatory properties of fungal β -glucans were studied and described almost 50 years ago. Shortly afterwards, in experimental animals were described their effects against a tumour. Finally, there is evidence that β-glucans may modulate other conditions (cholesterol level, glucose tolerance, etc.). [20-22]. As it is mentioned above, many studies have reported the ability of pleuran to activate innate immunity with effects also on adaptive immunity, inducing humoral and cell-mediated immune responses. Pleuran was found to increase the antimicrobial activity of mononuclear cells and neutrophils and enhance the functional activity of macrophages.

Findings of our studies showed significantly lower incidence, as well as the significantly shorter duration of exacerbations in COPD patients treated with pleuran besides regular treatment of stable disease as opposite to their incidence and duration in COPD patients treated only with the regular treatment of stable disease. Similar findings were reported by several studies which investigated effects of pleuran on incidence, duration and severity of respiratory infections in both children and adults. Results from the double-blinded, placebo-controlled, randomized, multicentric study carried out by Jesenak et al. including 175 children (mean age 5.65 ± 2.39 years) treated with pleuran over the 12 month-period indicated significant reduction of the frequency of recurrent respiratory tract infections in children treated with pleuran as compared to children who did not use it [23]. Similar findings were obtained from the study carried out by Pico Sirvent et al. including 166 children aged 1 to 10 years from 20 pediatric departments in Northeast Spain [24].

Results from the double-blind, placebo-controlled, randomized study carried out by Bergendiova et al. including 50 athletes treated with pleuran over the 3 month-period indicated significant reduction of the frequency of upper respiratory tract infections as compared to their frequency in athletes treated with placebo. Furthermore, the authors found significantly higher number of circulating natural killers cells in pleuran group as compared to their number in the placebo group [25]. In addition, in the study which investigated effects of pleuran on changes in the peripheral blood cells after acute, exhausting physical load in 22 elite athletes Bobovcak et al. found no statistically significant reduction in natural killers cell activity in the group treated with pleuran as opposite to the finding of significant reduction in natural killer cell activity after intensive exercise in the placebo group [26].

As it is mentioned above, pleuran as an insoluble substance is non-absorbable and non-digestible, so systemic adverse effects could not be expected. As in the case of the present study, local (i.e. gastrointestinal) adverse effects were not registered in all cited studies.

The present study must be interpreted within the context of its limitations. The results should be viewed with caution, since the study was neither blinded nor randomized 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. Also, the small number of the subjects in the examined groups could have certain implications on the data obtained and its interpretation. The short follow-up period could also have certain implications on the data obtained and its interpretation. Furthermore, in the study groups were included only Group D COPD patients that that could impact results of the study.

In conclusion, in an observational, non-randomized, open-label study including a Group D COPD patients who received the supplement combination containing pleuran 100 mg, vitamin C 60 mg and zinc 5 mg besides recommended chronic pharmacological treatment for stable disease over three months we found significantly lower incidence and significantly shorter duration of bacterial exacerbations as compared to their incidence and duration in a Group D COPD patients who took the supplement combination containing vitamin C 60 mg and zinc 5 mg besides the recommended treatment for stable disease in the same period. Our findings suggested that the use of pleuran may be beneficial in the prevention of exacerbations in the patients with COPD. Further investigations, as well as comparisons to the other therapeutic modalities, are needed for assessment of the preventive effects of pleuran regarding the COPD exacerbations.

Ethical Approval

The Ethical Committee of the Institute of Occupational Health of R. Macedonia, Skopje – WHO Collaborating Center and GA2LEN Collaborating Center approved for performing the study and publishing the results obtained (03-48/23.01.2017).

Authors Participations

JM participated in the study design, data collection, managing the analyses of the study, and writing all versions of the manuscript. JKB and TP participated in the study design and managing the analyses of the study. KV performed the statistical analysis and participated in the managing the analyses of the study. SS and DM participated in the data collection and the managing the analyses of the study. All authors read and approved the final manuscript.

References

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PloS Med. 2006; 3: e442. https://doi.org/10.1371/journal.pmed.0030442 PMid:17132052 PMCid:PMC1664601

2. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease: 2014 Update. Available at: http://www.goldcopd.org/ (Accessed 14.04.2017).

3. Global Initiative for Chronic Obstructive Pulmonary Disease. A pocket guide to COPD diagnosis, management and prevention: Updated 2015. Available at: http://www.goldcopd.org/ (Accessed 14.04.2017).

4. Bohn JA, BeMiller JN. (1-3)-beta-D-glucans as biological response modifiers: a review of structure-function relationships. Carbohydr Polymers. 1995; 28: 3-14. https://doi.org/10.1016/0144-8617(95)00076-3

5. Raa J. Immune modulation by non-digestible and non-

apsorbable beta-1,3/1,6-glucan. Microbial Ecology in Health & Disease. 2015; 26: 27824. https://doi.org/10.3402/mehd.v26.27824 PMid:26031679 PMCid:PMC4451094

6. Vannucci L, Krizan J, Sima P, et al. Immunomodulatory properties and antitumor activities of glucans (Review). International Journal of Oncology. 2013; 43: 357-364. https://doi.org/10.3892/ijo.2013.1974 PMid:23739801 PMCid:PMC3775562

7. Yap AT, Ng ML. An important method for the isolation of lentina from edible and medicinal shitake mushroom, Lentinus edodes (Berk.) Sing. (Agaricomycetideae). In J Med Mushr. 2001; 3: 6-19.

8. Brown GD, Gordon S. Immune recognition. A new receptor for beta-glucans. Nature. 2001; 413: 36-37. https://doi.org/10.1038/35092620 PMid:11544516

9. Goodridge HS, Wolf AJ, Underhill DM. Beta-glucan recognition by the innate immune system. Immunol Rev. 2009; 230 (1): 38-50. https://doi.org/10.1111/j.1600-065X.2009.00793.x PMid:19594628

10. Batbayar S, Lee DH, Kim HW. Immunomodulation of fungal β-glucan in host defense signaling by dectin-1. Biomol Ther. 2012; 20 (5): 433-445. https://doi.org/10.4062/biomolther.2012.20.5.433 PMid:24009832 PMCid:PMC3762275

11. Volman JJ, Ramakers JD, Plat J. Dietary modulation of immune function by beta-glucans. Physiol Behav. 2008; 94: 276-284. https://doi.org/10.1016/j.physbeh.2007.11.045 PMid:18222501

12. Spirometry Guide: 2010 Update. Available at: http://www.goldcopd.org/uploads/users/files/GOLD_Spirometry_2010. (Accessed: 16.04.2017).

13. Calculate your Body Mass Index. Available at: https://www.nhlbi.nih.gov (Accessed: 16.04.2017).

14. World Health Organization. Guidelines for controlling and monitoring the tobbaco epidemic. Geneva: WHO, 1998.

15. U.S. Department of Health and Human Services. The health consequences of smoking: chronic obstructive pulmonary disease. A report of the Surgeon General. US Department of Health and Human Services, Public Health Service, Office of the Assistant for Health, Office of Smoking and Health. DHHS Publication No. 84-50 205, 1984.

16. Janson C, Chinn S, Jarvis D, et al. Effects of passive smoking on respiratory symptoms, bronchial responsiveness, lung function, and total serum IgE in the European Community Respiratory Health Survey: a cross-sectional study. Lancet. 2001; 358: 2103-2109. https://doi.org/10.1016/S0140-6736(01)07214-2

17. 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

18. Minov J, Karadzinska-Bislimovska J, Nelovska Z, Vasilevska K, Risteska-Kuc S, Stoleski S, Mijakoski D. Smoking among Macedonian workers five years after anti-smoking campaign. Arh Hig Rada Toksikol. 2012; 63: 207-213. https://doi.org/10.2478/10004-1254-63-2012-2150 PMid:22728803

19. Minov J. Smoking among Macedonian Workers. Saarbrücken: LAP LAMBERT Academic Publishing, 2013.

20. Cassone A, Bistoni F, Cenci E, et al. Immunopotentiation of anticancer therapy by Candida albicans, other yeasts and insoluble glucan in an experimental lymphoma model. Sabouraudia. 1982; 20: 115-125. https://doi.org/10.1080/00362178285380191 PMid:7051368

21. Zhang Y, Xia L, Pang W, et al. A novel soluble β-1,3-d-glucan Salecan reduces adiposity and improve glucose tolerance in high-fat diet-fed mice. Br J Nutr. 2012; 13: 1-9.

22. Haggard L, Andersson M, Punga AR. β-glucans reduce LDL cholesterol in patients with myastenia gravis. Eur J Clin Nutr. 2013; 67: 226-227. https://doi.org/10.1038/ejcn.2012.191 PMid:23187951

23. Jesenak M, Majtan J, Rennerova Z, et al. Immunomodulatory effect of pleuran (β-glucan from Pleurotus ostreatus) in children with recurrent respiratory tract infections. Int Immunopharmacol. 2013; 15 (2): 395-399. https://doi.org/10.1016/j.intimp.2012.11.020 PMid:23261366

24. Pico Sirvent L, Sapena Grau J, Morera Ingles M, Rivero Urgell M. Effect of supplementation with β–glucan from Pleurotus ostreatus in children with recurrent respiratory infections. Ann Nurr Metab. 2013; 63 (1): 1378.

25. Bergendiova K, Tibenska E, Majtan J. Pleuran (β-glucan from Pleurotus ostreatus) supplementation, cellular immune response and respiratory tract infections in athletes. Eur J Appl Physiol. 2011; 111 (9): 2033-2040. https://doi.org/10.1007/s00421-011-1837-z PMid:21249381

26. Bobovcak M, Kuniakova R, Gabriz J, Majtan J. Effect of Pleuran ((β-glucan from Pleurotus ostreatus) supplementation on cellular immune response after intensive exercise in elite athletes. Appl Physiol Nutr Metab. 2010; 35 (6): 755-762. https://doi.org/10.1139/H10-070 PMid:21164546

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):899-903.
https://doi.org/10.3889/oamjms.2017.190
eISSN: 1857-9655
Clinical Science

Relationship between Vitamin D, Inflammation and Lung Function In Patients with Severe Uncontrolled Asthma

Elena Janeva-Jovanovska[1*], Dejan Dokic[1], Biserka Jovkovska-Kaeva[1], Gorica Breskovska[1], Zlatica Goseva[1], Jordan Minov[2], Dejan Trajkov[3], Magdalena Dimitrova-Genadieva[4], Beti Zafirovska-Ivanovska[5]

[1]PHI University Clinic of Pulmonology and Allergy, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]Institute for Occupational Health of RM - Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [3]Institute of Immunobiology and Human Genetics, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [4]PHI University Clinic of Gastroenterohepatology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [5]Institute of Epidemiology and Biostatistics, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Janeva-Jovanovska E, Dokic D, Jovkovska-Kaeva B, Breskovska G, Goseva Z, Minov J, Trajkov D, Dimitrova-Genadieva M, Zafirovska-Ivanovska B. Relationship between Vitamin D, Inflammation and Lung Function In Patients with Severe Uncontrolled Asthma. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):899-903. https://doi.org/10.3889/oamjms.2017.190

Keywords: asthma; Vitamin D; IL-33; FEV1; Th2-type cytokines.

***Correspondence:** Elena Jovanovska-Janeva, MD, MSc. PHI University Clinic of Pulmonology and Allergy, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Macedonia. Tel.: +389 72 232 513. E-mail: eli.janeva@yahoo.com

Received: 24-Oct-2017; **Revised:** 03-Nov-2017; **Accepted:** 04-Nov-2017; **Online first:** 19-Nov-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Recently epidemiological studies showed that low vitamin D is linked to airway hyperresponsiveness, decreased lung function, poor asthma control, and steroid–resistant asthma.

AIM: We investigated the relationship between Vitamin D, inflammation with circulating IL-33 and lung function in 30 patients with severe uncontrolled asthma.

MATERIALS AND METHODS: The study included 30 patients with severe uncontrolled asthma. In each of them were measured serum levels of IL-33 and Vitamin D by the ELISA method. The pulmonary function is measured by basic spirometry parameters, FEV1. The results were statistically elaborated according to the Pearson's Correlation Tests.

RESULTS: The results showed statistically insignificant correlation between Vitamin D and IL-33, and Vitamin D with FEV1 (Vit.D/IL-33; r = 0.11323, p = 0.551); (Vit.D/FEV1; r = -0.1005; p = 0.597) Correlation between IL-33 and FEV1 is negative but statistically significant (IL-33/FEV1; r = -0.5248; p = 0.003).

CONCLUSION: Because there are little studies about the link between vitamin D and asthma, further research to clarify the mechanism how vitamin D control the activity of CD4+ T cells and the related Th2-type cytokines in the parthenogenesis of asthma.

Introduction

Asthma is the leading chronic respiratory diseases which affect more than 334 million people worldwide [1-3]. It is estimated that the number of people with asthma will grow by more than 100 million by 2025. Women were more likely than men and boys more likely than girls to have asthma [4]. Approximately 500,000 annual hospitalisations are due to asthma, and 250 000 deaths annually [3]. Five percentages or 100,000 of the Macedonian population have asthma [5, 6].

Characteristic mechanisms of asthma include inflammation, hyperresponsiveness and structural changes in the airways and the lung. Many cellular elements and different cells play a role (T lymphocytes, neutrophils, eosinophils, mast cells, macrophages, and dendritic cells). Recurrent episodes of wheezing, chest tightness, coughing and breathlessness, particularly at night or in the early morning are a clinical expression of this disease. These episodes are usually variable, and the airflow obstruction within the lung is often reversible with treatment or spontaneously [7].

Chronic inflammation of the asthmatic airway leads to epithelial desquamation, infiltration of the airway wall with T cells especially dominated Th2 helper CD4+ lymphocytes, smooth muscle, hypertrophy and hyperplasia, vascular congestion, oedema due to plasma leakage and mucus plugging [8-11]. All these changes could lead to thickening of

the airway walls due to subepithelial fibrosis and reduction of their lumen [12, 13].

More than 100 mediators and markers of inflammation are involved in this inflammation. Cytokines take place in complex pathophysiological process, and they are produced by cells like T cells, Tc, Th, Th1, Th2, NK, dendritic cells, B cells, endothelial cells, plasma cells, mast cells, bone marrow, tumor cells and thymus, together with, leukocytes, macrophages, fibroblasts, and monocytes [14, 15]. The interleukins are cytokines which can stimulate the proliferation and differentiation of immune cells. In asthma, T cells take a central place in coordinating the immune response. Cytokines present during activation of naïve CD4 + T cells from pathogens and many types of antigens differentiate them into subpopulations of T cells in T-follicular effector cells, Th1, Th2, Th9, Th17, and Th22. Th2 cells synthesise IL-4, IL-5, IL-13, IL-25, IL-31, IL-33 which are associated with asthma and allergic diseases [16-19].

Interleukin 33 (IL-33) is a new cytokine which was found in 2005. It belongs to the IL-1 family consisting of 11 members, high proinflammatory cytokines which activate the function of inflammation cell in the early asthmatic responses. IL-33 is a potent type 2 inducing cytokine. It is bind to ST2 receptors, which are expressed on mast cells and Th2 cells, and some various cells including epithelial, bronchial and endothelial cells, fibroblasts and some immune cells, as well as dendritic cells and macrophages [20-23]. It is assumed that IL-33 is one of the earliest released mediators and can orchestrate the immune cascade of asthma and stands out as an attractive candidate for discovering various therapeutic modalities, especially a new targeted therapy.

Vitamin D plays a role in the pathogenesis of asthma. Vitamin D is a potent immune system modulator and in the form of 1,25-dihydroxy vitamin D has been shown that it can be involved in the suppression of dendritic cell maturation and the development of consecutive Th1 cell [24-27]. Vitamin D may suppress the production of IL-12, which reduces the production of Th1 cells and potentially leading to increased proliferation of Th2 cells [25]. Additionally, studies with treatment of 1,25-dihydroxy vitamin D in mice showed reduced secretion of Th1-type cytokines IL-2 and IFN-γ and an increase in Th2-type IL-4. In asthmatic children, low vitamin D levels are associated with airway hyperresponsiveness, decreased lung function, worse asthma control, and steroid-resistant asthma and exacerbations. It remains unknown whether vitamin D is an association with increased airway hyperresponsiveness (AWH), inflammatory markers of asthma, decreased lung function, poor asthma control and steroid resistance in adult asthma patients [28-30].

We aimed to investigate the relationship between Vitamin D, inflammation with circulating IL-33 and lung function in 30 patients with severe uncontrolled asthma.

Material and Methods

The study included 30 patients with asthma. They are diagnosis and treated at the Clinic of Pulmonology and Allergy in Skopje, Macedonia. All of them are a classification of uncontrolled moderate persistent asthma. In all patients, serum IL-33 level was measured by the ELISA, enzyme-linked immunosorbent assay method according to the manufacture's protocol at the Institute of Immunobiology and Human Genetics, Faculty of Medicine, in Skopje, Macedonia. Values within the reference range for IL-33 are 0pg/ml. Serum 25-hydroxyvitamin D was measured by ELISA method according to the manufacture's protocol at University Clinic of Clinical biochemist, Skopje, Macedonia.

At the Clinic of pulmonology and allergy in Skopje, was done the spirometry with turbine spirometer - Spirobank G, according to the standard methodology. In all patients was done and analysed FEV1 - Forced expiratory volume in the first second. Every patient is done three manoeuvres with three acceptable and three reproducible curves, according to guidelines for the measurement of Spirometry, with Flow-Volume and Time-Volume curve. The best curve has been automatically selected from the spirometer [31-33].

Inclusion Criteria: the patients are diagnosed and classified in uncontrolled moderate persistent asthma at PHI University Clinic of Pulmonology and Allergy according to the actual version of the Global Initiative for Asthma guidelines – GINA [7] and Guidelines for the Diagnosis and Management of Asthma (EPR-3) of NAEPP, National Asthma Education Prevention Program [33]. Uncontrolled asthma defined as at least one of the following [34].

Poor asthma symptom control: Asthma Control Questionnaire (ACQ) consistently >1.5, or Asthma Control Test <20 (or ''not well controlled'' by GINA - NAEPP guidelines over three months of evaluation:

- two or more bursts of systemic CS (3 days each) in the previous year - frequent severe exacerbations;
- at least one hospitalisation, required to stay on ICU or mechanical ventilation in the previous year - serious exacerbations; and
- after an appropriate bronchodilator withhold FEV1<80% predicted - airflow limitation.

All patients have stable asthma because there

has been no increase asthma symptoms or need for add another asthma medication for at least the past four weeks. Some of the patients have arterial hypertension, and they did not have other comorbidities, that could increase the IL-33 level. The age range of patients was 20-71 years old.

Exclusion criteria were severe diseases of renal, neurological, haematological, cardiac, gastrointestinal, endocrine or immune system, psychiatric disorders, and neoplastic diseases. Pregnancy is also exclusion criteria.

Statistical analysis

The results were statistically analysed by the statistical program SPSS for Windows 17.0. The results were statistically analyzed according to the Pearson's Correlation Tests. The significances values were taken $p < 0.05$.

Results

The study included 30 patients with severe uncontrolled asthma. The majority were females, 19 women (63.33%), and 11 were men (36.67%). The age of the patients was 20-71 years. The obtained values showed (Table 1):

Table 1: Mean values for Vitamin D, IL-33 and FEV1

N-30	mean	Std.Dv
Vitamin D	15.260	5.808
IL-33	33.461	8.851
FEV1	44.367	14.207

All patients had low serum vitamin D and their correlation with IL-33 showed statistically insignificant correlation (Vit.D/IL-33; r = 0.11323; p = 0.551) (Figure 1).

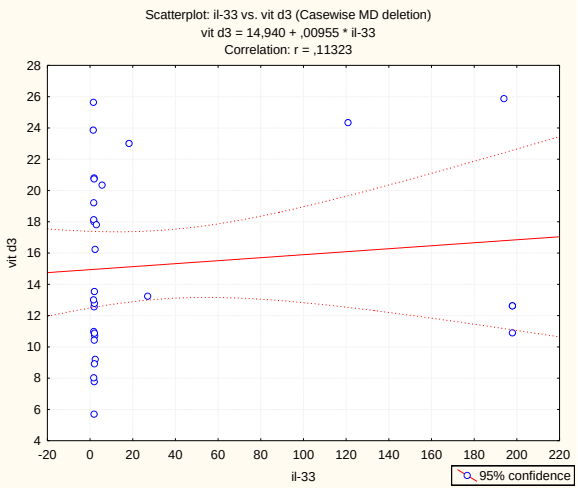


Figure 1: Correlation between Vitamin D and IL-33 in asthma patients. (r = -0.11323; p = 0.551)

Negative significant correlation between IL-33 and FEV1 (IL-33/FEV1; r = -0.5248; p = 0.003) (Figure 2).

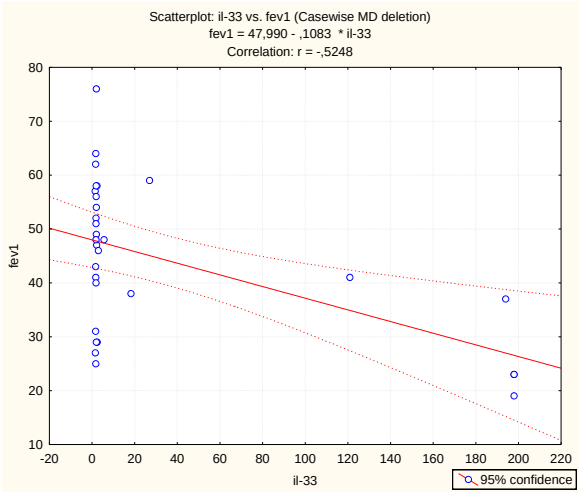


Figure 2: Correlation between IL-33 and FEV1 in asthma patients. (r = -0.5248; p = 0.003)

Negative insignificant correlation between serum levels of vitamin D and FEV1 (Vit.D/ FEV1; r = -0.1005; p = 0.597) (Figure 3)

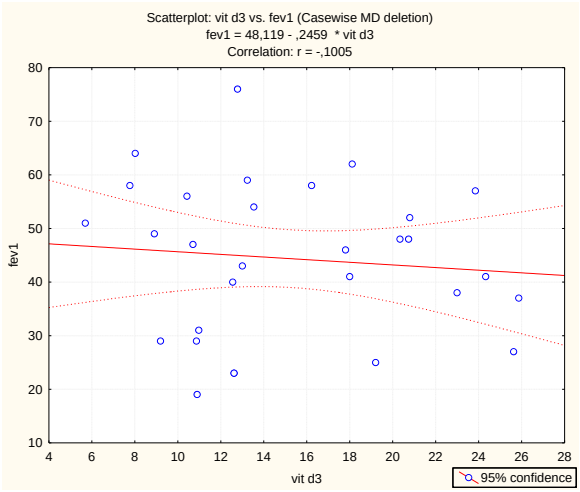


Figure 3: Correlation between Vitamin D and FEV1 in asthma patients. (r= -0.1005; p = 0.597)

Discussion

The studies which investigate vitamin D status, inflammatory markers of asthma and lung function are scarce. The studies which investigated the direct mechanistic links between vitamin D and lung diseases are limited. Cross section of data indicate that low level of vitamin D in patients with mild and moderate asthma is associated with more frequent exacerbations, poor asthma control, decreased lung function, steroid–resistant asthma, and consequent increased steroid use [24-26, 35-37].

A couple study presented that the low level of vitamin D has an association with airway obstruction and corticosteroid requirement, influencing the severity in asthmatic patients. Our study showed that serum vitamin D level was lower (level of vitamin D < 30 ng/ml) in these 30 patients with asthma but their correlation with IL-33 was statistically insignificantly. Anna Bonanno et al. in their study showed that Vitamin D it is not involved in IL-33/IL-31, Th2-type cytokines activity implicated in bronchial and nasal allergic disease [24]. Felicia M.A. et al. in their study they did not find a link between allergy markers (allergic rhinitis, eosinophil count, total IgE) and vitamin D levels [38].

Vitamin D inhibits the synthesis and releases cytokines which are Th1-associated and some other molecules, like IL-17, which lead to decreased inflammation and proliferation of smooth muscle cell [35, 38, 39]. Recent studies have shown that low level of vitamin D is linked with increased expression of the pro-inflammatory cytokine TNF-α, enhancing a pro-inflammatory effect in patients with asthma [35, 40]. This vitamin promotes regulatory T cells and also increases synthesis of IL-10, which lead to an inhibition of Th2 responses as well as airway inflammation and airway hyperresponsiveness [35, 41].

Larose et al. in HUNT, prospective cohort study, found that low serum 25(OH) D level was not correlated with airway obstruction in most asthmatics adults except men with asthma but without allergic rhinitis [42]. In our study, there was an insignificant correlation between serum levels of vitamin D and FEV1. Laura et al. also showed in their study that the vitamin D level did not correlate with lung function and markers of allergy in asthmatic patients [43]. Some studies show a significant direct relation between vitamin D level and both FEV1 and FEV1/FVC [43, 44].

In this study, we found that serum IL-33 was increased in patients with asthma and they positively correlated with asthma severity with the significant negative correlation between IL-33 and FEV1. In human studies of allergic asthma, serum IL-33, bronchoalveolar lavage fluid and lung tissue have found to be higher in patients with asthma compared to healthy controls and correlate with asthma severity [45-50], which was confirmed in our study

In conclusion, despite the limitation of this study with a small number of patients, we found an increased serum IL-33 in patients with asthma and it is positively related to the severity of asthma (FEV1), but we did not find a correlation between levels of vitamin D and FEV1 and IL-33. Because there are little studies about the link between asthma and vitamin D, further studies are necessary to explain the mechanism how vitamin D can control the activity of CD4+ T cells and the related Th2 type cytokines in the pathogenesis of the allergic disease, including asthma.

References

1. Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy. 2004; 59(5): 469-478. https://doi.org/10.1111/j.1398-9995.2004.00526.x PMid:15080825

2. Beasley R. The Global Burden of Asthma Report, Global Initiative for Asthma (GINA). Available from http://www.ginasthma.org 2004.

3. World Health Organization. Global surveillance, prevention and control of chronic respiratory diseases: a comprehensive approach, 2007.

4. Centers for Disease Control and Prevention, Vital Signs, May 2011.

5. Gjorcev A. Makedonski nacionalen plan i program za dijag-nostikai lekuvanje na bronhilajnata asthma-realnost i vizija, 1996.

6. Vladimir Cvetanov. Alergiskite bolesti vo R. Makedonija, 2006: 151-174.

7. Global Initiative for Asthma (GINA), GINA Report 2017, Global Strategy for Asthma Management and Prevention updated 2017. http://www.ginasthma.org

8. Holgate ST. Genetic and environmental interaction in Allergy and asthma. J Allergy Clin Immunol. 1999; 104(6): 1139-1146. https://doi.org/10.1016/S0091-6749(99)70005-9

9. Thomas MJ, Kemeny DM. Novel CD4 and CDS T-cell subsets. Allergy. 1998; 53: 1122-1132. https://doi.org/10.1111/j.1398-9995.1998.tb03831.x

10. Heard BE, Hossain S. Hyperplasia of bronchial muscle in asthma. J Pathol. 1973; 110: 319-331. https://doi.org/10.1002/path.1711100406

11. Shiels IA, Bowler SD, Taylor SM. Airway smooth muscle proliferation in asthma: the potential of vascular leakage to contribute to pathogenesis. Med Hypotheses. 1995; 45: 37-40. https://doi.org/10.1016/0306-9877(95)90198-1

12. Dirkje S Postma, Wim Timens. Remodeling in Asthma and Chronic Obstructive Pulmonary Disease. American Thoracic Society. 2006; 3(5): 434-439. https://doi.org/10.1513/pats.200601-006AW PMid:16799088

13. Christopher L. Grainge, Laurie C.K. Lau, Jonathon A. Ward, et al. Effect of bronchoconstriction on Airway Remodeling in Asthma. N Engl J Med. 2011; 364: 2006-2015. https://doi.org/10.1056/NEJMoa1014350 PMid:21612469

14. Barnes PJ. Pathophysiology of asthma. Eur Respir Mon. 2003; 23: 84-113.

15. Kuipers H, Lambrecht BN. The interplay of dendritic cells, Th2cells and regulatory T cells in asthma. Curr Opin Immunol. 2004; 16(6): 702-708. https://doi.org/10.1016/j.coi.2004.09.010 PMid:15511661

16. Abbas AK, Lichtman Ah, Pober JS. Cellular and Molecular Immunology. 4th ed., 2000: 235-69.

17. Roitt IM, and Delves PJ. Essential Immunology, 10th ed., 2001: 177-199.

18. Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. J Allergy Clin Immunol. 2003;111: 450-463. https://doi.org/10.1067/mai.2003.169 PMid:12642820

19. Zhu J, Hidehiro Y, William EP. Differentiation of effector CD4 T cell populations. Annu Rev Immunol. 2010;28: 445-489. https://doi.org/10.1146/annurev-immunol-030409-101212 PMid:20192806 PMCid:PMC3502616

20. Borish L, Steinke JW. Interleukin-33 in asthma: how big of a role does it play? Curr Allergy Asthma Rep. 2011; 11: 7-11. https://doi.org/10.1007/s11882-010-0153-8 PMid:20931364 PMCid:PMC3068600

21. Schmitz J, Owyang A, Oldham E., et al. et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T-helper type 2-associated cytokines.

Immunity. 2005; 23: 479-490. https://doi.org/10.1016/j.immuni.2005.09.015 PMid:16286016

22. Kakkar R, Lee RT. The IL-33/ST2 pathway: therapeutic target and novel biomarker. Nat Rev Drug Discov 2008; 7: 827-840. https://doi.org/10.1038/nrd2660 PMid:18827826 PMCid:PMC4277436

23. Miller AM. Role of IL-33 in inflammation and disease. J Inflamm. 2011; 8: 22. https://doi.org/10.1186/1476-9255-8-22 PMid:21871091 PMCid:PMC3175149

24. Anna Bonanno, Sebastiano G. Stefania La G. at al. 25-Hydroxyvitamin D, IL-31, and IL-33 in Children with Allergic Disease of the Airways Mediators Inflamm. 2014; 2014: ID 520241

25. Benson AA, Toh JA, Vernon N, Jariwala SP. The role of vitamin D in the immunopathogenesis of allergic skin diseases. Allergy. 2012;67(3):296–301. https://doi.org/10.1111/j.1398-9995.2011.02755.x PMid:22171613

26. Annesi-Maesano I. Perinatal events, vitamin D, and the development of allergy. Pediatric Research. 2002;52(1):3–5. https://doi.org/10.1203/01.PDR.0000017228.29635.EF

27. Meyts I, Hellings PW, Hens G, et al. IL-12 contributes to allergen-induced airway inflammation in experimental asthma. Journal of Immunology. 2006;177(9):6460–6470. https://doi.org/10.4049/jimmunol.177.9.6460

28. Matheu V, Bäck O, Mondoc E, Issazadeh-Navikas S. Dual effects of vitamin D-induced alteration of TH1/TH2 cytokine expression: enhancing IgE production and decreasing airway eosinophilia in murine allergic airway disease. The Journal of Allergy and Clinical Immunology. 2003;112(3):585–592. https://doi.org/10.1016/S0091-6749(03)01855-4

29. Wu AC, Kelan T, Lingling L, et al. The effect of vitamin D and inhaled corticosteroid treatment on lung function in children. Am J Respir Crit. Care Med. 2012; 186(6):508-513. https://doi.org/10.1164/rccm.201202-0351OC PMid:22798322 PMCid:PMC3480528

30. Hala Mohamed ShS, Amany RE, Eman N. Vitamin D and markers of airway inflammation in asthma. Egyptian Journal of Chest Diseases and Tuberculosis. 2015; 64:779–783. https://doi.org/10.1016/j.ejcdt.2015.03.027

31. Clinical Pulmonary Function Testing, Exercise Testing, and Disability Evaluation. In Chest Medicine: Essentials Of Pulmonary And Critical Care Medicine. 5th edition. Editors: George RB, Light RW, Matthay RA, Matthay MA, May 2005.

32. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J. 2005;26:319-338. https://doi.org/10.1183/09031936.05.00034805 PMid:16055882

33. National Asthma Education, Prevention Program (National Heart, Lung, Blood Institute). Second Expert Panel on the Management of Asthma. Expert panel report 2: Guidelines for the diagnosis and management of asthma. DIANE Publishing; 1998.

34. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, Adcock IM, Bateman ED, Bel EH, Bleecker ER, Boulet LP. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. European Respiratory Journal. 2013:erj02020-2013.

35. Sandhu MS, Casale TB. The role of vitamin D in asthma. Ann Allergy Asthma Immunol. 2010; 105:191-199. https://doi.org/10.1016/j.anai.2010.01.013 PMid:20800785

36. Stephanie K, Hübner M, Matthias J, Maria B, Roland B. Severe and uncontrolled adult asthma is associated with vitamin D insufficiency and deficiency, Respir Res. 2013; 14:25. https://doi.org/10.1186/1465-9921-14-25 PMid:23432854 PMCid:PMC3648461

37. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr. 2006;84:18-28. PMid:16825677

38. Felicia Montero-Arias,Giovanni Sedó-Mejía, Allan Ramos-Esquivel. Vitamin D Insufficiency and Asthma Severity in Adults From Costa Rica. Allergy Asthma Immunol Res. 2013;5(5):283-288. https://doi.org/10.4168/aair.2013.5.5.283 PMid:24003384 PMCid:PMC3756174

39. Matheu V, Bäck O, Mondoc E, Issazadeh-Navikas S. Dual effects of vitamin D-induced alteration of TH1/TH2 cytokine expression: enhancing IgE production and decreasing airway eosinophilia in murine allergic airway disease. J Allergy Clin Immunol 2003;112:585-92. https://doi.org/10.1016/S0091-6749(03)01855-4

40. Sutherland ER, Goleva E, Jackson LP, Stevens AD, Leung DY. Vitamin D levels, lung function, and steroid response in adult asthma. Am J Respir Crit Care Med. 2010;181:699-704. https://doi.org/10.1164/rccm.200911-1710OC PMid:20075384 PMCid:PMC2868500

41. Lange NE, Litonjua A, Hawrylowicz CM, Weiss S. Vitamin D, the immune system and asthma. Expert Rev Clin Immunol. 2009;5:693-702. https://doi.org/10.1586/eci.09.53 PMid:20161622 PMCid:PMC2812815

42. Larose TL, Arnulf L, Yue Chen, et al. Serum 25-hydroxyvitamin D levels and lung function in adults with asthma: the HUNT Study. Eur Respir J. 2015;45(4):1019-26. https://doi.org/10.1183/09031936.00069714 PMid:25395037

43. Laura T, Edita G, Simona L, et al. Evaluation of vitamin D levels in allergic and non-allergic asthma. Medicine. 2015; 51(6):321-327. https://doi.org/10.1016/j.medici.2015.11.003 PMid:26739673

44. Lin Z, Li W. The roles of vitamin D and its analogues in inflammatory diseases. Current topics in medicinal chemistry. 2016;16(11):1242-61. https://doi.org/10.2174/1568026615666150915111557 PMid:26369816

45. Li YM, Cheng XM. Clinical significance of IL-18 and IL-33 levels changes in the treatment of bronchial asthma with acute attack stage by glucocorticoids. Practical Pharmacy and Clinical Remedies. 2013; 16: 897-9.

46. Azazi EA, Elshora AE, Tantawy EA, Elsayd MA. Serum levels of Interleukin-33 and its soluble receptor ST2 in asthmatic patients. Egyptian Journal of Chest Diseases and Tuberculosis. 2014;63(2):279-84. https://doi.org/10.1016/j.ejcdt.2013.11.005

47. Liu SP, Ma R, Li XA. The relationship between interleukin 33 promotes and lung inflammation in asthmatic children. Chin J Diffic and Compl Cas. 2013; 12:422-4.

48. Préfontaine D, Nadigel J, Chouiali F, et al. Increased IL-33 expression by epithelial cells in bronchial asthma. J Allergy Clin Immunol. 2010; 125: 752-4. https://doi.org/10.1016/j.jaci.2009.12.935 PMid:20153038

49. Préfontaine D. Lajoie-Kadoch S, Foley Set al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. J Immunol. 2009; 183: 5094-103. https://doi.org/10.4049/jimmunol.0802387 PMid:19801525

50. Liao XM. Changes of plasma interleukin 33 in asthmatic patients and its clinical significance. Journal of Clinical Pulmonary Medicine. 2014; 19: 33-5.

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):904-908.
https://doi.org/10.3889/oamjms.2017.215
eISSN: 1857-9655
Clinical Science

Survival of Advanced Stage High-Grade Serous Ovarian Cancer Patients in the Republic of Macedonia

Igor Aluloski[1*], Mile Tanturovski[1], Rubens Jovanovic[2], Slavica Kostadinova-Kunovska[2], Gordana Petrusevska[2], Igor Stojkovski[3], Bojana Petreska[3]

[1]Department of Gynecologic Oncology, University Clinic of Gynecology and Obstetrics, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]Institute of Patology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [3]University Clinic for Oncology and Radiotherapy, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Aluloski I, Tanturovski M, Jovanovic R, Kostadinova-Kunovska S, Petrusevska G, Stojkovski I, Petreska B. Survival of Advanced Stage High-Grade Serous Ovarian Cancer Patients in the Republic of Macedonia. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):904-908. https://doi.org/10.3889/oamjms.2017.215

Keywords: Ovarian cancer; High grade; Survival; Surgical cytoreduction.

***Correspondence:** Igor Aluloski. Department of Gynecologic Oncology, University Clinic of gynaecology and obstetrics, University "Ss. Cyril and Methodius", Skopje, Macedonia. E-mail: ugakskopje@gmail.com

Received: 20-Aug-2017; **Revised:** 12-Oct-2017; **Accepted:** 28-Oct-2017; **Online first:** 20-Nov-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: The primary objective of the study was to evaluate the overall survival of women with advanced stage (Stage IIIA-IV) high-grade serous ovarian cancer in Macedonia

MATERIALS AND METHODS: The study was a cross-sectional medical record review of patients diagnosed with advanced stage HGSC. Patients were deemed eligible for inclusion if they were diagnosed with an advanced stage (Stage IIIA-IV) HGSC of the ovary, fallopian tube or peritoneum between 2009 and 2015. The data were analyzed in a descriptive fashion and summary statistics were provided, as appropriate. Survival was calculated using the Kaplan-Meier method.

RESULTS: A total of 81 eligible patients were identified and included in the study. The average overall survival in the studied cohort was 46.59 months (95%CI = 39.11-54.06). Patients that were optimally debulked and patients that had a platinum-free interval larger than 12 months had significantly longer survival in the current series ($p < 0.001$).

CONCLUSION: the average overall survival of advanced stage HGSC patients in the studied series was 46.59 months (95%CI = 39.11-54.06). Patients aged 65 years or younger tended to live approximately ten months longer than patients older than 65 years, but this difference was not statistically significant. There was no difference in HGSC survival in the groups of patients with grade 2 and grade 3 disease. However, optimal surgical debulking and platinum sensitivity were associated with significantly better overall survival.

Introduction

Epithelial ovarian cancer is the fifth most common cancer-related cause of death in women in the developed countries with no significant improvement in the survival rates in the last three decades in spite of numerous scientific efforts [1]. It is estimated that in Europe alone, a total of 65538 new cases and 42704 deaths were registered in 2012 [2]. Early stage disease (i.e. disease localised to the ovaries) is associated with an overall 5-year survival rate of over 90%; however, only a small proportion of patients (approximately 15%) present with early disease [3]. On the other hand, five-year survival rates for patients with metastatic ovarian cancer are currently estimated to be less than 30% [3]. The current consensus is that all patients with advanced or metastatic ovarian cancer should be treated with surgical debulking and subsequent systemic chemotherapy. First-line chemotherapy is usually a platinum-based regimen (i.e. carboplatin with or without paclitaxel) [4]. Most ovarian cancer patients have high initial response rates, but eventually, recur and require further treatment.

Tumor relapses can be classified, by the time interval between the completion of the initial platinum-based chemotherapy regimen and the diagnosis of the recurrence, as platinum-sensitive (recurrence detected after six months or longer) or platinum-resistant (recurrence detected within six months). Patients with platinum-resistant tumours have poorer response rates to the subsequent chemotherapy regimens and, consequently, shorter overall survival [5]. Ovarian cancer may occur at any age, but most patients are older than 50 years at the time of diagnosis. Advanced age at the time of diagnosis is associated with poorer outcomes: the 5-year survival

rate drops to below 30% in patients older than 65 years [6]. This is probably because older patients have at least one underlying comorbidity which is thought to be associated with poorer prognosis [7, 8].

The most common histological type of ovarian cancers is serous cancers. Once thought to arise from the ovarian surface epithelium, recent pathological and molecular studies have shifted the paradigm of the origin of serous epithelial ovarian cancers towards non-ovarian tissues, namely the secretory cells of the distal parts of the fallopian tubes [9].

Tumor grade, as a pathological index of cellular aberration, is an additional prognostic factor for serous ovarian cancer. It is widely accepted that low-grade tumours occur from tumours of borderline malignancy that acquire activating mutations in the member of the RAS pathway (KRAS, BRAF, and ErbB2) [10]. On the other hand, such mutations of the RAS pathway are rarely identified in high-grade serous cancers.

High-grade serous carcinomas (HGSCs) remain the most aggressive subtype of ovarian cancers and account for the vast majority of advanced stage cases [11].

To our knowledge, there is virtually no published data on the survival of advanced stage HGSC patients in the Republic of Macedonia. The primary objective of the study was to evaluate the overall survival of women with advanced stage (Stage IIIA-IV) high-grade serous ovarian cancer in Macedonia.

Material and Methods

The study was a cross-sectional medical record review of patients diagnosed with advanced stage HGSC. We searched the hospital registers of the University Clinic for gynaecology and obstetrics and the University clinic for oncology and radiotherapy at the University "Ss. Cyril and Methodius" in Skopje, Macedonia. Both participating academic centres are tertiary healthcare where the majority of these patients in Macedonia are referred for treatment. Patients were deemed eligible for inclusion if they were diagnosed with an advanced stage (Stage IIIA-IV) HGSC of the ovary, fallopian tube or peritoneum between 2009 and 2015. Patients with non-serous histology, borderline tumours, early-stage and low-grade serous subtypes, as well as patients with insufficient follow-up data were excluded from the study. Clinical and disease characteristics, including age at diagnosis, stage, residual disease after primary surgery, treatment history and recurrence history, were collected from the available medical records. All primary surgeries were performed at the Department of gynecologic oncology at the University clinic of gynaecology and obstetrics, and all patients received adjuvant chemotherapy at the University clinic of oncology and radiotherapy. Cytoreductive surgery was characterised as optimal if there was ≤1 cm of residual disease and suboptimal if >1 cm of disease remained at the end of the surgical procedure. The National statistics office of the Republic of Macedonia mortality registry was accessed in April 2017 to acquire the exact date of death of all patients. The same date was used as a cut-off for the surviving patients.

The data were analysed in a descriptive fashion and summary statistics were provided, as appropriate. Survival was calculated using the Kaplan-Meier method. For survival comparison, patients were grouped based on age into two groups: (1) 65 years and younger; (2) older than 65 years. Patients were further stratified in relation to the initial platinum-free interval as: (1) highly platinum-sensitive (recurrence after at least 12 months after stopping the initial adjuvant platinum-based therapy); (2) partially platinum, sensitive (recurrence within 6-12 months); and platinum resistant (recurrence or progression within 6 months of the initial platinum-based therapy). Differences in survival were also evaluated for the groups of patients with grade 2 and grade 3 tumours. The Mantel-Cox log-rank method was used to test for statistically significant differences in the survival. All statistical calculations were done using IBM SPSS Statistics software package, version 23. A probability value of $p \leq 0.05$ was considered significant.

Results

A total of 81 eligible patients were identified and included in the study. Table 1 summarises the demographic and clinical characteristics of the study group.

Table 1: Summary of patient characteristics

Characteristic	
Age (*median, [range]*)	60 years [24-78]
Tumor grade	
2 (*n, %*)	26 (32.1%)
3 (*n, %*)	55 (67.9%)
Tumor stage	
IIIA (*n, %*)	9 (11.1%)
IIIB (*n, %*)	24 (29.6%)
IIIC (*n, %*)	45 (55.6%)
IV (*n, %*)	3 (3.7%)
Residual disease	
≤1cm (*n, %*)	29 (35.8%)
>1cm (*n, %*)	52 (64.2%)
Platinum-free interval	
< 6 months	16 (19.8%)
6-12 months	24 (29.6%)
> 12 months	41 (50.6%)

The median age of the patients was 60 years (range 24-78). Twenty-eight (34.6%) patients were older than 65 years at the time of diagnosis. The majority of patients, 45 (55.6%), were diagnosed with

stage IIIC disease at the time of diagnosis, 9 (11.1%) patients were staged as IIIA, 24 (29.6%) patients had stage IIIB disease, and only 3 (3.7%) patients were stage IV. All stage IV patients were classified as stage IV by malignant pleural effusion. Twenty-six (32.1%) of the neoplasms were grade 2, and 55 (67.9%) were grade 3. Optimal debulking was achieved in 29 patients (35.8%). Forty-one (50.6%) of the patients were reported to be highly platinum sensitive, with a platinum-free interval larger than 12 months. Thirty-six patients (44.4%) from the cohort were still alive at the study cross-section point.

The average overall survival in the studied cohort was 46.59 months (95%CI = 39.11-54.06). Figure 1 shows the Kaplan-Meier survival function plot for the overall survival.

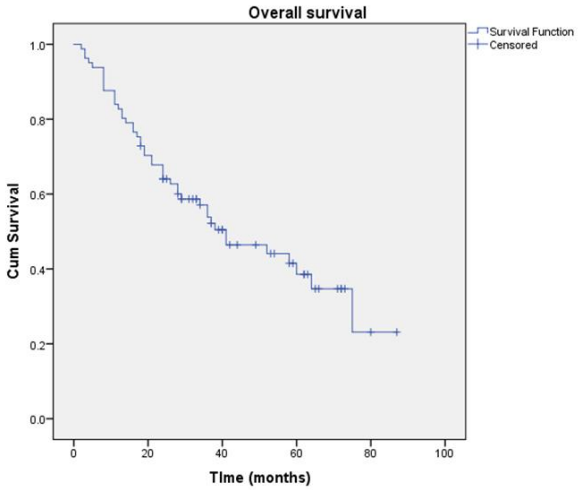


Figure 1: Overall survival of patients with an advanced stage high-grade serous cancer in the studied cohort

The estimated survival of patients 65 years old or younger was 49.18 months (95%CI = 40.85-57.51), compared to an average survival of 39.03 months (95%CI = 27.25-50.81) for patients older than 65 years (Figure 2), but the difference is not statistically significant (p = 0.099).

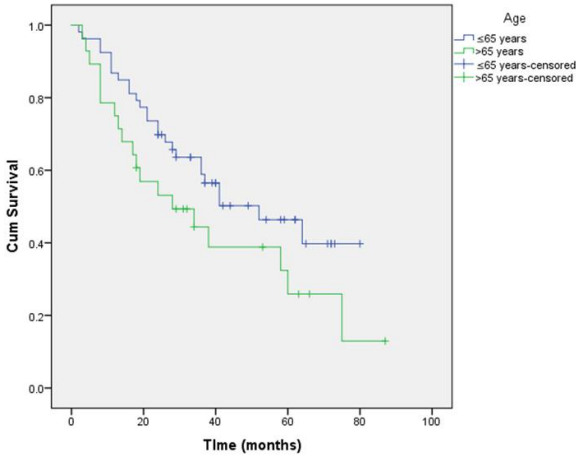


Figure 2: Comparison of survival in the different age groups

We also failed to document a statistically significant difference (p = 0.183) in the survival of patients with grade 2 and grade 3 disease, with survival averaging at 51.74 months (95%CI = 40.69-62.78) and 43.03 months (95%CI = 34.01-52.05) for grade 2 and grade 3 disease, respectively (Figure 3).

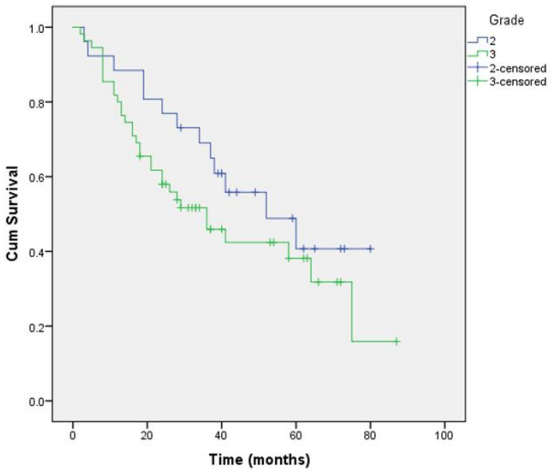


Figure 3: Comparison of survival in patients with grade 2 and grade disease

Optimal surgical debulking greatly influenced survival in the analysed series of patients. The estimated survival of patients that had the postoperative residual disease of ≤1cm was 75.55 months (95%CI = 66.47-84.63), while patients with suboptimal surgical debulking (i.e. a postoperative residual disease of >1 cm) had an estimated survival of 30.78 months (95%CI = 24.14-37.42) (p < 0.001).

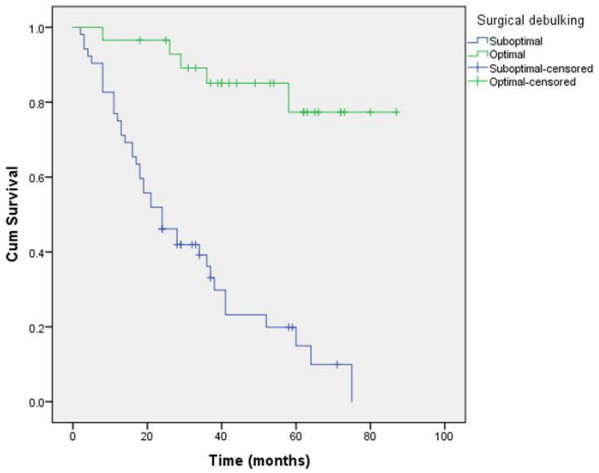


Figure 4: Comparison of survival in patients with optimal and suboptimal surgical debulking

Platinum sensitivity was also an important determinant of survival with estimated survival ranging from 8.75 months (95%CI = 6.55-10.95) for patients with a platinum-free interval less than 6 month, 27.89 months (95%CI = 23.38-32.39) for patients with a

platinum-free interval of 6-12 months and 67.05 months (95%CI = 58.83-75.27) for highly platinum-sensitive patients with a platinum-free interval longer than 12 months ($p < 0.001$). Figure 4 and 5 present the Kaplan-Meier plots for both comparisons.

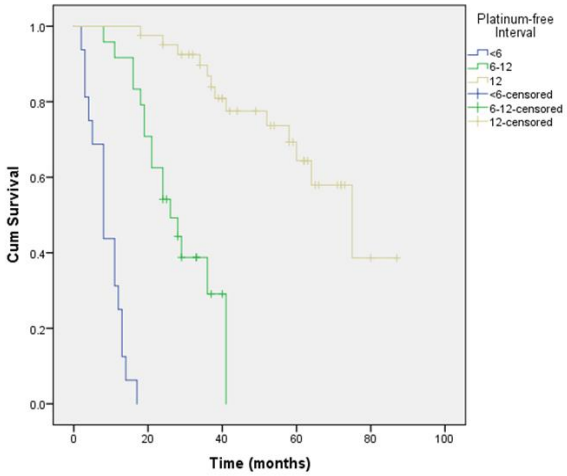


Figure 5: Comparison of survival depending on platinum resistance status

Discussion

In this study, we present the demographic and clinical (surgical and treatment-related) factors associated with survival in patients treated for advanced-stage HGSC in the Republic of Macedonia. We analysed the medical data of a total of 81 advanced stage HGSC patients. Patients with longer survival in our series appeared to be younger and were likely to have had optimal surgical cytoreduction, defined in the study as ≤ 1cm of residual disease. Patients that had longer platinum-free intervals also had better survival rates. In spite of that, a small fraction of patients who either had suboptimal debulking or primary platinum resistance achieved paradoxically long survival periods.

Age has been consensually accepted as a prognostic factor for ovarian cancer survival [12, 13]. Older women have an increased risk of treatment failure and hence higher rates of recurrences and lower survival rates [14]. In the series of patients analysed by Markman et al. [15], the median survival for patients younger than 65 years was at least two years longer, compared to the median survival of patients older than 65 years. Patients in the current cohort that were older than 65 years had shorter average survival times of ten months, compared to patients younger than 65 years, but the difference was not statistically significant. The lack of statistical significance could be attributed to selection bias (advanced stage HGSC patients only) and a small sample size.

The tumour grade, or the degree of cellular differentiation, has long been considered a determinant of survival. The theory is based on the understanding that poorly differentiated cells manifest a higher degree of aberrant mitoses, which translates into a more aggressive cancer cell behaviour. However, published data for the association between tumour grade and clinical outcome are conflicting, partly as a result of the lack of a universally accepted grading system [16, 17]. In the past decade, the well-established three-tier grading system introduced by FIGO in the 1970s that uses cellular architecture as the defining characteristic of tumour grade has been replaced by a two-tier system based on clinical, morphological and molecular studies [18, 19]. Indeed, our data showed no difference in survival between grades 2 and 3, which was to be expected since all grade 2 and grade 3 tumours met contemporary criteria to be classified as high-grade tumours.

The success of cytoreductive surgery, defined by the volume of residual disease, has consistently been heralded as one of the most significant factors influencing both progression-free and overall survival [20]. In this study, optimal surgical debulking (defined as a residual disease of ≤ 1 cm) more than doubled survival; patients that were optimally debulked had an estimated mean survival of 75.55 months versus and estimated mean survival of 30.78 months for patients with postoperative residual disease greater than 1 cm ($p < 0.001$). A Cochrane Database Review, published recently [21], was focused on analysing the effectiveness of optimal primary cytoreductive surgery in patients with advanced stage epithelial ovarian cancers. The authors demonstrated that overall survival and progression-free survival were significantly prolonged in patients who had no grossly visible residual disease following primary debulking surgery. The beneficial effects were also present in the groups of patients with residual diseases of less than 1 cm, but only a borderline difference in progression-free survival remained when the residual disease of more and less than 2 cm was used as the basis for patient comparison. Consequently, the authors conclude that the aim of every primary surgery for advanced-stage epithelial ovarian cancer should be complete cytoreduction to no grossly visible disease.

One of the most reliable predictors of response to chemotherapy, and consequently survival, is the platinum-free interval [15, 22]. This is mostly the result of the first-line adjuvant chemotherapy for ovarian cancers being relatively constant (carboplatin with paclitaxel) over the past two decades. Our data also showed that patients with a platinum-free interval longer than 12 months survived significantly longer ($p < 0.001$) when compared to patients with a platinum-free interval of 6-12 months and less than six months (67.05 months vs. 27.89 months and 8.75 months, respectively).

The prognosis in ovarian cancer patients is largely a function of disease stage, which represents the most important prognostic variable for ovarian cancer survival. In the current series, we analysed only data from advanced stage patients, in an attempt to highlight the prognostic importance of other parameters such as tumour characteristics, surgical and treatment-related conditions and demographic factors. Furthermore, the study was descriptive. Recently published studies have focused on the significance of molecular features of the tumours as a proxy for tumour biology, thus enabling the researchers to better predict the response to various therapeutic modalities and determine factors influencing survival. Our future studies will compare this group of patients and compare survival by the molecular determinants of the tumour.

In conclusion, the average overall survival of advanced stage HGSC patients in the studied series was 46.59 months (95%CI = 39.11-54.06). Patients aged 65 years or younger tended to live approximately ten months longer than patients older than 65 years, but this difference was not statistically significant. There was no difference in HGSC survival in the groups of patients with grade 2 and grade 3 diseases. However, optimal surgical debulking and platinum sensitivity were associated with significantly better overall survival.

References

1. Vaughan S, Coward JI, Bast RC Jr, Berchuck A, Berek JS, Brenton JD, et al. Rethinking ovarian cancer: recommendations for improving outcomes. Nat Rev Cancer. 2011;11:719–25. https://doi.org/10.1038/nrc3144 PMid:21941283 PMCid:PMC3380637

2. Incidence and Mortality Worldwide: IARC Cancerbase number 11. Lyon, France: International Agency for Research on Cancer 2013. Available from: http://globocan.iarc.fr. Accessed April 2017.

3. National Cancer Institute. SEER (Surveillance, Epidemiology and End Results Program). SEER Cancer Fact Sheets: Cancer of the Ovary. Available from: http://seer.cancer.gov/statfacts/html/ovary.html. Accessed April 2017.

4. du Bois A, Quinn M, Thigpen T, et al. 2004 consensus statements on the management of ovarian cancer: final document of the 3rd International Gynecologic Cancer Intergroup Ovarian Cancer Consensus Conference (GCIGOCCC 2004). Ann Oncol. 2005;16(Suppl 8):7–12. https://doi.org/10.1093/annonc/mdi961 PMid:16239238

5. Cannistra SA. Cancer of the Ovary. New Engl J Med. 2004;351: 2519–29. https://doi.org/10.1056/NEJMra041842 PMid:15590954

6. Lowe KA, Chia VM, Taylor A, et al. An international assessment of ovarian cancer incidence and mortality. Gynecol Oncol. 2013;130:107–14. https://doi.org/10.1016/j.ygyno.2013.03.026 PMid:23558050

7. Janssen-Heijnen ML, Houterman S, Lemmens VE, Louwman MW, Coebergh JW. Age and co-morbidity in cancer patients: a population-based approach. Cancer Treat Res. 2005;124:89–107. https://doi.org/10.1007/0-387-23962-6_5 PMid:15839192

8. Gulbech Ording A, Garne JP, Witt Nyström PM, Frøslev T, Toft Sørensen H, Lash TL. comorbid diseases interact with breast cancer to affect mortality in the first year after diagnosis – a Danish nationwide matched cohort study. PLoS One. 2013;8(10):e76013. https://doi.org/10.1371/journal.pone.0076013 PMid:24130755 PMCid:PMC3794020

9. Lee Y, Miron A, Drapkin R, Nucci MR, Medeiros F, Saleemuddin A, et al. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. J Pathol. 2007;211:26–35. https://doi.org/10.1002/path.2091 PMid:17117391

10. Vang R, Shih IeM, Kurman RJ. Ovarian low-grade and high-grade serous carcinoma: pathogenesis, clinicopathologic and molecular biologic features, and diagnostic problems. Adv Anat Pathol. 2009;16:267–82. https://doi.org/10.1097/PAP.0b013e3181b4fffa PMid:19700937 PMCid:PMC2745605

11. Bowtell DD, Bohm S, Ahmed AA, et al. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. Nat Rev Cancer. 2015;15:668–79. https://doi.org/10.1038/nrc4019 PMid:26493647 PMCid:PMC4892184

12. Hainsworth JD, Grosh WW, Burnett LS, Jones HW 3rd, Wolff SN, Greco FA. Advanced ovarian cancer: long-term results of treatment with intensive cisplatin-based chemotherapy of brief duration. Ann Intern Med. 1988;108(2):165-70. https://doi.org/10.7326/0003-4819-108-2-165 PMid:3124679

13. Neijt JP, ten Bokkel Huinink WW, van der Burg ME, et al. Long-term survival in ovarian cancer. Mature data from The Netherlands Joint Study Group for Ovarian Cancer. Eur J Cancer. 1991;27(11):1367-72. https://doi.org/10.1016/0277-5379(91)90011-2

14. Winter WE 3rd, Maxwell GL, Tian C et al. Prognostic factors for stage III epithelial ovarian cancer: a Gynecologic Oncology Group Study. J Clin Oncol. 2007;25(24):3621-7. https://doi.org/10.1200/JCO.2006.10.2517 PMid:17704411

15. Markman M, Lewis JL Jr, Saigo P et al. Impact of age on survival of patients with ovarian cancer. Gynecol Oncol. 1993;49(2):236-9. https://doi.org/10.1006/gyno.1993.1113 PMid:8504993

16. Rabban JT, Bell DA. Current issues in the pathology of ovarian cancer. J Reprod Med. 2005;50(6):467-74. PMid:16050571

17. Silverberg SG. Histopathologic grading of ovarian carcinoma: a review and proposal. Int J Gynecol Pathol. 2000;19(1):7-15. https://doi.org/10.1097/00004347-200001000-00003 PMid:10638449

18. Singer G, Kurman RJ, Chang H-W, et al. Diverse tumorigenic pathways in ovarian serous carcinoma. Am J Pathol. 2002;160:1223–28. https://doi.org/10.1016/S0002-9440(10)62549-7

19. Vang R, Shih Ie M, Salani R, et al. Subdividing ovarian and peritoneal serous carcinoma into moderately differentiated and poorly differentiated does not have biologic validity based on molecular genetic and in vitro drug resistance data. Am J Surg Pathol. 2008;32:1667–74. https://doi.org/10.1097/PAS.0b013e31816fd555 PMid:18769340

20. Fader AN, Rose PG. Role of surgery in ovarian carcinoma. J Clin Oncol. 2007;25(20):2873-83. https://doi.org/10.1200/JCO.2007.11.0932 PMid:17617518

21. Elattar A, Bryant A, Winter-Roach BA, Hatem M, Naik R. Optimal primary surgical treatment for advanced epithelial ovarian cancer. Cochrane Database Syst Rev. 2011;(8):CD007565. https://doi.org/10.1002/14651858.CD007565.pub2

22. Dao F, Schlappe BA, Tseng J et al. Characteristics of 10-year survivors of high-grade serous ovarian carcinoma. Gynecol Oncol. 2016;141(2):260-3. https://doi.org/10.1016/j.ygyno.2016.03.010 PMid:26968641 PMCid:PMC4844793

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):909-914.
https://doi.org/10.3889/oamjms.2017.194
eISSN: 1857-9655
Clinical Science

The Predictive Role of Procalcitonin On the Treatment of Intra-Abdominal Infections

Dafina Mahmutaj[1*], Shaip Krasniqi[2], Bedri Braha[1], Dalip Limani[1], Burim Neziri[2]

[1]Clinic of Surgery, University Clinical Center of Kosovo, Hospital and University Clinical Service of Kosovo, Prishtina, Kosovo; [2]Faculty of Medicine, University of Prishtina "Hasan Prishtina", Prishtina, Kosovo

Citation: Mahmutaj D, Krasniqi S, Braha B, Limani D, Neziri B. The Predictive Role of Procalcitonin On the Treatment of Intra-Abdominal Infections. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):909-914. https://doi.org/10.3889/oamjms.2017.194

Keywords: intraabdominal infections; procalcitonin; antibiotic; microbiological examination; CRP.

***Correspondence:** Bedri Braha, MD MsC. Clinic of Surgery, University Clinical Center of Kosovo, Hospital and University Clinical Service of Kosovo Prishtina, Kosovo. E-mail: dafinamahmutaj@gmail.com

Received: 07-Aug-2017; **Revised:** 20-Sep-2017; **Accepted:** 21-Oct-2017; **Online first:** 24-Nov-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Aim: This study aims to evaluate the algorithm of procalcitonin (PCT) and its role on the duration of antibiotics prescription for intra-abdominal infections.

Materials and Methods: This study is a prospective controlled study that is conducted in groups of 50 hospitalised patients and 50 controlled group patients.

Results: The results indicated that the average duration of antibiotic delivery to the PCT group was -10.6 days (SD ± 6.6 days), while in the control group -13.2 days (SD ± 4.2 days). These data showed a significant difference in the duration of antibiotic therapy and the monitoring role of PCTs in the prediction success of antibiotic treatment. The antibiotic delivery was longer in the septic shock 17 (SD ± 11.7) that corresponds to high PCT values of 67.8 (SD ± 50.9). Recurrence of the infection after the cessation of antibiotics occurred in 2 cases (4%) in the standard group, while it occurred in 3 cases (6%) in the control group.

Conclusion: The treatment of the intra-abdominal infections based on the PCT algorithm shortens the duration of antibiotic treatment and does not pose a risk for the recurrence of the infection.

Introduction

Intra-abdominal infections are a group of diseases with a relatively high degree of mortality and morbidity. Two determinants for the successful treatment of intra-abdominal infections are the identification of the source of the infection and the speed at which the empirical delivery of antibiotics begins, circumstances that reduce the risk of complications and mortality [1]. Reduction of mortality is not possible without effective control of the source of infection [2]. The mortality rate of intra-abdominal infections was about 90% at the end of the 19th century, where management was mostly non-operative. In recent years, surgical intervention in intra-abdominal infections has influenced the control of the source of infection by reducing the mortality rate about 30% in cases of severe sepsis and septic shock [3-5]. Reduction of mortality rate is achieved with the earliest diagnostics of these infections and timely and adequate treatment [6-8].

Antibiotic therapy should start immediately after the intraabdominal infection diagnosis, while the duration of administration of antibiotics is a matter of ongoing discussion and there is still no consensus [8]. Treatment protocols recommend empirical antimicrobial therapy that is effective against the enterococcus, staphylococcus and the candida [6, 7]. If the empirical antibiotic is inadequate, it is followed by failure of treatment, an extension of hospitalisation and death. Non-rational antibiotic delivery leads to increased resistance to antibiotics and the appearance of intrahospital infections [8]. Therefore it is recommended to restrict the use of antibiotics and apply the biomarker algorithm in treatment to monitor the success of antibiotic therapy and discontinuation of their ordination as earliest [9, 10]. In Intensive Care Department, the procalcitonin (PCT) serial measurement can be used as a guide to facilitate the

most rapid interruption of antibiotics [11]. PCT is the calcitonin prohormone produced by the C-cell of the thyroid gland. The PCT inflammatory processes do not happen in thyroid C cells and do not depend on the calcium concentration, but its production depends on the microbial endotoxins and indirect from specific cytokines such as IL-1, IL-6 and TNF [12]. Indeed, it has been reported that the PCT threshold of 0.25 ng/mL to 0.50 ng/mL or a reduction of at least 80% of the initial value [13, 14] allows the antibiotic to stop earlier without changing the clinical outcome [15-18]. The highest PCT values reach 24-48 h after infection, and proper management causes the PCT values to drop by about 50% within about one to one-half days after the infection decoction is interrupted. In some clinical cases, interferences with PCT values have been introduced, as in patients with renal failure, where half-life elimination is prolonged, but PCT accumulation does not occur [19]. Data from the literature suggests that if stimulation is discontinued its concentration decreases progressively and returns to baseline values on Day 5 or 7 [20]. An exceptional PCT situation is presented if clinical symptoms indicate possible sepsis but PCT values are low, then sepsis therapy should begin anyway, PCT measurement should be repeated (after 12, 24, 36 h) until the final diagnosis is clarified [19]. Despite the important information, the determination of PCT values that have a diagnostic and predictive role in the mentioned diagnoses is not yet definitive and is considered to be investigated further in other issues of surgical infections.

Indeed, most of the research has evaluated PCT's interest in determining the duration of antibiotic delivery to conservatively treated patients [9, 11], while there is no or insufficient research on the role of PCT in intra-abdominal infections diagnosed as peritonitis, therefore, the aim of this study is to evaluate the predictive role of PCT in the duration of antibiotic delivery and treatment priorities based on the PCT algorithm in relation to standard treatment.

Materials and Methods

This is a clinically prospective, controlled trial involving hospitalised patients in the Clinical Surgery department at University Clinical Hospital of Kosovo in Prishtina, registered in the period from March 2016 to January 2017. The study was performed with simple randomisation, and 100 patients were included out of which, 50 patients were used for testing the PCT algorithm efficacy in discontinuing antibiotics. The study included a control group of 50 patients as a comparator in the duration of antibiotic delivery while comparing the complications following the discontinuation of antibiotics in both groups. The Ethics Committee of the University of Prishtina – Faculty of Medicine, has approved the research protocol and all participants or their family members completed the consent form.

Inclusion and exclusion criteria: Patients over the age of 18 diagnosed with acute abdomen with Systemic Inflammatory Response Syndrome (SIRS), who underwent urgent surgical intervention and Index Manheim Peritonitis > 10 points, were eligible for enrollment in the study. Patients with a) autoimmune disease b) acute hepatic insufficiency c) diabetes d) immunosuppression e) pregnant women f) patients treated with corticoids g) when the patient or family members have refused to be included in the study were excluded.

Protocol and Treatment: PCT levels were measured before surgery on the day 1, 4, and 7 and the following days as needed. The measurement was made with the Elecsys BRAHMS PCT method. The restraints of the values measured by this method are from 0.02 to 100 ng/mL. Values below the detectable limit are reported as < 0.02 ng/mL, whereas those over the upper limit are reported >100 ng/mL [21]. PCT values in healthy people are low <0.1ng/mL [22]. To exclude sepsis and systemic inflammation PCT < 0.2 ng/mL concentration is a useful reference. As the cut off for the diagnosis of sepsis the PCT values > 0.5 ng/mL are interpreted as abnormal and suggest for sepsis [20].

Recommendations based on the PCT algorithm are discontinuation of the antibiotics when PCT decreases > 80% of the initial value or PCT < 0.5 ng/mL, reduction by the clinical condition and other laboratory parameters.

Antibiotic delivery has begun in all patients with acute abdominal symptoms and SIRS presence. Interventions have been performed by experienced surgeons. After the intervention, all participants continued empirically given antibiotics, to the group with SIRS/sepsis and severe sepsis ceftriaxone 1 gram was ordinated intravenously every 12 hours and metronidazole 500 mg intravenously every 8 hours, while in patients with septic shock imipenem 500 mg three times daily and metronidazole 500 mg intravenous every 8 hours.

After receiving the microbiological examination results, antibiogram treatment continued. Termination of the antibiotics in the study group was conducted according to the protocol of the study. The basic criteria for the antibiotic discontinuation in control group were the normalization of leukocyte values followed by improvement of the clinical condition. After the antibiotic discontinuation, the condition of all patients was monitored for the identification of possible complications.

Statistical Analysis

Stratification of the results was conducted based on the criteria of the severity of intraabdominal infections (SIRS/sepsis, Severe sepsis, Septic shock). We included age, gender, APACHE II, MODS, SOFA and INDEX MANNHEIM PERITONITIS.

The statistical processing of the data was conducted with the statistical package SPSS 22.0. The arithmetic average, the standard deviation, the minimum and the maximum value were calculated from statistical parameters. Qualitative data testing was done with the χ2-test and the exact Fisher test of quantitative data that had a normal distribution with T-test and One Way ANOVA, and those with a nonnormal distribution with the Mann-Whitney test or Kruskal Wallis test.

Results

In both study groups the male patients were slightly more than females but without a significant statistical difference (X2 = 0.176, P = 0.675). Patients of both groups were in different age groups - the youngest was 17 years old, and the oldest was 86 years old. The average age of the patients was 43.2 years (DS ± 19.5 years), ranging from 17 to 86 years.

According to the demographic characteristics of patients, there is no significant difference between the study and control groups. In both groups, according to the diagnosis structure, the acute perforative appendicitis and perforated duodenal ulcer were a more frequent diagnosis of patients.

The average duration of antibiotic delivery to all patients was 11.1 days (SD ± 5.5 days), ranging from 3.0 to 31.0 days. In the test group, the average duration was 10.6 days (SD ± 6.6 days), ranging from 3.0 to 31.0 days. In the control group, the average duration was 13.2 days (SD ± 4.2 days), ranging from 3.0 to 29.0 days. With t-test, we revealed significant statistical significance (t-test), (P = 0.028), (Table 1).

Table 1: Characteristics of Patients included in the study

	Study Group	Control Group	p
Age	39.4 ± 19.6	47.0 ± 18.8	0.064a
Female	16	19	0.675b
Male	34	31	0.675b
Etiology of the disease			
Appendicitis acuta perforativa	23	34	
Ulcus bulbi duodeni perforans	9	10	
Perforatio intestini (ilei,jejuni,sygmae)	11	5	
Cholecystitis acuta perforativa	4	1	
St. post op.Wiple (Dehiscencio anastomosis pancreatico-jejuni)	1		
Perforatio diverticulum Meckeli	2		
Duration of Antibiotic Delivery	10.6 ± 6.6	13.2 ± 4.2	0.028c
Reconnaissance of infection after antibiotic termination	2 (4%)	3 (6%)	

A = Man Whitney test; b = χ2-test; c-T-test.

According to duration of antibiotic administration in the study group, we have noted this structure of administration: 8% - 4 days, 4% -5 days, 16% - 6 days, 10% - 7 days, 62% > 7 days. The criteria of PCT decreased > 80% of the initial value was met in 8 patients; 8 (16%) patients did not receive the antibiotics as per PCT < 0.5 ng/mL algorithm. This group of patients had an average of 4 days more (SD ± 3) administered antibiotic due to unsatisfactory clinical condition and laboratory parameters, while in other 4 patients we had an initial decrease of PCT in < 0.5 ng/mL, and after that an increase of PCT values. Two of these patients had pulmonary problems, and the other two were indispensable for reintervention. Contrary, in the control group, only 4 (8%) patients had antibiotics < 7 days (Table 1).

Table 2: Duration of Antibiotics Delivery in report to SOFA, APACHE II, IMP, MODS, PCT, CRP, WBC in admission of patients to the study group

	Abdominal Urgencies (n=50)			
Parameters	**Septic Shock**	**Severe Sepsis**	**SIRS/Sepsis**	**p**
Duration of antibiotic administration	17.0 (SD ± 11.7)	15.9 (SD ± 8.4)	8.6 (SD ± 5.7)	<0.001[a]
SOFA	17.8 (SD ± 7.6)	10.5 (SD ± 3.8)	5.2 (SD ± 2.4)	<0.0001[a]
APACHE II	34 (SD ± 9.5)	19,5 (SD ± 6.3)	9.9 (SD ± 5.1)	<0.0001[a]
MODS	12.8 (SD ± 3.8)	7.3 (SD ± 3.6)	2.3 (SD ± 1.3)	<0.184[a]
PCT	67.8 (SD ± 50.9)	22.5 (SD ± 36.7)	3.7 (SD ± 5.6)	0.00015[a]
CRP	117.9 (SD ± 123.7)	154.2 (SD ± 72.3)	133.1 (SD ± 90.5)	0.431[a]
WBC	11.5 (SD ± 8.2)	18.4 (SD ± 11.7)	14.2 (SD ± 4.1)	0.132[b]

a = Kruskal Wallis test; b = one way ANOVA test.

MODS-Multiple Organ Dysfunction Score, IMP-Index Mannheim peritonitis, APACHE-Acute Physiology and Chronic Health Evaluation, SOFA-Sequential Organ Failure Assessment, PCT-procalcitonin, CRP-C reactive protein, TG-Triglycerides, WBC-White Blood Cell.

Based on the results we have found a significant correlation of the values of SOFA, APACHE II, MODS, PCT, and TG with the duration of antibiotic delivery, while the values of CRP and WBC do not correspond to the duration of antibiotic delivery (Table 2).

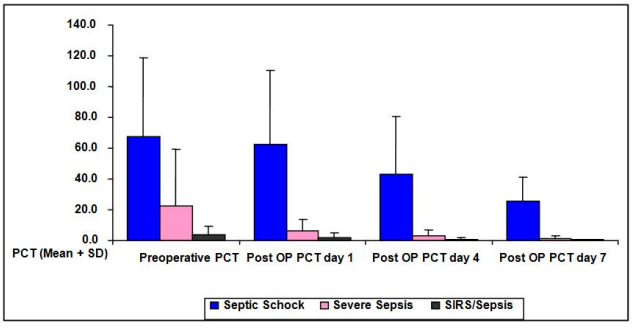


Figure 1: PCT algorithm according to the severity of the infection

PCT algorithm shows that after surgical treatment and initiation of antibiotic therapy in a group with SIRS/sepsis and severe sepsis the values are reduced until in the septic shock PCT values decreases about the initial values but remain high

enough to prevent the discontinuation of antibiotics (Fig. 1).

Table 3: Results of microbiological examination in the study group and control group

Microbiological results	Study Group		Control Group		Total	
	N	%	N	%	N	%
Escherichia coli	13	26.0	15	30.0-	28	28.0
Escherichia coli streptococcus pyogenes	2	4.0	3	6.0	5	5.0
Escherichia coli Pseudomonas aeruginosa	1	2.0	2	4.0	3	3.0
Candida SPP	4	8.0	2	4.0	6	6.0
Klebsiella pneumoniae	4	8.0	2	4.0	6	6.0
Pseudomonas aeruginosa	5	10.0	2	4.0	7	7.0
Staphylococcus aureus	2	4.0	3	6.0	5	5.0
Peptostreptococcus	1	2.0	2	4.0	3	3.0
Acinetobacter SP	1	2.0	-	-	1	1.0
No culture of bacteria is isolated	17	34.0	19	38.0	36	36.0
Total	50	100.0	50	100.0	100	100.0

In most of the patients of the study group and control group, *E. coli* in the form of mono-culture or co-infection with *Streptococcus pyogenes* and *Pseudomonas aeruginosa* were isolated. In the structure of isolated microorganisms, monocultures of *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Candida* were also identified microbiological cultures. However, the culture of bacteria was not isolated in 34.0% of cases in the study group and 38% in the control group (Table 3).

Table 4: Procalcitonin, duration, and types of antibiotics according to microbial infection

Parameters	Microbiological Infections					
	Gram positive and Gram negative	Gram negative	Gram positive	Mycotic	Not culture isolated	p
PCT before OP (ng/mL)	4.2 ± 0	18.8 ± 34.6	0.9 ± 0.3	29.5 ± 47.2	3.5 ± 5.2	0.001
Duration of antibiotic delivery (d)	12 ± 1.4	11.6 ± 5.4	19 ± 16.9	2.5 ± 0.7		0.41
Metronidazole	2 (5%)	25 (62.5%)	1 (2.5%)	4 (10%)	8 (20%)	
Ceftriaxone	-	25 (62.5%	1 (2.5%)	2 (5%)	12 (30%)	
Imipenem and enzyme inhibitor	3 (20%)	6 (40%)	3 (20%)	-	3 (20%)	
Gentamicin		1 (100%)				
Tazobactam		1 (100%)				
Lincomycin		2 (50%)			2 (50%)	
Amoxicillin/clavulanic acid		4 (100%)				

The PCT values in the diagnosis structure are quite variable with the signaling scale for $P < 0.001$ the highest values were recorded in mycotic infections, then the gram negative ones, mixed gram negative and gram positive infections and even lower in gram positive which show a significant statistical difference (Table 4). For the treatment of gram negative infections, seven different forms of antibiotics were administered where Metronidazole and Ceftriaxone were dominated, whereas other forms of infections were administered with fewer antibiotics.

Discussion

This randomized study evaluated the role of PCT algorithm in the duration of antibiotic delivery and monitored the success of antibiotics after surgeries of intra-abdominal infections with peritonitis. Our findings indicate a significant difference in the duration of antibiotic delivery between the study group and the control group, with a reduction in the duration of antibiotic delivery to patients of the study group by 2.6 days without risking a recurrence of infection. Our findings do not correspond to the study conducted by Juliette [21], which states that in general there is no significant difference in the duration of antibiotic delivery between the study group and the control group. They revealed the difference by structure of pathogenesis of infections, concluding that there is a difference in the duration of antibiotic delivery between groups of patients with peritonitis due to gastrointestinal perforation (7 days in the PCT and 10-days with control group for $p = 0.065$), while in patients with localized peritonitis and postoperative peritonitis caused by intestinal dehiscences, there was no reduction of antibiotics with PCT algorithm.

Other authors have stated that the PCT algorithm has a role in reducing the duration of antibiotic delivery of peritonitis. Ting Shuo Huang has concluded that this algorithm reduces the use of antibiotics for three days and provides a 50% relative reduction in the duration of antibiotic delivery [22].

Also, Schroeder et al. in a comparative study with 19 patients with peritonitis after abdominal surgery, stated that antibiotics were reduced to the research group in relation to the control group in significant values (6.6 ± 1.1 days versus 8.3 ± 0.7 days) [23]. Hochreiter et al. recorded patients with confirmed infection or high suspicions for a bacterial infection that were treated with antibiotics and were hospitalized in Intensive Care [24]. Both authors had established antibiotic discontinuity criteria when there was clinical improvement and PCT levels < 1.0 ng/mL or decreased by 25% to 35% of the initial value within three days by making measurements every day. In the results of Hochreiter with authors, the duration of antibiotic delivery was significantly shorter in 57 patients compared to the control group 53 patients (5.9 ± 1.7 days versus 7.9 ± 0.5 days: $p < 0.001$) without negative effects on results.

In our study, the discontinuation of the antibiotics in the control group was done based on the value of the leukocytes and the clinical condition of the patients. In our study, measurement was done every successive day, and this approach has had an impact on the duration of antibiotic delivery. The PCT algorithm does not affect the reduction of antibiotics in septic shock and severe sepsis. Neither the threshold of PCT of 0.5 ng/mL nor the reduction of at least 80% of the initial value can accurately predict the response to treatment for cases with intra-abdominal infections, where there are a septic shock and severe sepsis corresponding to the results of other authors [25].

Findings of our study show that relation between high PCT, TG and high scores of APACHE

II, SOFA, IMP, and MODS and antibiotic treatment duration, whereas preoperative leukocyte and C – reactive protein values do not correspond to the duration of antibiotic administration. Other studies report that age, hypoalbuminemia, malnutrition, comorbidities, APACHE II score ≥ 15 affect the failure of management of infection source and the appearance of infectious complications [26-28].

In the microbiological examination of intraperitoneal fluid, our findings indicate that PCT values have varied between infection groups and have been higher in mycotic infections than in gram-negative ones, mixed gram negative and positive infections, and lower in gram positive infections. However, our data regarding mycotic infections do not correspond to the findings of other authors Martini et al., [29] in his study of 48 surgical patients with mycotic infections risk found that the cut off of PCT for these infections was 2.0 ng/mL. According to Leli, a cut-off of PCT > 1.3 ng/mL can help us in diagnosing mycotic infection [30]. Considering that PCT in mycotic infections are not usually increased since the PCT values are too high in our results, we can conclude that these infections are accompanied by bacterial infections that have not been detected microbiologically in our facilities. In our study PCT cut-off of 18.8 ng/mL is important for predicting sepsis with gram negative bacteria, whereas some studies refer to data linking high PCT values with gram negative sepsis but with cut off values that differ from our values. In the study of Brodská et al. [31], it is described that the PCT cut-off of 15 ng/mL can affect the sepsis caused by gram negative bacteria from sepsis caused by gram positive bacteria and mycotic sepsis with 87.8% specificity. While in the study conducted by Leli, the findings indicate that the cut-off of PCT 10.8 ng/mL may be significant in predicting the infection caused by gram-negative bacteria with a specificity of 82.5%. Other authors have also found that PCT levels were higher in gram negative than in gram positive sepsis [32, 33]. In our study, the PCT average of sepsis with the gram positive bacteria was 0.64 ng/mL (SD = 0.3), corresponding to the data of the author Shuhua [33], where the PCT value was 0.48 ng/mL.

The results from our study and other authors suggest that PCT can help in the ordination of proper antibacterial initiation therapy, considering that 24-48 hours should be expected for microbiological results.

The structure of microorganisms isolated on microbiological samples is similar to the results of other authors, who reported similar infections of the gastrointestinal tract [34, 28].

Overall, we conclude that monitoring of PCT value may impact in shortening of treatment antibiotic duration in patients with intra-abdominal infections. Although PCT is considered to be a very important predictive factor in intra-abdominal infections, in the presence of clinical signs of sepsis, the role PCT is diminished, and antibiotic therapy should be continued according to clinical signs and other predictive parameters. Also, we conclude that further studies are needed to validate the complex role of PCTs in antibiotic monitoring through pharmacoepidemio-logical and pharmacoeconomic studies of antibiotic consumption in both groups that would enable valuable data to be obtained for the role of this algorithm in reducing resistance to antibiotics.

References

1. Marshall JC. Principles of source control in the early management of sepsis. Current infectious disease reports. 2010; 12(5):345–53. https://doi.org/10.1007/s11908-010-0126-z PMid:21308516

2. Grundmann RT, Petersen M, Lippert H, Meyer F. [The acute (surgical) abdomen - epidemiology, diagnosis and general principles of management]. Zeitschrift fur Gastroenterologie. 2010; 48(6):696–706. https://doi.org/10.1055/s-0029-1245303 PMid:20517808

3. Schoeffel U, Jacobs E, Ruf G, Mierswa F, B. von Specht U, Farthmann EH. Intraperitoneal micro-organisms and the severity of peritonitis. The European journal of surgery = Acta chirurgica. 1995; 161(7):501–8.

4. Wacha H, Hau T, Dittmer R, Ohmann CP, Peritonitis Study Group. Risk factors associated with intraabdominal infections: a prospective multicenter study. Langenbeck's archives of surgery. 1999;384(1):24-32. https://doi.org/10.1007/s004230050169 PMid:10367626

5. Mulier S, Penninckx F, Verwaest C, Filez L, Aerts R, Fieuws S, Lauwers P. Factors affecting mortality in generalized postoperative peritonitis: multivariate analysis in 96 patients. World journal of surgery. 2003;27(4):379-84. https://doi.org/10.1007/s00268-002-6705-x PMid:12658477

6. Mazuski JE. Antimicrobial treatment for intra-abdominal infections. Expert opinion on pharmacotherapy. 2007;8(17):2933-45. https://doi.org/10.1517/14656566.8.17.2933 PMid:18001254

7. Adkins AL, Robbins J, Villalba M, Bendick P, Shanley CJ. Open abdomen management of intra-abdominal sepsis. Am Surg. 2004;70(2):137-40. PMid:15011916

8. Stolz D, Smyrnios N, Eggimann P, Pargger H, Thakkar N, Siegemund M, Marsch S, Azzola A, Rakic J, Mueller B, Tamm M. Procalcitonin for reduced antibiotic exposure in ventilator-associated pneumonia: a randomised study. European Respiratory Journal. 2009;34(6):1364-75. https://doi.org/10.1183/09031936.00053209 PMid:19797133

9. Jensen JU, Heslet L, Jensen TH, Espersen K, Steffensen P, Tvede M. Procalcitonin increase in early identification of critically ill patients at high risk of mortality. Critical care medicine. 2006;34(10):2596-602. https://doi.org/10.1097/01.CCM.0000239116.01855.61 PMid:16915118

10. Hochreiter M, Köhler T, Schweiger AM, Keck FS, Bein B, von Spiegel T, Schroeder S. Procalcitonin to guide duration of antibiotic therapy in intensive care patients: a randomized prospective controlled trial. Critical Care. 2009;13(3):R83. https://doi.org/10.1186/cc7903 PMid:19493352 PMCid:PMC2717450

11. Dandona P, Nix D, Wilson MF, Aljada A, Love J, Assicot M, Bohuon CL. Procalcitonin increase after endotoxin injection in normal subjects. The Journal of Clinical Endocrinology & Metabolism. 1994;79(6):1605-8. PMid:7989463

12. Schroeder S, Hochreiter M, Koehler T, Schweiger AM, Bein B, Keck FS, Von Spiegel T. Procalcitonin (PCT)-guided algorithm

reduces length of antibiotic treatment in surgical intensive care patients with severe sepsis: results of a prospective randomized study. Langenbeck's archives of surgery. 2009;394(2):221-6. https://doi.org/10.1007/s00423-008-0432-1 PMid:19034493

13. Schuetz P, Chiappa V, Briel M, Greenwald JL. Procalcitonin algorithms for antibiotic therapy decisions: a systematic review of randomized controlled trials and recommendations for clinical algorithms. Archives of internal medicine. 2011;171(15):1322-31. https://doi.org/10.1001/archinternmed.2011.318 PMid:21824946

14. Schuetz P, et al, Procalcitonin to initiate or discontinue antibiotics in acute respiratory tract infections. Cochrane Database of Systematic Reviews. 2012; (9):CD007498. https://doi.org/10.1002/14651858.CD007498.pub2

15. Agarwal R, Schwartz DN. Procalcitonin to guide duration of antimicrobial therapy in intensive care units: a systematic review. Clinical Infectious Diseases. 2011;53(4):379-87. https://doi.org/10.1093/cid/cir408 PMid:21810753

16. Kopterides P, Siempos II, Tsangaris I, Tsantes A, Armaganidis A. Procalcitonin-guided algorithms of antibiotic therapy in the intensive care unit: a systematic review and meta-analysis of randomized controlled trials. Critical care medicine. 2010;38(11):2229-41. https://doi.org/10.1097/CCM.0b013e3181f17bf9 PMid:20729729

17. Rau BM, Frigerio I, Büchler MW, Wegscheider K, Bassi C, Puolakkainen PA, Beger HG, Schilling MK. Evaluation of procalcitonin for predicting septic multiorgan failure and overall prognosis in secondary peritonitis: a prospective, international multicenter study. Archives of Surgery. 2007;142(2):134-42. https://doi.org/10.1001/archsurg.142.2.134 PMid:17309964

18. Meisner M. Update on procalcitonin measurements. Annals of laboratory medicine. 2014;34(4):263-73. https://doi.org/10.3343/alm.2014.34.4.263 PMid:24982830 PMCid:PMC4071182

19. Meisner M, Tschaikowsky K, Schnabel S, Schmidt J, Katalinic A, Schüttler J. Procalcitonin-influence of temperature, storage, anticoagulation and arterial or venous asservation of blood samples on procalcitonin concentrations. Clinical Chemistry and Laboratory Medicine. 1997;35(8):597-602. https://doi.org/10.1515/cclm.1997.35.8.597

20. Omar AS, ElShawarby A, Singh R. Early monitoring of ventriculostomy-related infections with procalcitonin in patients with ventricular drains. Journal of clinical monitoring and computing. 2015;29(6):759-65. https://doi.org/10.1007/s10877-015-9663-1 PMid:25638513

21. Slieker JC, Aellen S, Eggimann P, Guarnero V, Schäfer M, Demartines N. Procalcitonin-Guided Antibiotics after Surgery for Peritonitis: A Randomized Controlled Study. Gastroenterology Research and Practice. 2017;2017.

22. Huang TS, Huang SS, Shyu YC, Lee CH, Jwo SC, Chen PJ, Chen HY. A procalcitonin-based algorithm to guide antibiotic therapy in secondary peritonitis following emergency surgery: a prospective study with propensity score matching analysis. PloS one. 2014;9(3):e90539. https://doi.org/10.1371/journal.pone.0090539 PMid:24594916 PMCid:PMC3942439

23. Schroeder S, Hochreiter M, Koehler T, Schweiger AM, Bein B, Keck FS, Von Spiegel T. Procalcitonin (PCT)-guided algorithm reduces length of antibiotic treatment in surgical intensive care patients with severe sepsis: results of a prospective randomized study. Langenbeck's archives of surgery. 2009;394(2):221-6. https://doi.org/10.1007/s00423-008-0432-1 PMid:19034493

24. Hochreiter M, Köhler T, Schweiger AM, Keck FS, Bein B, von Spiegel T, Schroeder S. Procalcitonin to guide duration of antibiotic therapy in intensive care patients: a randomized prospective controlled trial. Critical Care. 2009;13(3):R83. https://doi.org/10.1186/cc7903 PMid:19493352 PMCid:PMC2717450

25. Jung B, Molinari N, Nasri M, Hajjej Z, Chanques G, Jean-Pierre H, Panaro F, Jaber S. Procalcitonin biomarker kinetics fails to predict treatment response in perioperative abdominal infection with septic shock. Critical Care. 2013;17(5):R255. https://doi.org/10.1186/cc13082 PMid:24156734 PMCid:PMC4056026

26. Solomkin JS, Mazuski JE, Bradley JS, Rodvold KA, Goldstein EJ, Baron EJ, O'neill PJ, Chow AW, Dellinger EP, Eachempati SR, Gorbach S. Diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. Clinical infectious diseases. 2010;50(2):133-64. https://doi.org/10.1086/649554 PMid:20034345

27. Hennessey DB, Burke JP, Ni-Dhonochu T, Shields C, Winter DC, Mealy K. Preoperative hypoalbuminemia is an independent risk factor for the development of surgical site infection following gastrointestinal surgery: a multi-institutional study. Annals of surgery. 2010;252(2):325-9. https://doi.org/10.1097/SLA.0b013e3181e9819a PMid:20647925

28. Hakansson A, Molin G. Gut microbiota and inflammation. Nutrients. 2011;3(6):637-82. https://doi.org/10.3390/nu3060637 PMid:22254115 PMCid:PMC3257638

29. Martini A, Gottin L, Menestrina N, Schweiger V, Simion D, Vincent JL. Procalcitonin levels in surgical patients at risk of candidemia. Journal of Infection. 2010;60(6):425-30. https://doi.org/10.1016/j.jinf.2010.03.003 PMid:20226210

30. Leli C, Ferranti M, Moretti A, Al Dhahab ZS, Cenci E, Mencacci A. Procalcitonin levels in gram-positive, gram-negative, and fungal bloodstream infections. Disease markers. 2015;2015.

31. Brodská H, Malíčková K, Adámková V, Benáková H, Šťastná MM, Zima T. Significantly higher procalcitonin levels could differentiate Gram-negative sepsis from Gram-positive and fungal sepsis. Clinical and experimental medicine. 2013;13(3):165-70. https://doi.org/10.1007/s10238-012-0191-8 PMid:22644264

32. Charles PE, Ladoire S, Aho S, Quenot JP, Doise JM, Prin S, Olsson NO, Blettery B. Serum procalcitonin elevation in critically ill patients at the onset of bacteremia caused by either Gram negative or Gram positive bacteria. BMC infectious diseases. 2008;8(1):38. https://doi.org/10.1186/1471-2334-8-38 PMid:18366777 PMCid:PMC2289831

33. Li S, Rong H, Guo Q, Chen Y, Zhang G, Yang J. Serum procalcitonin levels distinguish Gram-negative bacterial sepsis from Gram-positive bacterial and fungal sepsis. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2016;21.

34. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, Suppes R, Feinstein D, Zanotti S, Taiberg L, Gurka D. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Critical care medicine. 2006;34(6):1589-96. https://doi.org/10.1097/01.CCM.0000217961.75225.E9 PMid:16625125

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):915-919.
https://doi.org/10.3889/oamjms.2017.202
eISSN: 1857-9655
Clinical Science

Correlation between the Montreal Cognitive Assessment-Indonesian Version (Moca-INA) and the Mini-Mental State Examination (MMSE) in Elderly

Aldy Safruddin Rambe*, Fasihah Irfani Fitri

Department of Neurology, Faculty of Medicine, Universitas Sumatera Utara, Indonesia

Citation: Rambe AS, Fitri FI. Correlation between the Montreal Cognitive Assessment-Indonesian Version (Moca-INA) and the Mini-Mental State Examination (MMSE) in Elderly. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):915-919. https://doi.org/10.3889/oamjms.2017.202

Keywords: MMSE; MoCA-INA; Cognitive screening; Elderly.

***Correspondence:** Aldy Safruddin Rambe, University of Sumatera Utara, Medan, North Sumatera, Indonesia. E-mail: aldyrambe84@gmail.com

Received: 21-Aug-2017; **Revised:** 24-Sep-2017; **Accepted:** 28-Sep-2017; **Online first:** 25-Nov-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: As the rapid growth of the elderly population and the increased prevalence of Alezheimer's Disease and related disorders, there is an increasing need for effective cognitive screening. The Mini Mental State Examination (MMSE) is the most frequently used screening test of cognitive impairment because of its convenience. The Montreal Cognitive Assessment-Indonesian Version (MoCA-INA) has been validated and recently been used as a cognitive screening tool.

OBJECTIVES: The aim of this study was to compare the MMSE and MoCA-INA scores and to determine the correlation between the MMSE and MoCA-INA scores in elderly.

MATERIAL AND METHODS: This was a cross-sectional study including 83 elderly subjects from November 2016 until June 2017. We performed MMSE and MoCA-INA for assessment of cognitive function and the time between each test was at least 30 minutes.

RESULTS: The study included 83 subjects which were consisted of 46 (55.4%) males and 37 (44.6%) females. The mean age was 69.19 ± 4.23 ranging from 65 to 79 years old. The average MMSE scores was 24.96 ± 3.38 (range 14 to 30). The average MoCA-INA scores was 21.06 ± 4.56 (range 5 to 30). The Pearson correlation coefficient between the scores was 0.71 ($p<0.005$). There were no significant differences of both scores based on history of hypertension, diabetes mellitus and previous stroke, but there was a significant difference in MMSE scores based on level of education.

CONCLUSION: The MoCA-INA score showed a good correlation with the MMSE score. Both tests showed comparable results but MoCA-INA showed lower average with wider range of scores.

Introduction

As our population grows older, the issue of screening for dementia and cognitive impairment will become increasingly important because of the increase incidence of Alzheimer's disease (AD) and related disorders. Improvements in survival rates following stroke will also increase the incidence of vascular and post-stroke cognitive impairment, as approximately 30% of stroke survivors will develop a progressive dementia [1].

Alzheimer's disease (AD) is a chronic, debilitating condition causing significant disease burden and mortality in elderly. The health and economic impact of AD has led to a pressing need to prevent or slow disease onset and progression, and recent research efforts have focused on the transitional period from normal cognitive aging to dementia. This transition period, namely mild cognitive impairment (MCI), is signified by a measurable deterioration in cognitive function that is greater than expected based on an individual's age and education but which has not meaningfully affected a person's daily functioning [2, 3].

Mild cognitive impairment is common in elderly patients and can impact on prognosis and quality of life. The areas of cognitive impairment that occur at this stage primarily involve attention, verbal fluency, executive function and visuo-spatial skills, which differs from the language and memory skills that are commonly associated with dementia [5].

Cognitive screening tools in the elderly are important for the purpose of identifying the presence of cognitive impairment. Neuropsychological testing is the gold-standard for assessing dementia and cognitive impairment, but it is time-consuming and requires highly trained assessors. The Mini Mental State Examination (MMSE) is the most frequently used screening test of cognitive impairmens of AD [5], mainly because of its convenience but not sensitive, as it is influenced by age, socio-economic status and level of education. It assesses primarily language and memory skills and has been found to be insensitive to detecting mild cognitive impairment [4]. Cognitive performance as measured by the MMSE varies within the normal population by age and education [6]. The Montreal Cognitive Assessment (MoCA), has been developed as a brief cognitive screening tool to detect mild-moderate cognitive impairment. It has been found to have high sensitivity and specificity for the detection of mild cognitive impairment [4, 7]. The MoCA assess several cognitive domains including executive fuction, visuospatial function, attention and concentration, memory, languange, calculation and orientation [7]. The Indonesian version of MoCA, namely MoCA-INA has been developed and validated in Indonesia and so it can be used as a cognitive screening tool [8]. The aim of this study was to compare the MMSE and MoCA-INA scores and to determine the correlation between the MMSE and MoCA-INA scores in elderly subjects.

Method

This was a cross sectional study involving 83 subjects which were recruited from the Memory Clinic Neurology Department Adam Malik General Hospital Medan North Sumatera Indonesia, between November 2016 and June 2017. Inclusion criteria were age more than 65 years-old, compos mentis and fully cooperative, speak Bahasa Indonesia fluently, able to read and write, and gave written consent to be included in the study. Subjects who were medically unstable (delirium) or other psychiatric disorders, had an aphasia were excluded from the study. All subjects underwent physical and neurologic examination and cognitive assessment including Mini Mental State Examination (MMSE) and Montreal Cognitive Assessment-Indonesian Version (MoCA-INA). The time between each test was at least 30 minutes. Demographic information was collected including age, sex, occupation, level of education, history of stroke, hypertension and diabetes mellitus. The MMSE and MoCA-INA Scores were obtained. All statistical procedures were performed with SPSS. The correlation between MMSE and MoCA-INA Scores was measured using the Pearson correlation. Both scores were also compared based on level of education. The study was performed with approval obtained from the Health Research Ethical Committee Medical Faculty of Universitas Sumatera Utara/H. Adam Malik General Hospital.

Results

A total of 83 subjects were studied. The average age of subjects was 69.19 years old, ranging from 65 to 79 years old. There were 46 (55.4%) males and 37 (44.6%) females. Most of the subjects had level of education of high school (43 subjects, 51.8%). There were 26 subjects (31.3 %) with history of hypertension, 17 (20.5%) with Diabetes Mellitus and 11 subjects (13.3%) with history of previous stroke. The demographic characteristics are shown in Table 1.

Table 1: Demographic characteristics

Characteristic	Number (%) (n = 83)
Sex	
Male	46 (55.4)
Female	37 (44.6)
Age,mean ± SD, years	69.19 ± 4.23
Age group, years	
65-69	53 (63.9)
70-74	18 (21.7)
75-79	12 (14.5)
Occupation	
Employee	16 (19.3)
Housewive	20 (24.1)
Entrepreneur	13 (15.7)
Farmer	4 (4.8)
Unemployed	30 (36.1)
Level of education	
Elementary School	15 (11.3)
Junior High school	19 (14.3)
High School	63 (47.4)
College/University	36 (27.1)
History of Diabetes Mellitus	
Yes	17 (20.5)
No	66 (79.5)
History of Hypertension	
Yes	26 (31.3)
No	57 (68.7)
History of Previous Stroke	
Yes	11 (13.3)
No	72 (86.7)
MMSE	
< 24	31 (37.3)
≥ 24	52 (62.7)
Moca-INA Score	
< 26	71 (85.5)
≥ 26	12 (14.5)

The average MMSE score was 24.96 ± 3.38 (range 14 to 30). The average MoCA-INA score was 21.06 ± 4.56 (range 5 to 30). Both scores showed comparable result but MoCA-INA showed lower average and a broader range of scores. Comparison between the MMSE and MoCA-INA Score is shown in Table 2.

Table 2: Comparisan between the MMSE and MoCA-INA Score

Score	Mean	SD	Median	Range
MMSE	24.96	3.38	25	14-30
Moca-INA	21.06	4.56	21	5-30

The Pearson's correlation coefficient between the scores was 0.71 ($p < 0.005$). A graph showing the comparison between the MMSE and MoCA-INA

scores based on age groups is shown in Figure 1. There were no significant differences of both scores based on history of hypertension, diabetes mellitus and previous stroke, but there was a significant difference in MMSE scores based on level of education, but not in MoCA-INA score (Table 3).

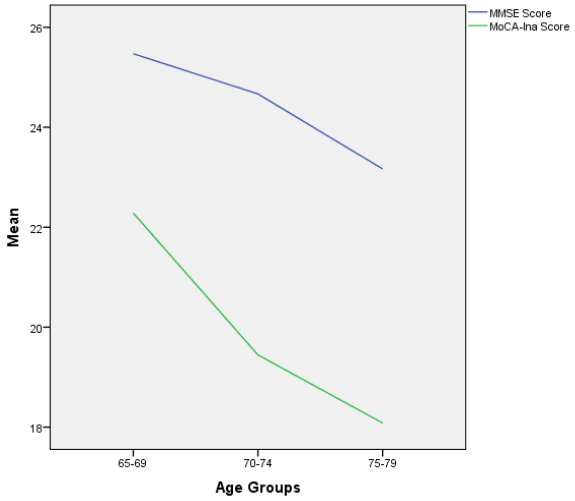


Figure 1: A graph showing the comparison between the MMSE and MoCA scores based on age groups

One-way ANOVA. *Post-hoc LSD: elementary vs. high school p = 0.003; high school vs. college p = 0.015.

Table 2: Comparison between the MMSE and MoCA-INA Score

Level of Education	MMSE Score			MoCA-INA Score		
	Mean	SD	P	Mean	SD	p
Elementary	21.40	2.96	0.007*	16.60	5.12	0.133
Junior High School	24.67	3.36		22.00	3.24	
High School	26.00	2.94		21.42	4.83	
College/University	23.96	3.58		20.87	4.24	

One-way ANOVA; *Post-hoc LSD: elementary vs high school p = 0.003; high school vs college p = 0.015.

Discussion

As the rapid growth of the elderly population and the increased prevalence of AD and related disorders, there is an increasing need for effective cognitive screening. This study compared the MMSE and MoCA-INA scores as cognitive screening tools. The Indonesian version of MoCA has been validated and said to be applicable for assessment of cognitive function. There are several adjusments of MoCA-INA compared to the original version in assessment of naming, memory and delayed recall and language function because of transcultural validation [8]. The results showed that The MoCA-INA and MMSE showed comparable results but MoCA-INA showed lower average with wider range of scores. This finding has also been observed in a study by Ohta et al which compared Japanese version of MMSE and MoCA scores in 304 patients with Parkinson's disease. They found the MMSE and MoCA scores were 26.3 ± 3.6 (range 12-30) and 20.9 ± 5.0 (range 5-30), respectively [9]. A study by Aggarwal et al also found lower MoCA score (22.2 ± 5.1) if compared with MMSE (26.5 ± 3.5) [4].

This finding reflects the MoCA-INA as a more challenging test that includes executive function, higher level languange and complex visuospatial processing that enable it to detect mild impairment or certain domain of cognitive function, if compared to MMSE. The MMSE has both a 'ceiling' and 'floor' effect: a score of 30 does not always mean normal cognitive function and a score of zero does not mean an absolute absence of cognition. It does not contain much capacity to test frontal/executive or viusospasial (typically right parietal) functions. The pentagon task of the MMSE simply requires the patient to copy the image and does not assess planning skills [10].

This is in line with several previous studies that compared the MMSE and MoCA as cognitive screening tool in differentiating dementia from MCI and normal cognitive aging. A study conducted by Roalf et al in 321 AD patients, 126 MCI and 140 healthy controls found that the MoCA is superior to the MMSE as a global assessment tool, particularly in discerning earlier stages of cognitive decline. In addition, the author found that overall diagnostic accuracy improved when the MMSE or MoCA was combined with an informant-based functional measure [2]. In a study involving 219 healthy control, 299 MCI and 100 AD cases, in which the author analyzed the relationship between the MoCA and MMSE scores, it was found that both tools were more similar for dementia cases, but MoCA distributed MCI cases across a broader score range with less ceiling effect [11]. The MOCA is a useful brief screening tool for the detection of mild dementia or MCI. With a cut-off score of 26, the MMSE had a sensitivity of 17% to detect subjects with MCI, whereas the MoCA detected 83% [12]. The MoCA showed a high sensitivity (0.94) compared to MMSE (0.66) in detecting post stroke cognitive impairment [13].

This study found the differences in MMSE score based on level of education, while the MoCA-INA did not show any significant difference. This finding could also support the superiority of MoCA-INA than MMMSE. MoCA has also been found to be superior to MMSE in assessing cognitive impairment in several other conditions. Wong et al carried out a prospective onservational and diagnostic accuracy study on aneurysmal subarachnoid hemorrhage, The MoCA and MMSE were administered 2-4 weeks and 1 year after ictus. They found that both tools were succesful in differentiating between patients with and without cognitive impairment but at 1 year post-ictus, the MoCA produced higher area under the curve scores for cognitive impairment than the MMSE [14]. MoCA was also found to be more sensitive and reliable than MMSE in testing the cognitive status in

epilepsy population with phenytoin monotherapy [15]. The MoCA, but not the MMSE, has adequate psychometric properties as a screening instrument for the detection of mild cognitive impairment or dementia in Parkinson disease [16].

Several advantages of the MMSE includes being the most widely used and studied worldwide and often used as reference for comparative evaluations of other assessments. Its limitations are : education/age/language/culture bias; ceiling effect; best performance for at least moderate cognitive performance. Advantages of the MoCA include being designed to test for mild cognitive impairment and tests many separate domains. Its limitations are: lacks of studies in general practice settings; education bias (≤12 years), limited use and evidence due to published data relatively new (2005) [17].

This study also found a good correlation between the MMSE and MoCA-INA scores ($r = 0.71$ ($p < 0.005$). This finding is consistent with several previous studies about correlation between these scores in different clinical setting, such as in an inpatient rehabilitation ($r = 0.695$, $p < 0.003$) 4 and in Parkinson's disease patients ($r = 0.74$, $p < 0.001$) 9 and in a clinical cohort ($r = 0.82$, $p < 0.001$) [18].

We used cut point of 24 for the MMSE and 26 for the MoCA in this study, so score equal to or less than 24 on the MMSE or less than 26 on the MoCA showed cognitive impairment. Although optimum sensitivity and specificity of the MMSE probably vary depending on the patien's age and education level, a large body of literature suggests that a general cut point of 23/24 or 24/25 is appropriate for most primary care populations [3, 6].

Interestingly in this study, the proportion of subjects with cognitive impairment found to be higher than normal subjects. Although this could be attributed to history of previous disease (stroke, hypertension and diabetes mellitus), we did not find any statistical differences between these groups indicating the importance of screening for cognitive function in every day clinical practice. Early detection of cognitive impairment that indicates transition to AD may improve diagnosis and lead to better management of the disease. The basic purpose of cognitive screening tests is to indicate a likelihood of cognitive impairment which can be inferred from comparing the patient's score to reference norms. A very impaired score, along with supporting history and clinical findings may lead a clinician to make a diagnosis, while a borderline score may need a further investigation Cognitive screening test is not intended to substitute a full neuropsychlogical assessment but it can be used to obtain a key for impaired cognitive domain in a patient.

References

1. Cullen B, O'Neill B, Evans JJ, et al. A review of screening tests for cognitive impairment. J Neurol Neurosurg Psychiatry. 2007; 78: 790-9. https://doi.org/10.1136/jnnp.2006.095414 PMid:17178826 PMCid:PMC2117747

2. Roalf DR, Moberg PJ, Xie SX, et al. Comparative accuracies of two common screening instruments for classification of Alzheimer's disease, mild cognitive impairment, and healthy aging. Alzheimer's & Dementia. 2012:1-9.

3. Moyer VA. Screening for Cognitive Impairment in Older Adults: U.S. Preventive Services Task Force Recommendation Statement. Ann Intern Med. 2014; 160:791-7. https://doi.org/10.7326/M14-0496 PMid:24663815

4. Aggarwal A, Kean E. Comparison of the Folstein Mini Mental State Examination (MMSE) to the Montreal Cognitive Assessment (MoCA) as a Cognitive Screening Tool in an Inpatient Rehabilitation Setting. Neuroscience & Medicine. 2010; 1: p. 39-42. https://doi.org/10.4236/nm.2010.12006

5. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975; 12:189–98. https://doi.org/10.1016/0022-3956(75)90026-6

6. Sjahrir H, Ritarwan K, Tarigan S, Rambe AS, Lubis ID, Bhakti I. The mini mental state examination in healthy individuals in Medan Indonesia by age and education level. Neurol J Southeast Asia. 2001;6:19-22.

7. Nasreddine ZS, Phillips NA, Bedirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005;53(4):695–9. https://doi.org/10.1111/j.1532-5415.2005.53221.x PMid:15817019

8. Husein N, Silvia L, Yetty R, dkk. Uji Validitas dan Reabilitas Montreal Cognitive Assessment Versi Indonesia (MoCA-Ina) Untuk Skrining Gangguan Fungsi Kognitif. Neurona Neuro Sains. 2010; 27(4):15-22.

9. Ohta K, Osada T, Shinohara Y, Suzuki N, Takahashi K, Seki M, et al. Comparing the Montreal Cognitive Assessment with Mini-Mental State Examination in japanese parkinson's disease patients. Neurology and Clinical Neuroscience. 2014;2:44-49. https://doi.org/10.1111/ncn3.80

10. Woodford HJ, George J. Cognitive assessment in the elderly: a review of clinical methods. Q J Med. 2007; 100:469-484. https://doi.org/10.1093/qjmed/hcm051 PMid:17566006

11. Trzepacz PT, Hochstetler H, Wang S, et al. Relationship between the Montreal Cognitive Assessment and Mini-mental State Examination for assessment of mild cognitive impairment in older adults. BMC Geriatrics. 2015; 15:107. https://doi.org/10.1186/s12877-015-0103-3 PMid:26346644 PMCid:PMC4562190

12. Smith, T.; Gildeh, N. and Holmes, C. The Montreal Cognitive Assessment: Validity and Utility in a Memory Clinic Setting. Can J Psychiatry. 2007; 52(5): 329-32. https://doi.org/10.1177/070674370705200508 PMid:17542384

13. Godefroy O, Fickl A, Roussel M, Auribault C, Bugnicourt JM, Lamy C, et al. Is the Montreal Cognitive Assessment superior to the Mini-Mental State Examination to detect poststroke cognitive impairment? A study with neuropsychological evaluation. Stroke. 2011;42:1712-1716. https://doi.org/10.1161/STROKEAHA.110.606277 PMid:21474808

14. Wong GK, Lam SW, Wong A, Ngai K, Poon WS, Mok V. Comparison of montreal cognitive assessment and mini-mental state examination in evaluating cognitive domain deficit following aneurysmal subarachnoid haemorrhage. PLoS One. 2013;8(4):e59946. https://doi.org/10.1371/journal.pone.0059946 PMid:23573223 PMCid:PMC3616097

15. Nathami R, Amirthalingam P, Mukunthu et al.Sensitivity of Montreal Cognitive Assessment in Comparison with Mini MentalStatus Examination in Testing Cognitive Status in Epilepsy

Patients with Phenytoin Monotherapy. AJPCT. 2015; 3(03): 237-244.

16. Hoops S, Nazem S, Siderowf AD, et al. Validity of the MoCA and MMSE in the detection of MCI and dementia inParkinson disease. Neurology. 2009; 73:1738-1745. https://doi.org/10.1212/WNL.0b013e3181c34b47 PMid:19933974 PMCid:PMC2788810

17. Cordell CB, Borson S, Boustani M, Chodosh J, Reben D, Verghese J, et al. Alzheimer's association recommendations for operationalizing the detection of cognitive impairment during the Medicare Annual Wellness Visit in primary care setting. Alzheimer's & Dementia. 2013;9:141-150. https://doi.org/10.1016/j.jalz.2012.09.011 PMid:23265826

18. Roalf DR, Moore TM, Hamilton DM, Wolk DA, Arnold SE, Weintraub DA, et al. Bridging cognitive screening tests in neurologic disorders: A crosswalk between the short Montreal Cognitive Assessment and Mini-Mental State Examination. Alzheimer's & Dementia. 2017;1-6. https://doi.org/10.1016/j.jalz.2017.01.015

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):920-924.
https://doi.org/10.3889/oamjms.2017.205
eISSN: 1857-9655
Clinical Science

Kosovo's Experience for Children with Feeding Difficulties after Cardiac Surgery for Congenital Heart Defect

Ramush Bejiqi[1*], Ragip Retkoceri[2], Hana Bejiqi[3], Arlinda Maloku[2], Armend Vuçiterna[1], Naim Zeka[2], Abdurrahim Gerguri[2], Rinor Bejiqi[4]

[1]University of Gjakovo, Paediatric Clinic, University Clinical Centre of Kosovo, Prishtina, Kosovo; [2]Paediatric Clinic, University Clinical Centre of Kosovo, Prishtina, Kosovo; [3]Main Center of Family Medicine, Prishtina, Kosovo; [4]Medical School, University or Kosovo, Prishtina, Kosovo

Citation: Bejiqi R, Retkoceri R, Bejiqi H, Maloku A, Vuçiterna A, Zeka N, Gerguri A, Bejiqi R. Kosovo's Experience for Children with Feeding Difficulties aOnlineFirstfter Cardiac Surgery for Congenital Heart Defect. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):920-924. https://doi.org/10.3889/oamjms.2017.205

Keywords: Cardiac surgery; Congenital heart defect; echocardiography; Chromosomal abnormalities; Feeding problems.

***Correspondence:** Ramush Bejiqi. University of Gjakovo, Paediatric Clinic, University Clinical Centre of Kosovo, Prishtina, Kosovo. E-mail: rbejiqi@hotmail.com

Received: 11-Jul-2017; **Revised:** 24-Sep-2017; **Accepted:** 27-Sep-2017; **Online first:** 27-Nov-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: A feeding disorder in infancy and during childhood is a complex condition involving different symptoms such as food refusal and faddiest, both leading to a decreased food intake.

AIM: We aimed to assess the prevalence and predictor factors of feeding difficulties in children who underwent cardiac open heart surgery in neonatal period and infancy. We address selected nutritional and caloric requirements for children after cardiac surgery and explore nutritional interdependence with other system functions.

METHODS: This was a retrospective study in a tertiary referral hospital, and prior approval from the institutional ethics committee was obtained. Information for 78 children (42 male and 36 female) was taken from patients charts. Data were analysed with descriptive statistics and logistic regression.

RESULTS: From a cohort of analysed children with feeding problems we have occurred in 23% of such cases. At the time of the study, refusal to eat or poor appetite was reported as a significant problem in 19 children and subnormal height and weight were recorded in 11 children. Early neonatal intervention and reoperation were identified as risk factors for latter feeding difficulties or inadequate intake. Children with feeding problems also tended to eat less than children without feeding problems. There was a trend towards more feeding problems in patients with chromosomal abnormalities or other associated anomalies.

CONCLUSION: Feeding disorder is often and a frequent long-term sequel in children after neonatal or early infancy heart surgery. Patients with chromosomal and associated anomalies who underwent multiple cardiac surgeries are at risk of developing feeding difficulties.

Introduction

A feeding disorder in infancy and during childhood is a complex condition involving different symptoms such as food refusal and faddiness, both leading to a decreased food intake. It often results from abnormal feeding development. Also, adequate nutrition is crucial and challenging for children after surgery for congenital heart defects. There is a worldwide reason for attention to the lesion or specific feeding problems, supplementation of trace elements and minerals, and an organised approach to pace, timing, and type of feeding are beneficial. These patients need to be selected for preventive strategies, and nutritional intervention should be offered in order to increase the caloric intake of the child and to develop a sound feeding relationship in the family.

Babies with congenital heart defects often need more calories per day than babies with normal hearts, particularly if they are struggling with symptoms of congestive heart failure. Feeding can be challenging for some reasons, so parents and other caregivers often work closely with the baby's healthcare team to make sure the baby is getting enough calories to gain weight and grow [1, 2].

What is known?

1. Children with congenital heart defects

have problems with feeding caused by heart insufficiency.

2. Patients with chromosomal and associated heart defects are at increasing risk for feeding problems.

What is new?

1. Patients with complex congenital heart defects and chromosomal anomalies who underwent multiple cardiac surgeries are at risk of developing feeding difficulties.

2. As a lack of paediatric cardiac surgeries in Kosovo, children with congenital heart defects have been surgically treated in many European and North American centres.

The aim of this retrospective study was to describe the prevalence of feeding disorders in infancy and children after open heart surgery.

Material and Methods

The study included 78 children undergoing open heart surgery for congenital heart defects in the neonatal period and infancy between 2005 and 2010. The study group included patients who had survived more than three years after surgery; all patients who did not survive the first three years of life after surgery were eliminated from the study.

The study was designed collecting data from medical records of cardiological diagnosis, reports from surgery intervention and outpatient correspondence as well as fellow assessment three years after surgery from a paediatric cardiologist at tertiary level. In the lack of cardiosurgical services in Kosovo, all children were sent abroad for surgery. Cardiological diagnosis from the local cardiologist was compared with diagnosis at the centre where the surgery was done, and there we found full compliance. Differently, from the other centres which used two years for reassessing the feeding behaviour, we have chosen three years as a reason that few children have been sent abroad under treatment with Prostaglandins where the possibility for developing neurological consequences is much higher. Otherwise, the period of 2 years is taken from more centres in the world as the ideal period to reassess feeding behaviour and only severe and relevant feeding disorders persist until that age, because the prevalence of the feeding disorders in the normal population has been well defined at the age of 2 years [7, 10, 11].

Analysed data included pre-operative data: birth weight, type of congenital heart defects, associated anomalies and syndromes, need for giving Prostaglandins and long-term of treating, based on the type of CH. The Cardiac surgery data included: the centre where the surgery was done, the type of surgery, the duration of extra-corporal circulation or the duration of the operation in off bypass operations. Post-operative data included: duration of the mechanical ventilation, total hospital stay, in-hospital feeding parameters which included the duration of tube feeding, the onset of oral food intake, and whether the child was referred to the speech pathologist on account of severe difficulties in swallowing or sucking. In the post-operative data, we also have attached neurological findings documented during the routine neurological examination after the operation was labelled as neurological abnormalities.

To simplify data on cardiac defects included in the study, all these were divided into two groups based on cardiological findings before the surgery and the intra-cardiac morphology during the surgery:

Group 1: "simple cardiac defects", in which a complete anatomical repair is possible by one intervention.

Group 2: "complex congenital disabilities" in which are necessary two or three cardiac surgery interventions achieve on anatomical or physiological repair.

Most patients from the Group 2 underwent two interventions and whereas few of them are preparing for third-stage of palliation.

Based on the age of children when they underwent surgery all patients are divided into three groups:

Group 1: Children underwent complete cardiac surgery on the neonatal period;

Group 2: Children where the first surgery was on the neonatal period and the second was in infancy;

Group 3: Patients where cardiac surgery was done on the period of infancy period.

The questionnaire was designed to obtain information for quality and quantity of the nutrition, on feeding behaviour and food intake, whether it was appropriate for the age of 3 years old. The questionnaire also includes body weight gain, needs for artificial feeding, present of gastro-oesophageal reflux and frequent respiratory infection (aspirate pneumonia).

Feeding disorder was defined as the presence of one or more of the following criteria at the age of 3 years, based on the information given by care provider.

Group 1: Child is partially or completely dependent on tube feeding;

Group 2: Feeding is not adequate for the following age and mostly is based on the drink or takes pureed food;

Group 3: Child manifests delays in obtaining foods, there is a failure of thriving, the body weight is in the third percentile, child manifests anaemia etc.

In the absence of cardiac surgery service in Kosovo, all children were sent abroad. Based on the country where surgery was done, all children can be divided into four groups:

Group 1: Children operated in Italy (mostly Genoa, few of them in Bergamo, Padua, Bologna and Verona) – 54/78 (69 %);

Group 2: Children operated in Albania – 12/78 (15.4 %);

Group 3: Children operated in Turkey - 6/78 (7.7 %);

Group 4: Children operated in other countries - 6/78 (7.7 %).

Statistical analysis

Data were analysed using the SPSS 15.0 for Windows statistical software. We analysed continuous variables which are expressed as the median (range) and dichotomous variables as numbers and percentage. Multivariate logistic regression analysis was performed to determine the independent influence of risk factors on abnormal feeding problems. Univariate analyses were performed using the chi-square test or Mann – Whitney U-test. Also, Spearman's correlation coefficient was calculated to determine the correlation between different risk factors.

Results

The study group consisted of 78 patients. Median birth weight was 3.35 kilograms, with a range from 2.8 to 4.6; the median gestational period was 39 weeks (range from 32 to 41 weeks). The patients underwent surgery for CHD at a median age of 16 days, ranging from 8 to 27 days (Group 1), 18 days, ranging from 12 to 31 days (Group 2) and 5 month and 16 days, ranging from three months and 22 days to 7 months and 12 days (Group 3). Clinical signs of heart failure were presented in 43/78 (55 %) patients. Open heart surgery with the use of cardiopulmonary bypass was performed in 62 patients (79 %). The most frequent surgery was resection of the aortic coarctation 21/78 (27 %), large ventricular septal defect 17/78 (22 %) and arterial switch operation for transposition of the great arteries 13/78 (16.6 %) (Table 1). Malformations syndromes were present in 11/78 (14 %) children (Table 2).

Initially, feeding through the nasogastric tube was in 43/78 (55 %) children (all neonates and six infancies). After three years of feeding through the nasogastric tube, only three patients continued.

Table 1: Type of congenital heart defect, number of patients and percentage

	N	%
Aortic coarctation	21	27
Ventricular septal defect	17	22
Transposition of the great arteries	13	16.6
Tetralogy of Fallot	8	10
Complete atrioventricular canal	6	7.7
Pulmonary atresia with ventricular septal defect	5	6
Total anomalous pulmonary venous return	4	5
Double outlet right ventricle	3	3.8
Double inlet left ventricle	1	1.3

The remaining patients obtained a nasogastric tube on the introduction of the anaesthesia as a routine procedure to start early feeding within the first few post-operative days. None of them needed a gastroscopic tube.

Table 2: Patients with malformation syndromes and with normal feeding, feeding disorders (FD) and neurological abnormalities (NA)

	Normal	FD	NA
Trisomy 21	2	2	4
Microdeletion 22q11	2	1	0
Turner syndrome	2	0	0
Unclassified dysmorphic syndrome	0	1	1

Feeding status after three years

From the study group of 78 children, nine patients (11.5 %) were diagnosed with feeding disorders. There was noted a strong relationship between the type of the surgery, duration of mechanical ventilation, age at the surgery, duration of perioperative tube feeding and centre where surgery was done (all $R > 0.8$, $p > 0.01$). Patients who undergo complex surgery (univentricular heart palliation, double outlet right ventricle), with small age at the time of surgery and longer ventilation were more frequent in the group with abnormal feeding compared with those with normal feeding behaviour. Also, patients with malformations syndromes manifested higher rate of neurological and feeding difficulties. The multivariate logistic regression analysis included the variables that were significant in the univariate analysis since there was a very high correlation between the three variables: type of CHD, age at operation and reoperation of the univentricular heart.

Discussion

Retrospective analysis of the data of children who underwent open heart surgery shows that feeding disorders are a relevant problem in this population.

This study has not included all aspects of energy balance as we have not attempted to assess time spent and energy expended in activity, thermogenesis, or other non-resting metabolism. Using a similar definition of feeding problems and age of children at the time of the study, the prevalence of severe feeding problems is much higher in a population of children who underwent open heart surgery (23 %) in compare with healthy children (1.42 %) [4, 12].This prevalence is almost as frequent and in correlation with age at the time of cardiac surgery and type-complexity of CHD. Cardiac defects are a significant constitutional factor which contributes to the development of defects in other organs and systems including secondary feeding difficulties. Simultaneously, our study shows that at the age of 3 years of feeding, difficulties were not depended from birth and gestational age, hemodynamic status pre and postoperatively but the greatest impact on the development of feeding disorders have general medical condition such are: age of children who go through the surgery, duration of the medical ventilation and type of surgery, reoperation. Since these three variables were strongly interrelated, only early feeding disorders and multiple surgeries remained significantly associated with feeding problems at the age of three years in the multivariate regression analysis [6].

Adequate enteral nutrition may be difficult to achieve early in neonates after cardiac surgery, but it is essential for growth, wound healing, and immune function. Feeding difficulties in infancy and childhood is a complex condition involving different symptoms, such as food refusal or inadequate intake leading to a decreased food intake and malnutrition. Child's feeding development is determined by its constitution, the environment and the child's learning process [3, 4]. Pathology in one or more of these components can lead to a feeding disorder. Factors of constitutional origin can be organic defects, such as defects of organs directly related to food intake or transport, or defects of other organ systems that disturb the child's feeding and digestion process by impacting on its general health [5]. The child's environment is defined by the parent's behaviour and the family's cultural and social background. Some children start with a purely organic problem, that is, constitutional or mixture of organic and non-organic components. Any imbalance between parental expectations and the child's feeding progress could cause an interaction problem, generating feeding disorders, such as food refusal, avoidance of aversion, on the part of the child. In most patients with feeding disorders, there are combinations of different factors that give rise to the disorder [6, 7].

Recent advances in cardiac surgery techniques and progress in the pre- and postoperative care of new-borns and low weighing children have substantially improved the survival of infants with CHD [8]. This trend is creating a growing "population at risk" for neurodevelopmental and behavioural problems as well as for the development of feeding disorders. However, feeding disorders tend to be increasingly common since advances in technology are allowing more very ill children to survive.

Early identification of deficient oropharyngeal motor skills and vocal cord dysfunction is crucial to establish enteral nutrition safely and has been demonstrated to improve clinical outcomes. The use of prealbumin as a marker of nutritional state should be accompanied by C-reactive protein given the influence of inflammation on its levels. Insulin infusions may improve outcomes in patients with postoperative hyperglycaemia. Trace element abnormalities and early identification of immune-compromised states can aid in reducing morbidity in children after cardiac surgery. Use of feeding protocols and a home surveillance system for hypoplastic left heart syndrome improves outcomes of those children [4, 9].

Besides other relevant influences on the development of feeding disorders in our study the fact that children are treated in several different Europeans Centres, mostly in Italian's, makes a significant implication and in some of the cases cardiovascular system was affected as a consequence of that some children have been longer treated by Prostaglandins (one 38 and the other one 36 days). From this, we can conclude that severe and long hypoxemia, caused by the primary defects and long-term Prostaglandin therapy are crucial for developing neurological and feeding abnormalities.

There was a high variability of the cardiac diagnoses in our study group. We found that univentricular repair was associated with a higher risk of feeding and neurological disorders in compare with simplex and at once corrected anomalies. This can be explained by the various degrees of intracardiac mixing and volume overload, various degree and duration of hypoxemia which is present in children with univentricular heart. These children often require palliative surgery within the first few days of life, followed by at least two other open-heart surgeries [13].

In our study the group with types of malformation syndromes was heterogeneous. It is known that not all syndromes are associated with feeding disorders; in our study in patients with trisomy 21 and those with microdeletion 22q11, the prevalence of feeding disorders is high, whereas in Turner's syndrome the prevalence is not present. The presence of feeding disorders in children with chromosomal and malformations syndromes are reported to be higher than in children without such syndromes due to the associated developmental delay, oral malformation and neurological comorbidity. In most children with malformation syndromes, several of the above-listed risk factors co-occur, which increases the probability of the manifestation and

persistence of a feeding disorder [14].

There is considerable inter-individual variability in the manifestation of feeding disorders within the same syndrome category. In our study group, children with chromosomal abnormalities had a higher prevalence of abnormal feeding development at the age of 3 years. The effect of malformation syndrome on latter feeding difficulties can also be mediated by other risk factors such as more complex cardiac defects and neurological comorbidity. The association between neurological disorders and feeding problems is a well-known phenomenon [8]. Neurological abnormalities such as muscular hypotonia are frequent in children with congenital heart defects and often are diagnosed before cardiac surgery [15]. Among those neurobehavioral abnormalities, there was also an absent suck or poor feeding efficiency. In our study, we found that neurological abnormalities at the time of surgery were associated with abnormal feeding behaviour at 3 years of age. This association persists after correction for other factors: children with neurological abnormalities were six times more likely to manifest later feeding disorders than those without neurological problems. Thus, confirmed neurological abnormalities before the surgery can contribute to the development of feeding disorders as an independent risk factor.

In conclusion, babies with congenital heart defects often need more calories per day than babies with normal hearts, particularly if they are struggling with symptoms of congestive heart failure. Feeding can be challenging for some reasons, so parents and other caregivers often work closely with the baby's healthcare team to make sure the baby is getting enough calories to gain weight and grow. Simultaneously, children who require cardiac surgery in the neonatal period and early infancy are at increasing risk of developing a feeding disorder at three years of age. This is a result of a complex multi-factorial process. Independent risk-factors include severity of CHD, the age of the child who goes through the surgery, type of operation and re-operation, duration of mechanical ventilation, previously diagnosed neurological abnormalities and presence of malformations syndromes. These factors provide key evidence as to which children need to be referred to multidisciplinary teams who will care for elimination or minimisation of feeding problems on these sensitive categories. Whenever feeding problems are reported, nutritional intervention should be offered to increase the caloric intake of the child and to develop a sound feeding relationship in the family.

References

1. Thommessen M, Heiberg A, Kase BF. Feeding problems in children with congenital heart disease: the impact on energy intake and growth outcome. Eur J Clin Nutr. 1992;46:457-64. PMid:1623850

2. Moller JH, Taubert KA, Allen HD, et al. Cardiovascular health and disease in children: current status. A Special Writing Group from the Task Force on Children and Youth, American Heart Association. Circulation. 1994;89:923-30. https://doi.org/10.1161/01.CIR.89.2.923 PMid:8313589

3. Perloff JK, Warnes CA. Challenges posed by adults with repaired congenital heart disease. Circulation. 2001;103:2637-43. https://doi.org/10.1161/01.CIR.103.21.2637

4. Lipsitt LP, Crook C, Booth CA. The transitional infant: behavioural development and feeding. Am J Clin Nutr. 1985;41:485 – 496. PMid:3969954

5. Deller SF, Hyams JS, Treem WR et al Feeding resistance and gastroesophageal reflux in infancy. J Pediatr Gastroenetrol Nutr. 1993;17:66 – 71. https://doi.org/10.1097/00005176-199307000-00009

6. Reilly S, Skuse D, Poblete X. Prevalence of feeding problems and oral motor dysfunction in children with cerebral palsy: a community survey. J Pediatr. 1996;129:877 – 882. https://doi.org/10.1016/S0022-3476(96)70032-X

7. Dahl M, Sundelin C. Early feeding problems in affluent society. I. Categories and clinical signs. Acta Pediatr Scand. 1986;75:370 – 379. https://doi.org/10.1111/j.1651-2227.1986.tb10217.x

8. Ilona M, Beatrice L, Hilda G et al. Prevalence and predictors factors of letter feeding disorders in children who underwent neonatal cardiac surgery for congenital heart disease. Card in the Young. 2011;21:303 – 309. https://doi.org/10.1017/S1047951110001976 PMid:21272426

9. Bejiqi R, Retkoceri R, Zeka N, et al. Treatment of children with protein - losing enteropathy after Fontan and other complex congenital heart disease procedures in condition with limited human and technical resources. Mater Sociomed. 2014;26:39-42. https://doi.org/10.5455/msm.2014.26.39-42 PMid:24757400 PMCid:PMC3990394

10. Dahl M, Eklund G, Sundelin C. Early feeding problems in an affluent society. II. Determinants. Acta Pediatr Scand. 1986;75:380 – 387. https://doi.org/10.1111/j.1651-2227.1986.tb10218.x

11. Dahl M. Early feeding problems in an affluent society. III. Follow-up at two years: natural course, health behaviour and development. Acta Pediatr Scand. 1987;76:872 – 882. https://doi.org/10.1111/j.1651-2227.1987.tb17257.x PMid:3480683

12. Stein A. Barnes J. Feeding and sleep disorders. In: Rutter M (ed) Child and Adolescent Psychiatry, 4th edn. Blackwell Science, Oxford, 2002: 754 – 775.

13. Morris CD, Maneshe VD. 25 year mortality after surgical repair of congenital heart defect in childhood. A population based study. JAMA. 1991;266:3447 – 3452. https://doi.org/10.1001/jama.1991.03470240069035 PMid:1744959

14. Boneva RS, Botto LD, Moore CA et al. Mortality associated with congenital heart defects in the United States. Trends and racial disparities 1979 – 1997. Circulation. 2000; 103: 2376 – 2381. https://doi.org/10.1161/01.CIR.103.19.2376

15. Limperopolous C, Majnemer A, Shevell MI, et al. Neurodevelopmental status of new-borns and infants with congenital heart defects before and after open heart surgery. J Pediatr. 2000; 137:638 – 645. https://doi.org/10.1067/mpd.2000.109152 PMid:11060529

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):925-928.
https://doi.org/10.3889/oamjms.2017.192
eISSN: 1857-9655
Clinical Science

Compare Of the West Syndrome with Other Syndromes in the Epileptic Encephalopathy - Kosovo Experience

Naim Zeka[1*], Abdurrahim Gërguri[2], Ramush Bejiqi[2], Ragip Retkoceri[2], Armend Vuciterna[1]

[1]Department for Neurology, Paediatric Clinic, University Clinical Centre of Kosovo, Prishtina, Kosovo; [2]Department for Cardiology, Paediatric Clinic, University Clinical Centre of Kosovo, Prishtina, Kosovo

Citation: Zeka N, Gërguri A, Bejiqi R, Retkoceri R, Vucitërna A. Compare Of the West Syndrome with Other Syndromes in the Epileptic Encephalopathy - Kosovo Experience. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):925-928. https://doi.org/10.3889/oamjms.2017.192

Keywords: West Syndrome; Hypsarythmia; Epileptic encephalopathy; Infantile spasm.

***Correspondence:** Naim Zeka. Department of Neurology, Paediatric Clinic, University Clinical Centre of Kosovo, Prishtina, Kosovo. E-mail: zekanaim@yahoo.com

Received: 20-Sep-2017; **Revised:** 22-Oct-2017; **Accepted:** 28-Oct-2017; **Online first:** 28-Nov-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: West Syndrome (WS) represents as a specific epileptic encephalopathy characterised with a unique type of attacks, called infantile spasms, severe forms of abnormalities in electroencephalographic (EEG) records as a hypsarythmias and delays in the psychomotoric development. The characteristics of the disease, mostly affecting male gender, are infantile spasms and typical findings in EEG as a hypsarythmia. Infantile spasms are a consequence of many factors in the undeveloped brain.

AIM: We aimed: (1) to see the incidence of the illness and the spreading out because of gender in rapport with other syndromes in the epileptic encephalopathies group; (2) to show principles of the treatment for the illness; and (3) to present the effects of the disease in the psycho-motoric development of affected children.

METHODS: The study was designed as a cross-sectional study of the patients with epileptic encephalopathies, treated in Paediatric Clinic in Prishtina, from 1st of January 2013 until the 31st of December 2015.

RESULTS: From the cohort group of 97 children diagnosed with epileptic encephalopathies, in 14 of them clinical and EEG signs of WS were noted. The earliest age of disease manifestation was 74 days (± 63.8 days). On the group of children with WS, 13 of them with Natrium Valpropat were treated, with the doses of 301.9 mg (± 64.1). From the cohort group, in 89 children (91.8%) psychomotoric retardation was documented, within the higher reoccurrence in the undifferentiated epileptic encephalopathies (96%) and the WS (78.6%).

CONCLUSION: WS is a frequent disease of the encephalopathies with the epileptogenic framework. The resistance in anticonvulsive therapy is huge, and psychomotoric retardation follows a big percentage of children with this syndrome.

Introduction

West Syndrome is specific epileptic encephalopathies groups characterised by a unique type of attacks, called infantile spasms, severe forms of abnormalities in the EEG - hypsarythmia and delays in the psychomotoric development. In 2004, there was a consensus by the experts in this subject, published about the West syndrome. Mostly the syndrome starts from 3 until 12 months of age, with a ratio 1:2000 - 4000 born alive, predominantly affecting male gender (60-70%) [1].

In 2001, ILEA classified the Syndrome West in the epileptic encephalopathies group.

We aimed: (1) to see the incidence of the illness and the spreading out because of gender in rapport with other syndromes in the epileptic encephalopathies group; (2) to show principles of the treatment for the illness; and (3) to present the effects of the disease in the psycho-motoric development of affected children.

Material and Methods

The study was designed as a cross-sectional of the patients with epileptic encephalopathies, treated from 1 of January 2013 until 31 of December 2015. In this presentation from the cohort group of 97 children,

14 of them with WS were diagnosed.

Diagnosis of the disease was based on the anamnestic data, clinical examination, lab analysis, and imagery examinations as a brain echosonography, computerised tomography (CT), magnetic resonance imaging (MRI), neurological examination, electroencephalographic examinations (EEG), tests on the aspect of psycho-motoric developments, therapeutic methods like physiotherapy exercises and medicaments.

For the statistical analysis, we used the program SPSS 20.0, Sigma Stat - Sigma Plot 11.0 and Microsoft Excel 2010. From the statistic parameters, we used the structure index, cumulative structure, simple arithmetic average, standard deviation, standard mistake and confidence interval with the significance level 95% (95%CI). For the testing of the differences of the nonparametric data, we used chi-cubic test (chi-test), for the exact significant level (p).

Results

The vast majority of cases were with undifferentiated epileptogenic encephalopathy (77.3%) and with Syndrome West (14.4%). With syndrome Lennox-Gastaut was diagnosed 4 cases (4.1%), 2 cases with CSWS (2) and 1 case was diagnosed with syndrome Dravet and syndrome Landau-Kleffner.

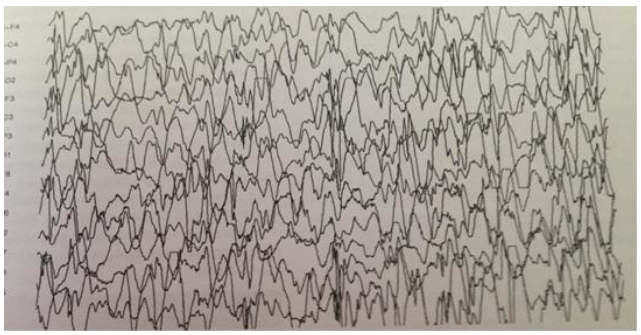


Figure 1: EEG records in the severe form of West Syndrome.

A similar structure was concluded analyzing the gender group; the difference of the structure in cases with undifferentiated epileptogenic encephalopathy was higher in females with (79.5% versus 75.9%). Also, with syndrome Lennox-Gastaut, we noted same relation (5.1% versus 3.4%). Moreover, in children with WS happens more often in males, with result of17.2% versus 10.3%.

Table 1: Demographic structure of the patients with diagnosis and genders

Diagnosis	F		M		Grand Total		
	No.	(%)	No.	(%)	No.	(%)	Report M:F
CSWS	1	2.6	1	1.7	2	2.1	1 : 1
Enceph.EPI	31	79.5	44	75.9	75	77.3	1.4 : 1
Sy Dravet (SD)		0.0	1	1.7	1	1.0	-
Sy Landau-Kleffner	1	2.6		0.0	1	1.0	-
Sy Lennox-Gastaut	2	5.1	2	3.4	4	4.1	1 : 1
Sy West	4	10.3	10	17.2	14	14.4	2.5 : 1
Grand Total	39	100	58	100	97	100	1.5 : 1

The manifestation of the clinical signs approximately in our group started on the 245 days (245.5 ± 522.9), earlier on the male than the female (214.1 ± 450.9 vs 301.2 ± 634.4) (Table 2).

The earliest signs were in Syndrome West (74.1 ± 63.8 days). Clinical signs of the Syndrome Dravet usually are manifested after the first year, Syndrome Landau-Klenner approximately after 2.5 years (913 days), Syndrome Lennox-Gastau after 1.1 years (410.5 ± 704.7 days) and the WS after 2.5 months (74.1 ± 63.8 days).

Table 2: Beginning of the disease according to diagnosis

Diagnosis	F		M		Grand Total	
	Mean	SD	Mean	SD	Mean	SD
CSWS	180.0	-	1095.0	-	637.5	647.0
Enceph.EPI	257.7	638.9	240.9	501.4	247.6	556.1
SD			365.0	-	365.0	-
Sy Landau-Kleffner	913.0	-			913.0	-
Sy Lennox-Gastaut	820.0	905.1	1.0	0.0	410.5	704.7
Sy West	120.0	-	70.5	64.9	74.1	63.8
Grand Total	301.2	634.4	214.1	450.9	245.5	522.9

Mean = arithmetical average; SD = Standard deviation.

From the group of 14 cases with WS, 13 of them with NA-Valproate were treated as a primary therapy, approximate doses of 301.9 ± 64.1 mg. In fourth children Clonazepam, as additional therapy, in an approximate dose of 0.5 mg were given. As a reason for recurrent seizures, one case with Levetiracetam was treated.

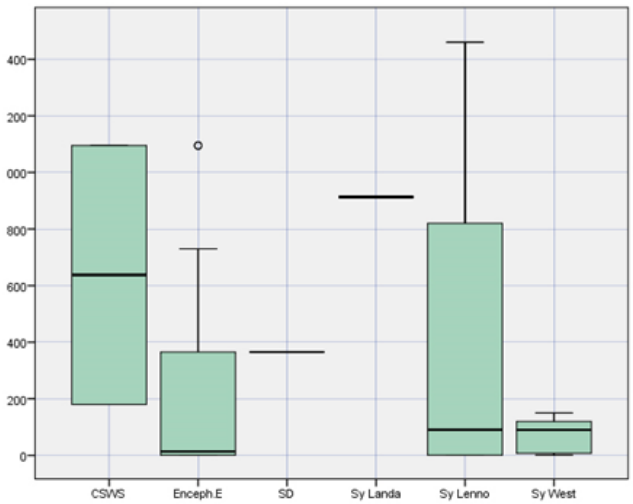


Figure 1: Approximate age of the disease manifestation

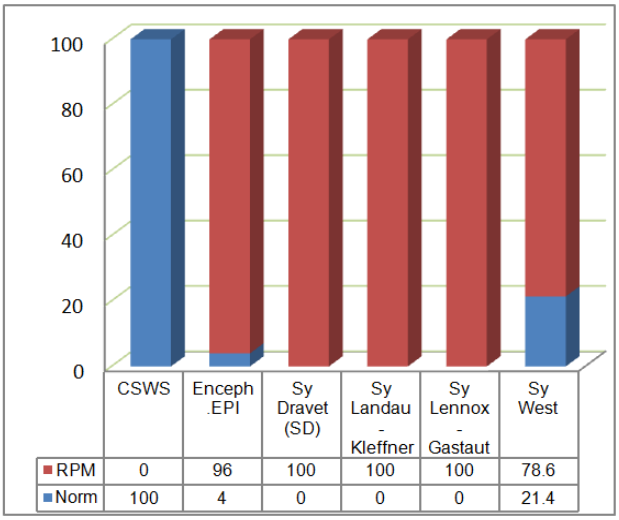


Figure 2: Prevalence of psychomotoric awkwardness according to the diagnosis

Topiramate was given in two cases; in the approximate dose of 50 mg. Clinical signs of psychomotoric retardation were concluded in 89 patients (91.8%), with a higher reoccurrence of undifferentiated epileptic encephalopathies (96 %) and the with WS in (78.6%).

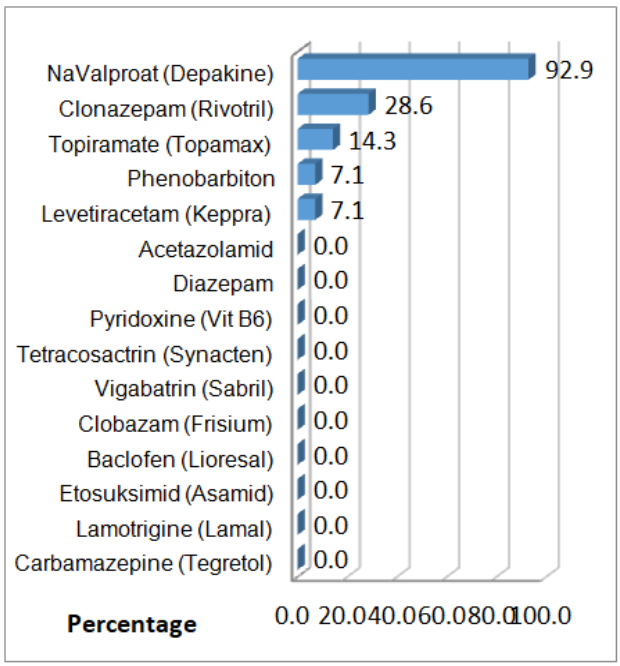


Figure 3: Structure of cases with West Syndrome according to the medical therapy

Discussion

According to the publication of Primec ZR from Slovenia during the 11 years, there were diagnosed 47 cases with infantile spasms. The cumulative incidence was 2.06 in 10.000 births. The age of first attacks was from 2 to 10 months [2].

However, Brna PM with colleague's reported the average incidence of infantile spasms in Canada with the incidence of 30.7/100.00 at births [3]. Boys were affected more than girls (60%).

In the presentation from Salonga, Filipine the ratio male-female was about 1:1. In the research done by Chiemc Hanya S with bp. in the paediatric department in Bangkok, Thailand, from 628 patients with epilepsy, 31 patients (4.9%) were diagnosed with Syndrome West. The report of gender was close 1:07:1, whereas the approximate age of the manifestation of disease is 9.7 months (range 4-32 months), while the seizures are from 5.7 months (range 2-11 months) [4].

According to the Wong V et all. in Hong Kong, from 1970 to 2000, 105 cases were diagnosed with WS. The number of new cases is 108 per year. The average incidence of WS to the new epileptic cases is approximately 0.8- 4.8% [5].

In Malaysia, during the period 1997-1999, WS was reported in 3% of new cases diagnosed with epilepsy [6].

Depending on the age of children with WS, the attacks usually start between 3 and 12 months, reaching the peak of attacks at the age of 5th month in 90% of the cases. In 70% of the cases, a first convulsive attack starts on the age of 6 months; it is very rare for WS to start in the middle of the 1st and 3rd year (around 5%). The location of the focal cortical lesion can impact the age of epileptic spasms at the beginning [7].

In the results of our research, the male is represented in a higher percentage than girls (10/4), ratio M: F is 2:5:1. Average of the presentations of WS is 74 days, (around 2.5 months). In our group, the incidence WS is 4.05 in 10000 live births. Mental and development signs were seen in 13 cases or 78.6% of cases with WS.

There are many causes of WS. Infantile spasms are as a consequence of many factors in the undeveloped brain. WS can occur in the children with cognitive delays or a healthy child up till then. The symptomatic form is known in 80% of cases while the idiopathic form remains in 20 % of the cases.

Idiopathic forms appear in families with hereditary character, whereby family members suffer from various forms of epilepsy or febrile convulsions.

Symptomatic forms happen more often, wherein 80% of the cases are connected alongside with other problems such are tuberous sclerosis, pre and perinatal damages, causing hypoxia, ischemia, premature birth, infections or disability of migration in the neurons in case of hemimegalencephalia,

agenesis of corpus callosum and lissencephaly.

Clinical manifestations: WS usually starts with an infantile spasm of lights that occur two or three times in a row, which come being added in the coming weeks with many cases of stroke in various attacks per day from 20 to 150 times. The attacks are short tonic or clonic and they happen before getting up or during, which follows crying that doesn't present any ictal signs. Usually, attacks are symmetric, rapid, presenting with mix forms of the flexor or extensor type [8].

Diagnosis: A correct anamnesis data and clinical neurological evaluation is a big step in the diagnosing of disease. The lab analyses usually are within the normal limits. Neurometabolic examinations have an important role in eliminating metabolic diseases. Neuroradiological examinations as a CT and brain MRI shows atrophy of the brain mass. Characteristic changes in the EEG between two attacks presented as a hypsarythmia, with predominate of a slow elementary activity with a high voltage which is characterised with multiple peaks, steep waves and complex SW with a lot of hearth's focal. The EEG changes can be symmetric and asymmetric. During the sleep, EEG is discontinued, and it reminds us of the suppression of the basic brain activity. In some cases, all EEG manifestations of hypsarythmia can be absent, and the findings of the EEG are characterised from a fragmental and modified hypsarythmia.

Prognosis and treatment: Prognosis of WS is variable. In most cases, it comes to motoric and mental regression, more often as an axial hypotonic. The continuous epileptic activity often is the cause of a cognitive deficit, loss of visual contact is a bad prognostic sign [9]. The attacks and hypsarythmia are stopped before the age of 3, in 40 to 50% of cases. The other cases evolve in the syndrome Lennox-Gastaut. 5 to 10% of children have a normal psychometric development. Children with idiopathic or cryptogenic form have a better prognosis. The children with Syndrome West have a fatal ending in 5 to 20% of cases. The deaths can happen because of the basic reason for the illness or the treatment with ACTH and corticosteroids.

Treatment: There is no consensus on the effective long-term treatment of WS. Treatment of choice is ACTH and Vigabatrin, however. As a reason for the multiple side effects, we used Valproate as a therapy of choice. In the absent of good control, we used on of the next medications as Lamotrigine, Levetiracetam, Nitrazepam, Pyridoxine, Sulthiam, Topiramate or Zonisamide. Vigabatrin was used relatively successfully in the children with WS, but, because of side effects in the damaging of the retina, the usage is limited. For the children with the Syndrome West and tuberous sclerosis, the use of Vigabatrin is more effective than the ACTH. In some cases, imunoglobin I.V was successful used. Also, the high doses of Pyridoxine 100-300 mg/kg during the first and second were given, and good results arrived. The ketogenic diet was successful in some cases of symptomatic/cryptogenic forms. In some cases when the attacks were very resistant calosototmia was applied but without satisfactory results.

In conclusion, West Syndrome is a frequent disease at the encephalopathic epileptogenic framework. The disease often involves syndrome Lennox-Gastaut. The resistance in anticonvulsive therapy is huge, and psychomotoric retardation follows a big percentage of children with this syndrome. The characteristics of the illness are infantile spasms and characteristics differences in EEG like hypsarythmia. This disease happens more often to the boys than the girls.

References

1. Chugani HT. Pathophysiology of infantile spasms. Adv Exp Med Biol. 2002;497:111–121.https://doi.org/10.1007/978-1-4615-1335-3_13 PMid:11993727

2. Primec ZR, Kopac S, Neubauer D. Epidemiologic features of infantile spasms in Slovenia. Epilepsia. 2002;43(2):183-7. https://doi.org/10.1046/j.1528-1157.2002.26201.x PMid:11903466

3. Brna PM, Gordon KE, Dooley JM, Wood EP. The epidemiology of infantile spasms. Can J Neurol Sci. 2001;28(4):309-12. https://doi.org/10.1017/S0317167100001517 PMid:11766774

4. Chiemchanya S, Visudtibhan A, Visudhiphan P. West syndrome in Thailand: a hospital-based survey. Brain Dev. 2001;23(7):605-8. https://doi.org/10.1016/S0387-7604(01)00294-7

5. Wong V.West syndrome – The University of Hong Kong experience (1970-2000). Brain Dev. 2001; 23(7):609-15. https://doi.org/10.1016/S0387-7604(01)00296-0

6. Thambyayah M. Early epileptic encephalopathies including West syndrome: a 3-year retrospective study from Klang Hospital, Malaysia. Brain Dev. 2001;23(7):603-4. https://doi.org/10.1016/S0387-7604(01)00293-5

7. Hrachovy RA, Frost JD. Severe Encephalopathic Epilepsy in Infants: Infantile Spasms (West Syndrome). In: Pellock JM, ourgeois BFD, Dodson WE. Undefined. Pediatric Epilepsy. Third Edition. New York, NY: Demos Medical Publishing, 2008:16.

8. Lux AL, Osborne JP. A proposal for case definitions and outcome measures in studies of infantile spasms and west syndrome: consensus statement of the west Delphi group. Epilepsia. 2004;45:1416–28. https://doi.org/10.1111/j.0013-9580.2004.02404.x PMid:15509243

9. Pavone P, Striano P, Falsaperla R, Pavone L, Ruggieri M. Infantile spasms syndrome, West syndrome and related phenotypes: what we know in 2013. Brain Dev. 2014;36(9):739-51. https://doi.org/10.1016/j.braindev.2013.10.008 PMid:24268986

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):929-933.
https://doi.org/10.3889/oamjms.2017.211
eISSN: 1857-9655
Clinical Science

Assessment of the Correlation between Severity of Coronary Artery Disease and Waist–Hip Ratio

Premtim Rashiti[1,2], Ibrahim Behluli[2], Albiona Rashiti Bytyqi[3*]

[1]Cardiology Clinic, University Clinical Center of Kosovo, Prishtina, Kosovo; [2]Department of Anatomy, University Clinical Center of Kosovo, Prishtina, Kosovo; [3]Department of Epidemiology, National Institute of Public Health in Kosovo, Prishtina, Kosovo

Citation: Rashiti P, Behluli I, Bytyqi AR. Assessment of the Correlation between Severity of Coronary Artery Disease and Waist–Hip Ratio. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):929-933. https://doi.org/10.3889/oamjms.2017.211

Keywords: waist/hip ratio; coronary artery disease; Gensini.

***Correspondence:** Albiona Rashiti Bytyçi, MD. Faculty of Medicine, University of Prishtina, Kosovo. Phone: +37744578822; Email: albionar@gmail.com

Received: 05-Oct-2017; **Revised:** 30-Oct-2017; **Accepted:** 05-Nov-2017; **Online first:** 30-Nov-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: This study was conducted to investigate the correlation between waist/hip ratio (WHR) as a measurement of obesity and severity of coronary artery disease (CAD) assessed by angiography in Kosovo.

METHODS: The study included 82 patients with suspected or known CAD who were referred for coronary angiography. All patients were subjected to full individual medical history, clinical examination including measurement of arterial blood pressure, body weight, height, body mass index (BMI), waist circumference, hip circumference, waist/hip ratio, and waist/height ratio. Coronary angiography was performed using standard techniques to determine the presence and severity of coronary artery lesions with the Gensini score.

RESULTS: Among the 82 patients in the study, the mean age in the CAD group was 66.76 ± 9.12 years and the mean age in the non-CAD group was 64.80 ± 8.30 years. Patients in the CAD group had a mean BMI of 28.17 ± 3.32 kg/m^2 and those in the non-CAD group had a mean BMI of 28.76 ± 4.68 kg/m^2. Patients in the CAD group had a mean waist/height ratio of 1.76 ± 7.56 and those in the non-CAD group had a mean waist/height ratio of 0.57 ± 0.08. Patient in the CAD group had a mean waist/hip ratio of 0.93 ± 0.06 and those in the non-CAD group had a mean waist/hip ratio of 0.88 ± 0.07. Thirty-seven patients (45.1%) had no coronary artery disease (Gensini score = 0), 15 (18.3%) had mild disease (Gensini score = 1-32), 14 (17.1%) had moderate disease (Gensini score = 32-58), and 16 (19.5%) had severe disease (Gensini score ≥ 58).

CONCLUSION: There was a significant positive correlation between waist/hip ratio and presence of CAD in Kosovar patients.

Introduction

Obesity is a growing health problem in most developed and in some developing countries. It is a very important risk factor for cardiovascular diseases as well as for type 2 diabetes mellitus and hypertension. Different methods exist for clinical evaluation of obesity. The body mass index (BMI), waist circumference (WC), waist/hip ratio (WHR), and waist/height ratio (WHtR) are some of the clinical tools enabling health teams to evaluate obesity and fat distribution. A central fat distribution is considered more atherogenic than peripheral obesity and attention has been paid to methods that can clinically evaluate central obesity [1]. The incidence of obesity is increasing in developing countries because of the westernization of diet and lifestyle [2].

Obesity is a proven independent risk factor for CAD in both sexes, and is in general a growing health problem [3-5]. Body weight, BMI, WC, and WHR are primary methods used to determine obesity. While BMI reflects general obesity, WC and WHR are related to central-type obesity, where body fat is primarily located in the abdomen. Prospective epidemiological studies have revealed that central obesity (determined by WC and WHR) is more relevant to CAD risk compared to general obesity (determined by BMI). While WHR is used commonly to evaluate central obesity, WC is shown to have a better correlation with abdominal fat localization (6-9). However, it also has been argued that BMI does not adequately reflect body fat distribution, and abdominal obesity, which captures the distribution of fat mass, may be an even more important predictor of CAD [10, 11].

In epidemiological settings WC, WHR, and WHtR are used as markers of visceral fat mass or abdominal adiposity to assess CAD risk.

To the best of our knowledge, this is the first study performed in Kosovo to focus on the correlation between WHR and the presence of CAD.

Material and Methods

Subjects of the study

The study was approved by the Ethics Committee of the Faculty of Medicine in the University of Prishtina, in accordance with the Declaration of Helsinki Guidelines. Written consent was obtained from all participants in the study. The prospective study consisted of 82 patients who underwent coronary angiography for suspected or known coronary atherosclerosis. Patients had to be clinically stable without major concomitant non-cardiovascular disease on assessment at the University Clinical Center of Kosovo. Patients with acute coronary syndrome, heart valve disease, elevated serum troponin levels, severe heart failure, chronic renal failure, and chronic inflammatory disease were excluded from the study.

Coronary angiography

Single-vessel disease was defined as > 50% luminal stenosis in a minimum of two views. Two experienced reviewers scored the angiograms. In case of disagreement between the reviewers, the average value of both angiograms was chosen. Reviewers were not aware of the diagnosis. Narrowing of the coronary artery lumen is assigned a Gensini score [12]. The score is 1 for 1-25% narrowing, 2 for 26-50%, 4 for 51-75%, 8 for 76-90%, 16 for 91-99%, and 32 for a completely occluded artery. The primary score is multiplied by a factor that takes into account the importance of the position of the lesion in the coronary arterial tree. For example, 1.5 is the multiplication factor for a mid-left anterior descending artery (LAD) lesion, with 1 for distal LAD, 2.5 for proximal LAD, and 5 for left main stem lesions. The Gensini score was expressed as the sum of the scores for all three coronary arteries to evaluate the overall extent of coronary artery disease [12].

Measurements

Patients were assessed in an examination gown, with upper body clothing and shoes removed. Each measurement was performed twice and the average was used for the analysis. We used the nearest 0.1 units of measurement for height and weight. BMI and WHtR were expressed in corresponding units, i.e., weight (kg) divided by the square of height (m^2), and waist circumference divided by height. WC was also categorized according to World Health Organization (WHO) criteria [13]. For the WHR, the waist is measured between the lowest rib and iliac crest, and the hip circumference is taken at the widest area of the hips at the greatest protuberance of the buttocks. The waist measurement is then divided by the hip measurement. For the decisive benchmark of metabolic syndrome, the WHO uses the ratio of > 9.0 in men and > 8.5 in women.

Laboratory measurements and blood samples were taken in the morning after an overnight fast and were stored immediately at -70°C after centrifugation until being assayed.

Statistics

The data were entered and analyzed with Statistical Package for the Social Science (SPSS), version 22 (SPSS Inc., Chicago, IL, USA). Continuous variables were primarily analyzed using a t-test, while binary data were primarily analyzed using a chi-squared test. Continuous variables were presented as means ± standard deviation (SD). Additionally, single (unadjusted) and multiple (adjusted) binary logistic regression analysis was carried out with the CAD group as the dependent variable, and the other significant variables as independent predictors. A p value of 0.1 was used as a criterion to identify variables for inclusion in the binary logistic regression models. Other adjusting variables included diastolic blood pressure (DBP), WHR, male sex, and smoking.

Results

Among the 82 patients in the study, the mean age was 65.78 ± 12.86 years, 46 patients (56.1%) were males, and 36 (43.9%) were females. Sixty-two patients (75%) had hypertension, 29 (35%) had diabetes mellitus, and 23 (28%) had dyslipidemia. Family history of CAD was positive in 42 patients (51%).

Patients in the CAD group had a mean BMI of 28.17 ± 3.32 kg/m^2 and those in the non-CAD group had a mean BMI of 28.76 ± 4.68 kg/m^2. Patients in the CAD group had a mean WC of 96.97 ± 8.63 cm and those in the non-CAD group had a mean WC of 95.13 ± 11.52 cm. Patients in the CAD group had a mean WHtR of 1.76 ± 7.56 and those in the non-CAD group had a mean WHtR of 0.57 ± 0.08. The mean WHR was 0.93 ± 0.06 in the CAD group and 0.88 ± 0.07 in the non-CAD group. The number of male patients in the study group was significantly higher (n = 29, 70.7%) than in the control group (n = 17, 41.5%) (p = 0.008).

WHR values in the study group (mean 0.93 ±

0.06) and control group (mean 0.88 ± 0.07) were significant (p-value: 0.0001), as shown in Table 1.

Table 1: Demographic and clinical characteristics of non-CAD and CAD groups

Variables	Group I Non-CAD (n = 41)	Group II CAD (n = 41)	p value*
BMI (kg/m^2) (mean ± SD)	28.76 ± 4.68	28.17 ± 3.32	0.51
WC (cm)	95.13 ± 11,52	96.97 ± 8.63	0.44
WHtR (mean ± SD)	0.57 ± 0.08	1.76 ± 7.56	0.32
WHR (mean ± SD)	0.88 ± 0.07	0.93 ± 0.06	0.0001*
SBP (mmHg1 (mean ± SD)	145.61 ± 13.29	145.85 ± 11.98	0.93
DBP (mmHg2 (mean ± SD)	81.34 ± 7.75	84.88 ± 7.79	0.042*
Age (mean ± SD)	64.80 ± 8.30	66.76 ± 9.12	0.31
Sex (M), n (%)	17 41.5%	29 70.7%	0.008*

*, Statistically significant differences; n=Number.

Table 2 presents the unadjusted and adjusted odd ratios (ORs) of CAD according to waist/hip ratio. After adjusting for confounder variables, the odds of having CAD were increased 14-fold (95% confidence interval [CI], 0.94-207.60), compared to those with a WHR <0.8.

In patients with a WHR of 0.8-0.99, the odds of having CAD significantly increased by 3.77-fold (95% CI, 0.40-35.51) compared to those with a WHR <0.8.

Significant differences were seen in binary logistic regression models for CAD analysis according to WHR (OR 14; 95% CI 0.94-207.60; p = 0.06), DBP (OR 2.75; 95% CI 1.12-6.87; p = 0.03), male sex (OR 3.41; 95% CI 1.37-8.52; p = 0.01), and smoking (OR 4.15; 95% CI 1.65-10.44; p = 0.001).

Table 2: Binary Logistic Regression Models for CAD (Unadjusted and Adjusted), DBP, WHR

	Unadjusted Odds Ratio (95% CI)	p value	Adjusted Odds Ratio (95% CI)	p value
DBP ≥ 90 mmHg2	2.75 (1.12-6.87)	0.03	3.35 (1.05-10.69)	0.04
WHR < 0.8	1.00 (Reference)	0.13	1.00 (Reference)	0.67
0.8 - 0.99	3.77 (0.40-35.51)	0.25	3.42 (0.23-50.38)	0.37
≥ 1	14 (0.94-207.60)	0.06	3.03 (0.13-71.20)	0.49
Sex (Male)	3.41 (1.37-8.52)	0.01	2.9 (0.81-10.35)	0.10
Smoker	4.15 (1.65-10.44)	0.00	3.51 (0.98-12.52)	0.05

Thirty-seven patients (45.1%) had no CAD (Gensini score = 0), 15 (18.3%) had mild disease (Gensini score = 1-32), 14 patients (17.1%) had moderate disease (Gensini score = 32-58), and 16 (19.5%) had severe disease (Gensini score ≥ 58). There was non-significant correlation between Gensini score and body mass index (p-value= 0.115) as shown in (Table 3).

Table 3. Correlation between BMI and Gensini

Gensini	BMI						Total	
	Normal 18-24.9 (kg/m^2)		Overweight 25-29.9 (kg/m^2)		Obese ≥30 (kg/m^2)			
	N	%	N	%	N	%	N	%
None (0)	7	46.7	13	35.1	17	56.7	37	45.1
Mild (1-32)	3	20	11	29.7	1	3.3	15	18.3
Moderate (32-58)	1	6.7	6	16.2	7	23.3	14	17.1
Severe (≥58)	4	26.7	7	18.9	5	16.7	16	19.5
Total	15	100	37	100	30	100	82	100
chi-square test, P-value				0.115				

Discussion

The current study showed a significant correlation between WHR and CAD (p = 0.0001) but not between BMI and CAD. This may be explained by the fact that BMI quantifies general adiposity; although individuals who are overweight or obese are likely to have excess fat, BMI does not give an indication of how this fat is distributed in the body. However, fat distribution is an important determinant of CAD, independent of BMI and other classic risk factors for CAD [14]. Welborn and Dhaliwal [15] and Srikanthan, Seeman, and Karlamangla [16] confirmed that WHR was a superior clinical measurement for predicting all-cause and cardiovascular disease mortality. Furthermore, one report [15] stated that the hip circumference indicated a lower risk for body fat accumulation, and thus including it in the WHR equation enhances the accuracy of measurement. It should be emphasized that most of the cross-sectional studies that compared WHR and WC as markers of risk factors have demonstrated superiority of WHR [6, 17].

The International Diabetes Federation has recommended waist circumference thresholds for increased risk of cardiovascular disease and diabetes: ≥ 94 cm in men and ≥ 80 cm in women. The American Heart Association (AHA) and National Heart, Lung, and Blood Institute (NHLBI) set the thresholds at ≥ 102 cm in men and ≥88 cm in women [7]. Cameron et al. [8] reported that higher WC was associated with increased risk in men but not in women, when adjusted for BMI and other covariates. That report was part of a prospective study of 6,072 Australian men and women during a follow-up period of 5 years [8]. Our study did not show a significant correlation of sex with WC but demonstrated that male sex is a significant risk factor for CAD (p = 0.008). In a large cohort study across Europe that involved patients of both sexes with a follow-up period of 9 years, Pischon et al. reported that higher WC (in men and women) and WHR (in women but not in men) were associated with higher mortality due to ischemic heart disease [11].

In a large cohort study of 1,346 middle-aged men free of CVD at baseline followed for 10.6 years, WHR, WC, and BMI were all directly associated with the risk of coronary events, with WHR providing additional information beyond BMI in predicting CAD, whereas BMI did not add to the predictive value of WHR [18].

WC, WHR and WHtR are used as measures of central obesity, while BMI is generally used to measure overall obesity.

Molarius and Seidell [19] emphasized the need to examine whether there could be age-related differences in the contribution of the pattern of fat distribution to risk factors. However, the WHO and

NHLBI reports barely address the question of the applicability of BMI and WC standards for older persons.

Several authors have reported the CAD risk associated with BMI, WC, WHR, or WHtR and have systematically compared some or all of these indicators to predict CAD [20-25].

Current studies confirm that obesity measured by any index usually is associated with increased risk of CAD. However, the findings comparing different measurements of total obesity (BMI) and abdominal obesity (WC, WHR, WHtR) in predicting CAD events have not been consistent. Some have suggested that total obesity rather than abdominal obesity is a better predictor of CAD [24, 25]. However, some investigators have found the reverse to be true [20, 23], and others did not find any significant difference (21, 22). This inconsistency in findings could be due to a number of reasons. There can be errors in self-reported measurements in some studies that can either cause spurious associations or can bias results towards the null. Inadequate or over adjustment of confounders and other cardiovascular risk factors also play a role in determining the nature of this association. Fat distribution and susceptibility to CAD vary by age, sex, and ethnicity and can cause these differences in the results as well.

Similar to our study, the Health Professionals Follow-up Study showed that WHR was a better predictor of CAD risk than BMI among elderly subjects; however, for young patients, BMI was a better predictor [26]. BMI was not a significant factor in our study, reinforcing the findings of Rimm et al. A possible explanation for our findings could be that the mean age was 65.78 ± 12.86 years in our population, and we did not include young patients.

Longer follow-up in the Health Professionals Study and the Nurses' Health Study also showed that WC was a better predictor of CAD risk than BMI among men and women above age 60, and BMI was more strongly associated with risk of CAD in younger compared to older participants [10]. WHR was proposed as the preferred measure of obesity for prediction of cardiovascular disease, with more universal application in individuals and population groups of different body builds. Benchmark studies of WHR as a dominant cardiovascular risk factor were reported in Swedish men and women in 1984 [10, 27].

Although a relatively small sample of patients were selected randomly from the general population, we showed a significant relationship between WHR and CAD. Further studies with larger patient groups are necessary to show statistical differences between BMI, WC, WHtR, WHR, systolic blood pressure (SBP), DBP, age, and sex.

In conclusion, there is a significant positive correlation between WHR and the presence and severity of CAD among Kosovar patients. Abdominal obesity is an independent risk factor for CAD and is more relevant than overall obesity. Since both total obesity and abdominal adiposity were associated with development of CAD, and since measurement of WC, WHR, WHtR, and BMI are inexpensive; we propose to include these in the general clinical setting for CAD risk assessment.

References

1. Siavash M, Sadeghi M, Salarifar F, et al. Comparison of body mass index and waist/height ratio in predicting definite coronary artery disease. Ann Nutr Metab. 2009; 53 (3-4):162-6. https://doi.org/10.1159/000172977 PMid:19011279

2. Zhang Y, Hong J, Gu W, et al. Impact of the metabolic syndrome and its individual components on risk and severity of coronary heart disease. Endocrine. 2009; 36(2): 233-8. https://doi.org/10.1007/s12020-009-9214-y PMid:19618299

3. Malik M, Razig SA. The prevalence of the metabolic syndrome among the multiethnic population of the United Arab Emirates: A report of a national survey. Metab Syndr Relat Disord. 2008; 6(3):177-86. https://doi.org/10.1089/met.2008.0006 PMid:18699721

4. DECODA Study Group. Prevalence of the metabolic syndrome in populations of Asian origin. Comparison of the IDF definition with the NCEP definition.Diabetes Res Clin Pract. 2007; 76(1):57-67. https://doi.org/10.1016/j.diabres.2006.07.020 PMid:17010470

5. Qiao Q, Pitkäniemi J, Tuomilehto J, et al. Comparison of different definitions of the metabolic syndrome in relation to cardiovascular mortality in European men and women. Diabetologia. 2006; 49(12):2837-46. https://doi.org/10.1007/s00125-006-0438-6 PMid:17021922

6. Wildman RP, Gu D, Reynolds K, et al. Are waist circumference and body mass index independently associated with cardiovascular disease risk in Chinese adults? Am J Clin Nutr. 2005; 82(6): 1195-202. PMid:16332651

7. Alberti KGMM, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; National heart, lung, and blood institute; American heart association; World heart federation; International atherosclerosis society and international association for the study of obesity. Circulation. 2009; 120(16):1640-5. https://doi.org/10.1161/CIRCULATIONAHA.109.192644 PMid:19805654

8. Cameron AJ, Dunstan DW, Owen N, et al. Health and mortality consequences of abdominal obesity: Evidence from the AusDiab study. Med J Aust 2009; 191(4):202-8. PMid:19705980

9. Pischon T, Boeing H, Hoffmann K, et al. General and abdominal adiposity and risk of death in Europe. N Engl J Med. 2008; 359(20): 2105-20. https://doi.org/10.1056/NEJMoa0801891 PMid:19005195

10. Larsson B, Svärdsudd K, Welin L, Wilhelmsen L, Björntorp P, et al. Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913. Br Med J (Clin Res Ed). 1984; 288: 1401–1404. https://doi.org/10.1136/bmj.288.6428.1401

11. Rimm EB, Willett WC, Hu FB, Sampson L, Colditz GA, et al. Folate and vitamin B6 from diet and supplements in relation to risk of coronary heart disease among women. JAMA. 1998; 279: 359–364. https://doi.org/10.1001/jama.279.5.359 PMid:9459468

12. Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. American Journal of Cardiology. 1983; 51:606. https://doi.org/10.1016/S0002-9149(83)80105-2

13. World Health Organization. Waist Circumference and Waist-Hip

Ratio Report of a WHO Expert Consultation. Geneva, 8-11 December 2008. WHO Press, World Health Organization; Geneva, Switzerland, 2011.

14. Després JP. CVD risk assessment: Do we need the metabolic syndrome or better global cardiometabolic risk calculators? International Journal of Obesity 32 2008; 32 (Suppl. 2): S1-4.

15. Welborn TA, Dhaliwal SS. Preferred clinical measures of central obesity for predicting mortality. European Journal of Clinical Nutrition. 2007; 61:1373-1379. https://doi.org/10.1038/sj.ejcn.1602656 PMid:17299478

16. Srikanthan P, Seeman TE, Karlamangla AS. Waist-hip-ratio as a predictor of all-cause mortality in high functioning older adults. Annals of Epidemiology. 2009; 19:724-731. https://doi.org/10.1016/j.annepidem.2009.05.003 PMid:19596204 PMCid:PMC3154008

17. Folsom AR, Kushi LH, Anderson KE, et al. Associations of general and abdominal obesity with multiple health outcomes in older women: The Iowa Women's Health Study. Arch Intern Med. 2000; 160 (14): 2117-28. https://doi.org/10.1001/archinte.160.14.2117 PMid:10904454

18. Lakka HM, Lakka TA, Tuomilehto J, et al. Abdominal obesity is associated with increased risk of acute coronary events in men. Eur Heart J. 2002; 23(9):706-13. https://doi.org/10.1053/euhj.2001.2889 PMid:11977996

19. Molarius, A., Seidell, J. C. Selection of anthropometric indicators for classification of abdominal fatness—a critical review. Int J Obes Relat Metab Disord. 1998; 22:719–27. https://doi.org/10.1038/sj.ijo.0800660 PMid:9725630

20. Canoy D, Boekholdt SM, Wareham N, Luben R, Welch A, et al. Body fat distribution and risk of coronary heart disease in men and women in the European Prospective Investigation Into Cancer and Nutrition in Norfolk cohort: a population-based prospective study. Circulation. 2007; 116: 2933–2943. https://doi.org/10.1161/CIRCULATIONAHA.106.673756 PMid:18071080

21. Flint AJ, Rexrode KM, Hu FB, Glynn RJ, Caspard H, et al. Body mass index, waist circumference, and risk of coronary heart disease: a prospective study among men and women. Obesity research & clinical practice. 2010; 4: e171–e181. https://doi.org/10.1016/j.orcp.2010.01.001 PMCid:PMC2992336

22. Gray RS, Fabsitz RR, Cowan LD, Lee ET, Welty TK, et al. Relation of generalized and central obesity to cardiovascular risk factors and prevalent coronary heart disease in a sample of American Indians: the Strong Heart Study. Int J Obes Relat Metab Disord. 2000;24:849–860. https://doi.org/10.1038/sj.ijo.0801243 PMid:10918531

23. Page JH, Rexrode KM, Hu F, Albert CM, Chae CU, et al. Waist-height ratio as a predictor of coronary heart disease among women. Epidemiology. 2009; 20: 361–366. https://doi.org/10.1097/EDE.0b013e31819f38f1 PMid:19289960 PMCid:PMC4012298

24. Rexrode KM, Buring JE, Manson JE. Abdominal and total adiposity and risk of coronary heart disease in men. Int J Obes Relat Metab Disord. 2001; 25: 1047–1056. https://doi.org/10.1038/sj.ijo.0801615 PMid:11443505

25. Taylor AE, Ebrahim S, Ben-Shlomo Y, Martin RM, Whincup PH, et al. Comparison of the associations of body mass index and measures of central adiposity and fat mass with coronary heart disease, diabetes, and all-cause mortality: a study using data from 4 UK cohorts. The American journal of clinical nutrition. 2010; 91: 547–556. https://doi.org/10.3945/ajcn.2009.28757 PMid:20089729

26. Rimm EB, Stampfer MJ, Giovannucci E, Ascherio A, Spiegelman D, Colditz GA, Willett WC. Body size and fat distribution as predictors of coronary heart disease among middle-aged and older US men. Am J Epidemiol. 1995; 141(12):1117-27. https://doi.org/10.1093/oxfordjournals.aje.a117385 PMid:7771450

27. Lapidus L, Bengtsson C, Larsson B, et al. Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, Sweden. BMJ. 1984; 289: 1257-1261. https://doi.org/10.1136/bmj.289.6454.1257 PMid:6437507 PMCid:PMC1443498

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):934-939.
https://doi.org/10.3889/oamjms.2017.204
eISSN: 1857-9655
Clinical Science

Apelin, Nitric Oxide and Vascular Affection in Adolescent Type 1 Diabetic Patients

Soha M. Abd El Dayem[1*], Ahmed A. Battah[2], Abo El Maged El Bohy[3], Rash Nazih Yousef[4], Azza M. Ahmed[1], Ahmed A. Talaat[1]

[1]Pediatrics Department, National Research Centre, Cairo, Egypt; [2]Critical Care Department, Cairo University, Cairo, Egypt; [3]Radiology Department, Cairo University, Cairo, Egypt; [4]Clinical Pathology Department, National Research Centre, Cairo, Egypt

Citation: Abd El Dayem SM, Battah AA, El Bohy AEM, Yousef RN, Ahmed AM, Talaat AA. Apelin, Nitric Oxide and Vascular Affection in Adolescent Type 1 Diabetic Patients. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):934-939. https://doi.org/10.3889/oamjms.2017.204

Keywords: Apelin; nitric oxide; FMD; adolescent; type 1 diabetes.

***Correspondence:** Soha M. Abd El Dayem. Professor of Pediatrics, Consultant of diabetes and Endocrinology, Pediatrics Department, Medical Research Division, National Research Centre, Cairo, Egypt. Telephone: +2 01006716852. E-mail: s_eldayem@yahoo.com

Received: 01-Sep-2017; **Revised:** 26-Nov-2017; **Accepted:** 27-Nov-2017; **Online first:** 04-Dec-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: To evaluate the relationship of apelin and nitric oxide (NO) to endothelial dysfunction in type 1 diabetics.

PATIENTS AND METHODS: Sixty two type 1 diabetics and 30 healthy age and sex matched controls were included. Blood samples for apelin, NO, glycosylated hemoglobin (HbA1c), and lipid profile were collected. Albumin/creatinine ratio was assessed in urine. Flow mediated dilatation (FMD) via ultrasound was done.

RESULTS: The mean age of diabetics were 16.3 ± 1.5 yrs (14.0 – 19.0 yrs), and duration of disease, were 9.4 ± 2.9 yrs (5.0 – 16.5 yrs). FMD and FMD/nitrate mediated dilatation (NMD) ratio were lower in diabetics. NO was decreased, while apelin and albumin/creatinine ratio were increased significantly in diabetics. There was a positive correlation between apelin and HbA1c. On the contrary, NO had a negative correlation with HbA1c, albumin/creatinine ratio, LDL-c and OxLDL.

CONCLUSION: Diabetic patients had endothelial dysfunction and high apelin level, with no related to each other. High level of apelin is associated with bad glycemic control. Obesity had no role to increase in apelin level. NO is related to diabetic nephropathy and atherosclerosis. We recommend a further large study to evaluate the relationship of apelin with endothelial dysfunction.

Introduction

Apelin, a recently described adipocytokine, is abundantly expressed in adipose tissue and produced in many body parts by the endothelial cells [1, 2]. It is synthesized as a prepropeptide then modified into smaller peptides with higher potency. It produces its effects through a cell surface G protein-coupled receptor called APJ, which is structurally similar to angiotensin receptor [3]. In obese persons with hyperinsulinemia, apelin levels are increased [4]. Levels are decreased in patients with dyslipidemia and newly diagnosed and untreated type 2 diabetes mellitus (T2DM) [5, 6].

Apart from insulin deficiency, insulin resistance is found in type 1 diabetes mellitus (T1DM), both at onset and course of the disease [7, 8]. Insulin resistance is also found in obesity making it a risk factor for T1DM in children in addition to type II DM [9-13]. Obese infants have higher risk for T1DM in chidhood [14]. This may be due to the harmful effects to the beta cells early in life as a result of the relative hyperinsulinemia induced by the increased demands in obesity [13, 15, 16].

There is endothelial dysfunction with increased cardiovascular risk in type 1 diabetes. L-Arginine is converted to nitric oxide (NO), which is an important mediator of vascular homeostasis due to its central role in the maintenance of the endothelial milieu [17, 18].

Cohen [19], reported endothelial dysfunction is the earliest event in the atherosclerotic process and Järvisalo et al. [20], found impaired FMD response is a common manifestation in children with type 1 diabetes and associated with high carotid artery IMT,

suggesting that endothelial dysfunction in with type 1 diabetics may predispose them to the development of early atherosclerosis.

We are aim to evaluate apelin and nitric oxide (NO) in type 1 diabetic patients and its relation to vascular affection as well as to evaluate the relationship between apelin and the glycemic balance.

Patients and Methods

Patients

The study included 62 adolescent patients with type 1 diabetes mellitus (DM) among those attending to the endocrine clinic, National Research Centre. The control group consisted of 30 age and sex matched healthy normal volunteers. Control group was the healthy friends or relatives of our patients.

Inclusion criteria: children with type 1 DM, duration of disease > 5 years, patients age > 14 and < 19 yrs old. We selected this young age group with short duration of diabetes firstly, to explore whether early atherosclerotic changes starts at this early age shortly after onset of diabetes or needs longer exposure to the diabetic milieu and secondly because in younger age group (< 14 yrs old) atherosclerotic lesions are expected to be in the form of microscopic intimal fatty streaks that is too minute to be resolved by ultrasonography.

Exclusion criteria were: patients during acute diabetic complications e.g. diabetic ketoacidosis (DKA) or hypoglycemia, patients suffering from cardiac diseases e.g. congenital, rheumatic heart, left ventricular dysfunction, patients on metformin or multivitamins and smokers.

Study design and protocol

It is a cross-sectional observational study done after obtaining approval from the ethical committee of the National Research Centre, Cairo, Egypt. Registration number is 11052. Written informed consent was obtained from all patients or their parents and controls after full discussion about the aim of the study. This study is a part of a project done in the National Research Centre for evaluation of cardiac, vascular and endothelial function in adolescent type 1 diabetic patients.

All the studied patients were subjected to: history taking including: age of patients, sex, age of onset of diabetes, duration of diabetes, type and dose of insulin therapy, family history of diabetes; and we asked about presence of any symptoms of cardiac, renal, neurological affection or presence of any type of autonomic dysfunction. We also asked about history of taking drugs other than insulin.

Clinical examination

I. Patients and controls were subjected to general, cardiac, chest and neurological examination.

II. Blood pressure was measured three times for patients and controls after 5-minute rest in the sitting position on both upper limbs with the use of automatic manometer (Omron M4 Plus, Omron Health care Europe, Hoof drop, and Holland). The mean value of the second and the third measurement was calculated. The measurements taken on the dominant limb were analyzed.

III. Anthropometric measurements in the form of weight, height, waist circumference (WC), and hip circumference (HC) were taken for each participant. The weight and height of the participants were measured up to 0.01 kg and 0.1 cm using a Seca Scale Standing Balance and a Holtain Portable Anthropometer (Holtain, Ltd, Crymmych, Wales, U.K.). Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Waist circumference was measured at the level of the umbilicus with the participant standing and breathing normally; hip circumference was measured at the level of the iliac crest, using non stretchable plastic tape to the nearest 0.1 cm. The waist / hip ratio and waist / height ratio (cm/ cm) were calculated. Each measurement was taken as the mean of three consecutive measurements, using standardized equipment [21, 22]. The landmarks, instruments used, and techniques followed were those recommended by the international biological program [21, 22].

Laboratory investigation

Simultaneously all patients and controls underwent the following tests:

All patients and controls underwent the following tests: For cholesterol measurements, venous blood was sampled after a 12-h fast. Serum total cholesterol was determined by a commercial kit (Boehringer-Mannheim, Germany) [23]. High-density lipoprotein (HDL) cholesterol was separated from the serum by precipitation of the other lipoproteins with a heparin/manganese procedure [24]. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. The concentration of triglycerides(Tg) was measured in a TechnoCon AutoAnalyzer II (TechnoCon Instruments, Tarrytown, NY, USA).

Glycosylated hemoglobin (HbA1c) was done every 3 months and the mean value was calculated per year. It was measured using high pressure liquid chromatography (Nichols Institute, Van Nuys, CA, USA) [25].

Screening for microalbuminuria was assessed

in fresh morning urine samples by measuring albumin/creatinine ratio by enzyme linked immunosorbent assay (ELISA) kit provided by Orgentec Diagnostika, Gmbh (Mainz, Germany) [26].

Serum concentrations of oxidized low-density lipoprotein (OxLDL) were detected by commercially available solid phase two-site enzyme immunoassay kit (Mercodia AB, Uppsala, Sweden). Measurements of the OxLDL levels in the sera were performed according to the recommendations of the manufacturer. The intra and interassay coefficients of variations were 5.5% – 7.3% and 4.0% – 6.2%, respectively, and the sensitivity was < 1 mU/L.

Nitric oxide production in sera was measured using a nitric oxide colorimetric assay kit (Roche cat No. 11756281001). Sera was irradiated and then filtered in a 10,000 kd MWCO column (Satorious, Vivascience cat No. 13239-E). The assay was conducted as per the manufacturer's instructions using a NO control with a standard curve plotted and samples were measured at a 550 nm wavelength. Serum apelin-12 concentration were measured using a commercial enzyme – liked immunosorbent assay (Phoenix Pharmaceuticals Inc. Burlingame,CA,USA).

Flow mediated dilatation (FMD)

All imaging studies were performed by the same vascular sonographer & the same ultrasonographic machine using (General Electric medical ultrasonographic machine model: Vivid 7 Pro, GE Vingmed ultrasound AS-Nl90, Horton-Norway equipped with 7.5–10 MHz linear-array transducer) after the published protocols [27-29]. With the subject lying in the supine position, ECG electrodes were placed on the chest; the machine automatically measured and recorded results of the electrocardiogram. All measurements were made at end diastole to avoid possible errors resulting from variable arterial compliance. A sphygmomanometer cuff was placed on the proximal right arm. The right brachial artery images were obtained 3 cm proximal to the elbow crease using B-mode imaging in the longitudinal plane of the artery. A baseline image was acquired using a resolution box function to magnify this part of the artery. Blood flow was estimated by the pulsed Doppler velocity signal obtained from a mid-artery sample volume. The cuff was inflated to 100 mm Hg above the systolic pressure to occlude arterial flow for 5 min. The cuff was then deflated, and the longitudinal image of the brachial artery was recorded immediately & continuously for 60 seconds after cuff deflation for greatest response guided by the hyperemic flow detected by pulsed Doppler. Flow-mediated dilation (FMD) was assessed by measurement of the greatest brachial artery diameter that was detected at 60 seconds after release of the cuff in most cases. The subject then had a rest for 30 min, after which a sublingual dose of nitroglycerin tablet (Dinitra, isosorbidedinitrate 5 mg manufactured by Egyptian int. pharmaceutical industries co., tenth of Ramadan city, A.R.E., under license of RHONE POULENC, PARIS FRANCE) was then administered, and the brachial artery response (endothelium-independent dilation) was assessed by imaging the artery continuously for 3 min after the nitroglycerin dose for greatest response.

Measurements of the brachial artery luminal diameter were performed on-line at end-diastole, coinciding with the onset of the R-wave on the ECG. For each phase (baseline, endothelium-dependent dilation, and endothelium-independent dilation), three brachial artery diameter measurements were obtained manually online with electronic calipers and averaged from the longitudinal image by identifying the lumen-intima interface. The largest reading for FMD post-ischemia {100 × [diameter (1 min)–diameter (basal)]/diameter (basal)} was used to represent spontaneous endothelial function. In addition, nitroglycerine-mediated dilatation (NTG) {100 × [the largest reading of diameter (after sublingual isosorbidedinitrate–diameter (basal)]/diameter (basal)} was assessed. The diameter percent change caused by endothelium-dependent flow-mediated dilatation (%FMD) and non-endothelium dependent dilatation (%NMD) were expressed as the percent change relative to that at the initial resting scan.

Significant endothelial dysfunction was defined as FMD < 10% and NMD > 10% [30]. In order to increase the sensitivity and specificity of the technique for endothelial dysfunction, FMD over NMD of the brachial artery < 0.70 defined endothelial dysfunction [31].

Statistical Analysis

Statistical analysis was conducted using Statistical Package for Social Science (SPSS) program version 15.0 (Chicago, Illinois, USA). t –test or Mann Whitney-U (for none symmetrically distributed data) for independent variables was done. Pearson's correlation was also done.

Results

Our research included 62 type1 diabetic patients (31 females and 31 males) and 30 normal controls (15 females and 15 males). Their mean age were 16.3 ± 1.5 yrs and mean duration of diabetes were 9.4 ± 2.9 yrs. HbA1, albumin/.creatinine ratio, cholesterol, Tg, LDL, OxLDL and apelin were significantly increased, in the contrary, nitric oxide, FMD, and FMD/NMD were decreased significantly in diabetics (Table 1).

Table 1: Comparison between demographic, laboratory data, carotid intimal medial thickness and resistivity index of diabetic patients and controls

	Patients		Controls		
Variables	Mean	SD	Mean	SD	P-value
Age (yrs)	16.32	1.52	16.13	2.63	0.70
Anthropometric data:					
BMI (kg/m^2)	24.91	4.20	24.76	5.67	0.8
BMI (SDS)	24				
Waist circumference (cm)	83.60	9.39	84.78	12.25	0.60
HIP circumference (cm)	91.69	8.37	91.20	11.93	0.80
Waist / hip ratio	0.91	0.06	0.93	0.05	0.20
Waist / height ratio	0.51	0.06	0.52	0.08	0.90
Blood pressure:					
Systolic blood pressure (mmHg)	119.35	12.53	118.21	14.42	0.70
Diastolic blood pressure (mmHg)	81.94	9.20	78.57	6.51	0.05
Laboratory data:					
HbA1 (%)	9.55	1.90	5.43	0.65	0.0001
	28.4		10.7		
Albumin/ creatinine ratio (µg/ g creatinine)	78.33 ± 100.65 (5.8 – 384.2)		11.28 ± 4.23 (5.4 – 23.2)		0.0001#
Total cholesterol (mg/dl)	188.81	63.77	100.54	20.41	0.0001
Triglyceride (mg/dl)	103.46	78.29	68.89	28.39	0.03
HDL-c (mg/dl)	51.77	20.58	52.21	11.12	0.90
LDL-c (mg/dl)	118.66	47.53	62.50	19.88	0.0001
OxLDL (mg/l)	17.56	6.45	9.06	3.92	0.0001
Nitric oxide (µmol/l)	28.42	7.06	40.33	6.32	0.0001
Apelin	223.95	185.83	70.45	27.74	0.0001
Doppler on brachial artery					
Brachial artery at rest, mm	3.42	0.39	3.26	0.79	0.2
Brachial artery (Shill test) (FMD), %	10.35	7.16	15.64	10.77	0.005
Brachial artery after nitrate (NMD), %	14.48	6.89	18.14	10.77	0.1
FMD/NMD	0.82	0.66	2.26	6.29	0.03

t- test for independent variables; # Mann Whitney U test was used; Median, mean ± SD (range). BMI = body mass index; HbA1 = glycosylated hemoglonin; LDL = Low density lipoprotein; HDL = high density lipoprotein; OLDL = oxidized low density lipoprotein.

Apelin had a significant positive correlation with HbA1c (Table 2).

Table 2: Correlation between demographic, anthropometric, laboratory data and flow mediated dilatation and Apelin of diabetic patients

	Apelin	
Variables	r	P-value
Age (yrs)	0.14	0.30
Duration of diabetes (yrs)	-0.05	0.72
Systolic blood pressure (mmHg)	0.17	0.22
Diastolic blood pressure (mmHg)	0.21	0.12
BMI (kg/m^2)	-0.05	0.72
BMI (SDS)	-0.06	0.65
Waist/ hip ratio	0.15	0.28
Waist / height ratio	-0.03	0.81
HbA1 (%)	0.26	**0.04**
Albumin / creatinine ratio (µg/g creatinine)	-0.05	0.74
Cholesterol (mg/dl)	0.02	0.91
Triglycerid (mg/dl)	-0.03	0.84
HDL-c (mg/dl)	0.06	0.67
LDL-c (mg/dl)	0.07	0.64
VLDL (mg/dl)	0.42	0.10
oxldl (mg/dl)	-0.11	0.44
Nitric oxide	0.05	0.74
Doppler on brachial artery at rest (mm)	0.08	0.55
Doppler on brachial artery (Shill test) (FMD)(%)	-0.04	0.76
Doppler on brachial artery after nitroglycerine (NMD) (%)	0.17	0.22

On the other hand, NO had a negative correlation with HbA1c, albumin/creatinine ratio, LDL-c and OxLDL (Table 3).

Discussion

In the current study, diabetic patients had higher HbA1c, albumin/creatinine ratio, lipid profile, OxLDL and lower NO, FMD and FMD/NMD. This is comparable with the study of Schulze el al., [32], who reported that the earliest functional atherosclerotic changes in the arterial wall is the endothelial dysfunction due to impaired endothelial release of nitric oxide detected by measuring flow mediated dilatation (FMD) of the brachial artery for measuring arterial diameter in response to increased flow.

Reduction of NO may be a result of either decreased production because of decreased activity and/.or reduced expression of eNOS, or low activity of NO, or a result of high degradation by high production of superoxide ions or reactive oxygen species [17, 33].

In the present study, NO showed a negative correlation with HbA1c, albumin/creatinine ratio, LDL-c, and OxLDL. NO in type 1 diabetics affect heart and kidney in the previous studies. Endothelial function affected by several factors associated with diabetes, including severity of hyperglycemia, duration of diabetes, and increase of advanced glycosylated end products, microalbuminuria and nephropathy [18].

In the present study, the attenuated FMD response in diabetic children coincide with the results of Wiltshire et al., [34] and Donaghue et al., [35], who studied flow-mediated dilation in diabetic children and demonstrated attenuated endothelial function in diabetic children compared with controls.

In our study, apelin is increased in diabetic patients with a positive correlation with HbA1c. On the other hand, it had no significant correlation with anthropometric measurement, lipid profile, NO and FMD.

Apelin mRNA is found in many tissues including central nervous system (CNS), lung, heart, placenta, mammary gland and gastrointestinal tract (GIT) [37, 38]. Apelin is an adipokine secreted and produced by white adipose tissue in mice and humans. It is also included in cardiovascular function. Apelin plays a role in the CNS and regulation of food intake and water balance. In the contrary, results of the other studies are confusing [39, 40].

Apelin has a role in energy metabolism: as it improves sensitivity of insulin in insulin-resistant obese mice, and it is related to an increase in glucose uptake in skeletal muscle [41, 42]. Synthesis of apelin is affected by insulin and plasma apelin levels and it is increased in obesity in association with hyperinsulinaemia [43]. In our study, apelin had a significant correlation with glycosylated hemoglobin.

The relationship of apelin and diabetes in humans are still controversial. On the other hand, increased apelin level in obese subjects and type 2 diabetics were reported in some studies. Whereas other authors revealed decrease apelin in obese subjects with newly-diagnosed type 2 diabetes [44-48].

We found that a very limited study was done

to assess apelin concentrations in type 1 diabetes. Two studies reported increase in apelin in type 1 diabetics, one of them was in children [49] and the other one was in adults [50]. On the other hand, a new study revealed that apelin levels is the same in diabetics and no diabetics [51]. In our study, we found that although apelin concentrations were increased, it had no relation to body mass index in diabetic patients. This means that obesity is not the main factor affecting apelin levels in diabetic patients which is in agreement with other studies [38, 44, 45, 52].

We conclude that apelin concentrations were increased in diabetic patients and it is affected by obesity. It is related to glycaemic balance and even insulin sensitivity. Diabetic patients had endothelial dysfunction and elevation of apelin, but they does not related to each other. NO is related to diabetic nephropathy and atherosclerosis.

Further large study is recommended to detect the relationship of apelin with vascular affection by assessing large number of diabetics with and without complication. Apelin is a beneficial adipokine and is a promising therapeutic target in metabolic disorders as it had anti diabetic properties.

References

1. Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, Kawamata Y, Fukusumi S, Hinuma S, Kitada C, Kurokawa T, Onda H, Fujino M. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem Biophys Res Commun. 1998;251:471-476. https://doi.org/10.1006/bbrc.1998.9489 PMid:9792798

2. Kleinz MJ, Davenport AP. Immunocytochemical localization of the endogenous vasoactive peptideapelin to human vascular and endocardial endothelial cells. Regul Pept 2004;118:119-125. https://doi.org/10.1016/j.regpep.2003.11.002 PMid:15003827

3. Lee DK, Cheng R, Nguyen T, Fan T, Kariyawasam AP, Liu Y, Osmond DH, George SR, O'Dowd BF. Characterization of apelin, the ligand for the APJ receptor. J Neurochem. 2000;74:34-41. https://doi.org/10.1046/j.1471-4159.2000.0740034.x PMid:10617103

4. Boucher J, Masri B, Daviaud D, Gesta S, Guigne C, Mazzucotelli A, Castan-Laurell I, Tack I, Knibiehler B, Carpene C, Audigier Y, Saulnier-Blache JS, Valet P. Apelin, a newly identified adipokine up-regulated by insulin and obesity. Endocrinology. 2005;146:1764-1771. https://doi.org/10.1210/en.2004-1427 PMid:15677759

5. Tasci I, Erdem G, Ozgur G, Tapan S, Dogru T, Genc H, Acikel C, Ozgurtas T, Sonmez A. LD cholesterol lowering increases plasma apelin in isolated hypercholesterolemia. Atherosclerosis. 2009;204:222-228. https://doi.org/10.1016/j.atherosclerosis.2008.08.030 PMid:18845302

6. Erdem G, Dogru T, Tasci I, Sonmez A, Tapan S. Low plasma apelin levels in newly diagnosed type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes. 2008;116:289-292. https://doi.org/10.1055/s-2007-1004564 PMid:18484561

7. DeFronzo RA, Hendler R, Simonson D. Insulin resistance is a prominent feature of insulin-dependentdiabetes. Diabetes. 1982;31:795-801. https://doi.org/10.2337/diab.31.9.795 PMid:6761214

8. Pang TT, Narendran P. Addressing insulin resistance in Type 1 diabetes. Diabet Med. 2008;25:1015-1024. https://doi.org/10.1111/j.1464-5491.2008.02493.x PMid:19183305

9. Bonadonna RC, Groop L, Kraemer N, Ferrannini E, Del Prato S, DeFronzo RA. Obesity and insulin resistance in humans: a dose-response study. Metabolism. 1990;39:452-459. https://doi.org/10.1016/0026-0495(90)90002-T

10. Caprio S, Bronson M, Sherwin RS, Rife F, Tamborlane WV. Co-existence of severe insulin resistance and hyperinsulinaemia in pre-adolescent obese children. Diabetologia. 1996;39:1489-1497. https://doi.org/10.1007/s001250050603 PMid:8960831

11. Sinaiko AR, Jacobs DR, Jr., Steinberger J, Moran A, Luepker R, Rocchini AP, Prineas RJ. Insulin resistance syndrome in childhood: associations of the euglycemic insulin clamp and fasting insulin with fatness and other risk factors. J Pediatr. 2001;139:700-707. https://doi.org/10.1067/mpd.2001.118535 PMid:11713450

12. Waldhor T, Schober E, Rami B. Regional distribution of risk for childhood diabetes in Austria and possible association with body mass index. Eur J Pediatr. 2003;162:380-384. PMid:12756559

13. Kibirige M, Metcalf B, Renuka R, Wilkin TJ. Testingthe accelerator hypothesis: the relationship between body mass and age at diagnosis of type 1 diabetes. Diabetes Care. 2003;26:2865-2870. https://doi.org/10.2337/diacare.26.10.2865 PMid:14514593

14. Baum JD, Ounsted M, Smith MA. Letter: Weight gain in infancy and subsequent development ofdiabetes mellitus in childhood. Lancet. 1975;2:866. https://doi.org/10.1016/S0140-6736(75)90250-0

15. Wilkin TJ. The accelerator hypothesis: weight gain as the missing link between Type I and Type II diabetes. Diabetologia. 2001;44:914-922. https://doi.org/10.1007/s001250100548 PMid:11508279

16. Meral C, Tascilar C, Karademir F, Tanju IA, Cekmez F, Ipcioglu OM, Ercin CN, Gocmen I and Dogru T. Elevated Plasma Levels of Apelin in Children with Type 1 Diabetes Mellitus. Journal of Pediatric Endocrinology & Metabolism. 2010, 23, 497-502. https://doi.org/10.1515/jpem.2010.081 PMid:20662349

17. Soha M. Abd El Dayem, Ahmed A. Battah, Amal El-shehaby and Abo El Maged El Bohy. Asymmetric dimethyl L-arginine, nitric oxide and cardiovascular disease in adolescent type 1 diabetics. J Pediatr Endocr Met. 2014; 27(5-6): 437–444.

18. Sibal L, Agarwal SC, Schwedhelm E, Lüneburg N, Böger RH, et al. A study of endothelial function and circulating asymmetric dimethylarginine levels in people with Type 1 diabetes without macrovascular disease or microalbuminuria. Cardiovasc Diabetol. 2009;8:27. https://doi.org/10.1186/1475-2840-8-27 PMid:19486510 PMCid:PMC2698883

19. Cohen RA. Dysfunction of vascular endothelium in diabetes mellitus. Circulation. 1993; 87: V-67–V-76.

20. Järvisalo MJ, Raitakari M, Toikka JO, Putto-Laurila A, Rontu R, Laine S, Lehtimäki T, Rönnemaa T, Viikari J, Raitakari OT. Endothelial dysfunction and increased arterial intima-media thickness in children with type 1 diabetes. Circulation. 2004;109(14):1750-5. https://doi.org/10.1161/01.CIR.0000124725.46165.2C PMid:15023875

21. Tanner JM, Hiernaux J, Jarman S. Growth and physical studies. In: Weiner JS, Lourie JA, editors. Human biology: a guide to field methods. Oxford: Blackwell Scientific Publ. 1969:3–41.

22. Cameron N. The methods of auxological anthropology. In: Falkner F, Tanner JM, editors. Human growth 3 Methodology. New York: Plenum Press, 1986:3–46. PMid:3956717

23. Flegg HM. An investigation for the determination of serum cholesterol by an enzymatic method. Ann Clin Biochem. 1973;10:79–84. https://doi.org/10.1177/000456327301000125

24. Marques-Vidal P, Ferrario M, Kuulasmaa K, Grafnetter D, Moltchanov V, for the WHO MONICA Project. Quality assessment of data on HDL cholesterol in the WHO MONICA Project (1999). Available at: URL:

http://www.thl.fi/publications/monica/hdl/hdlqa.htmURN:NBN:fi-fe19991137.

25. Danilova LA, Lopatina NI. Colorimetric method of determining glycosylated haemoglobin. Lab Delo. 1986;5:282–3.

26. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. N Eng J Med. 1984;310:356–60. https://doi.org/10.1056/NEJM198402093100605 PMid:6690964

27. Singh TP, Groehn H, Kazmers A. Vascular function and carotid intimal-medial thickness in children with insulin-dependent diabetes mellitus. J Am CollCardiol. 2003; 41: 661–665. https://doi.org/10.1016/S0735-1097(02)02894-2

28. Deanfield J, Donald A, Ferri C, Giannattasio C, Halcox J, Halligan S, Lerman A, Mancia G, Oliver JJ, Pessina AC, Rizzoni D, Rossi GP, Salvetti A, Schiffrin EL, Taddei S, Webb DJ. Endothelial function and dysfunction: part I: methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. J Hypertens. 2005; 23: 7–17. https://doi.org/10.1097/00004872-200501000-00004 PMid:15643116

29. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force [published correction appears in J Am Coll Cardiol. 2002;39:1082]. J Am Coll Cardiol. 2002; 39: 257–265. https://doi.org/10.1016/S0735-1097(01)01746-6

30. Kuvin JT, Patel AR, Sliney KA, Pandian NG, Rand WM, et al. Peripheral vascular endothelial function testing as a non invasive indicator of coronary artery disease. J Am Coll Cardiol. 2001;38:1843–1849. https://doi.org/10.1016/S0735-1097(01)01657-6

31. Palmieri V, Migliaresi P, Orefice M, Lupo T, Di Minno MN, et al. High prevalence of subclinical cardiovascular abnormalities in patients with systemic lupus erythematosus in spite of a very low clinical damage index. Nutr Metab Cardiovasc Dis. 2009;19:234–240. https://doi.org/10.1016/j.numecd.2008.09.009 PMid:19157818

32. Schulze F, Wesemann R, Schwedhelm E, Sydow K, Albsmeier J, et al. Determination of asymmetric dimethylarginine (ADMA) using a novel ELISA assay. Clin Chem Lab Med. 2004;42:1377–83. https://doi.org/10.1515/CCLM.2004.257 PMid:15576299

33. Altinova AE, Arslan M, Sepici-Dincel A, Akturk M, Altan N, et al. Uncomplicated type 1 diabetes is associated with increased asymmetric dimethylarginine concentrations. J Clin Endocrinol Metabol. 2007;92:1881–5. https://doi.org/10.1210/jc.2006-2643 PMid:17311854

34. Wiltshire EJ, Gent R, Hirte C, et al. Endothelial dysfunction relates to folate status in children and adolescents with type 1 diabetes. Diabetes. 2002; 51: 2282–2286. https://doi.org/10.2337/diabetes.51.7.2282 PMid:12086961

35. Donaghue KC, Robinson J, McCredie R, et al. Large vessel dysfunction in diabetic adolescents and its relationship to small vessel complications. J Pediatr Endocrinol Metab. 1997; 10: 593–598. https://doi.org/10.1515/JPEM.1997.10.6.593 PMid:9467129

36. Soha M Abd El Dayem, Abo El Maged El Bohy, Mohamed Ali, and Enass Moktar. Assessment of Endothelial Dysfunction, Coronary and Carotid Atherosclerosis In Type I Diabetics. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2017;8 (3):68-79.

37. Masri B, Knibiehler B, Audigier Y. Apelin signalling: a promising pathway from cloning to pharmacology. Cellular Signalling. 2005;17:415–426. https://doi.org/10.1016/j.cellsig.2004.09.018 PMid:15601620

38. Habchi M, Duvillard L, Cottet V, Brindisi MC, Bouillet B, Beacco M, Crevisy E, Buffier P, Baillot-Rudoni S, Verges B, Petit JM. Circulating apelin is increased in patients with type 1 or type 2 diabetes and is associated with better glycaemic control. Clin Endocrinol (Oxf). 2014;81(5):696-701. https://doi.org/10.1111/cen.12404 PMid:24417455

39. Valle A, Hoggard N, Adams AC, et al. Chronic central administration of apelin-13 over 10 days increases food intake, body weight, locomotor activity and body temperature in C57BL/6 mice. Journal of Neuroendocrinology. 2008; 20: 79–84. PMid:18081555

40. Clarke KJ, Whitaker KW, Reyes TM. Diminished Metabolic Responses to Centrally-Administered Apelin-13 in Diet-Induced Obese Rats Fed a High-Fat Diet. Journal of neuroendocrinology. 2009;21(2):83-9. https://doi.org/10.1111/j.1365-2826.2008.01815.x PMid:19076266

41. Dray C, Knauf C, Daviaud D, et al. Apelin stimulates glucose utilization in normal and obese insulin-resistant mice. Cell Metabolism. 2008; 8: 437–445. https://doi.org/10.1016/j.cmet.2008.10.003 PMid:19046574

42. Yue P, Jin H, Aillaud M, et al. Apelin is necessary for the maintenance of insulin sensitivity. American Journal of Physiology-Endocrinology and metabolism. 2010; 298: E59–E67. https://doi.org/10.1152/ajpendo.00385.2009 PMid:19861585 PMCid:PMC2806109

43. Boucher J, Masri B, Daviaud D, et al. Apelin, a newly identified adipokine up-regulated by insulin and obesity. Endocrinology. 2005; 146: 1764–1771. https://doi.org/10.1210/en.2004-1427 PMid:15677759

44. Li L, Yang G, Li Q, et al. Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. Experimental and Clinical Endocrinology & Diabetes. 2006; 114: 544–548. https://doi.org/10.1055/s-2006-948309 PMid:17177135

45. Soriguer F, Garrido-Sanchez L, Garcia-Serrano S, et al. Apelin levels are increased in morbidly obese subjects with type 2 diabetes mellitus. Obesity Surgery. 2009; 19: 1574–1580. https://doi.org/10.1007/s11695-009-9955-y PMid:19756893

46. Dray C, Debard C, Jager J, et al. Apelin and APJ regulation in adipose tissue and skeletal muscle of type 2 diabetic mice and humans. American Journal of Physiology-Endocrinology and metabolism. 2010; 298: E1161–E1169. https://doi.org/10.1152/ajpendo.00598.2009 PMid:20233941

47. Erdem G, Dogru T, Tasci I, et al. Low plasma apelin levels in newly diagnosed type 2 diabetes mellitus. Experimental and Clinical Endocrinology & Diabetes. 2008;116:289–292. https://doi.org/10.1055/s-2007-1004564 PMid:18484561

48. Zhang Y, Shen C, Li X, et al. Low plasma apelin in newly diagnosed type 2 diabetes in Chinese people. Diabetes Care. 2009; 32: e150. https://doi.org/10.2337/dc09-1146 PMid:19940213

49. Meral C, Tascilar E, Karademir F, et al. Elevated plasma levels of apelin in children with type 1 diabetes mellitus. Journal of Pediatric Endocrinology & Metabolism. 2010; 23: 497–502. https://doi.org/10.1515/jpem.2010.081

50. Alexiadou K, Kokkinos A, Liatis S, et al. Differences in plasma apelin and visfatin levels between patients with type 1 diabetes mellitus and healthy subjects and response after acute hyperglycemia and insulin administration. Hormones (Athens, Greece). 2012; 11: 444–450. https://doi.org/10.14310/horm.2002.1376

51. Cavallo MG, Sentinelli F, Barchetta I, et al. Altered glucose homeostasis is associated with increased serum apelin levels in type 2 diabetes mellitus. PLoS ONE. 2012; 7: e51236. https://doi.org/10.1371/journal.pone.0051236 PMid:23227256 PMCid:PMC3515542

52. Reinehr T, Woelfle J, Roth CL. Lack of association between apelin, insulin resistance, cardiovascular risk factors, and obesity in children: a longitudinal analysis. Metabolism. 2011; 60:1349–1354. https://doi.org/10.1016/j.metabol.2011.02.005 PMid:21489579

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):940-944.
https://doi.org/10.3889/oamjms.2017.177
eISSN: 1857-9655
Clinical Science

The Prevalence and Risk Factors of Early Arrhythmias Following Pediatric Open Heart Surgery in Egyptian Children

Tarek Ahmed Abdel Gawad[1], Waleed Mohamed Elguindy[1], Omneya Ibrahim Youssef[1*], Tamer Ashraf Abosalem[2]

[1]Pediatric Department, Faculty of Medicine, Ain Ainshams University, Cairo, Egypt; [2]Coronary Care Unit, Abu El Reesh Hospital, Cairo University, Giza, Egypt

Citation: Abdel Gawad TA, Elguindy WM, Youssef OI, Abosalem TA. The Prevalence and Risk Factors of Early Arrhythmias Following Pediatric Open Heart Surgery in Egyptian Children. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):940-944. https://doi.org/10.3889/oamjms.2017.177

Keywords: Postoperative arrhythmias; Children; Cardiac surgery; inotropic drugs.

***Correspondence:** Omneya Ibrahim Youssef. Pediatric Department, Faculty of Medicine, Ain Ainshams University, Cairo, Egypt. E-mail: ibrahim_omneya@yahoo.com

Received: 20-Mar-2017; **Revised:** 19-Sep-2017; **Accepted:** 22-Sep-2017; **Online first:** 05-Dec-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: This study aimed to assess the prevalence of early postoperative arrhythmias after cardiac operation in the pediatric population, and to analyse possible risk factors.

MATERIAL AND METHODS: Cross-sectional study included 30 postoperative patients, with age range four up to 144 months. They were selected from those admitted to the Cardiology Unit in the Pediatric department of Ain Shams University hospitals, after undergoing cardiopulmonary bypass (CPB) surgery for correction of congenital cardiac defects. All patients had preoperative sinus rhythm and normal preoperative electrolytes levels. All patients' records about age, weight, type of surgery, intraoperative arrhythmias, cardiopulmonary bypass time, ischemic time and use of inotropic drugs were taken before they were admitted to the specialised pediatric post-surgery intensive care unit (ICU).

RESULTS: Arrhythmia was documented in 15 out of 30 patients (50%). Statistically significant difference between the arrhythmic and non-arrhythmic group were recorded in relation to the age of operation (23 vs 33 months), weight (12 vs. 17 kg), ischemic time (74.5 vs. 54 min), cardiopulmonary bypass time (125.5 vs. 93.5min), inotrope use (1.6 vs. 1.16) and postoperative ICU stay (5.8 vs. 2.7 days), P<0.05.

CONCLUSION: Early postoperative arrhythmias following surgery for congenital heart disease are relatively frequent in children (50%).Younger age, lower body weight, longer ischemic time and bypass time, and more inotrope use are all risk factors for postoperative arrhythmias and lead to increase the hospital stay.

Introduction

Immediate postoperative arrhythmias are a widely recognised complication of cardiothoracic surgery in both the adult and pediatric populations [1]. Although they are transient and manageable, they are considered a major cause of morbidity and mortality after cardiac surgery for congenital heart disease during the phase of postoperative hemodynamic instability [2-4].

There are many factors known to increase the risk of post congenital heart repair surgeries. Cardiopulmonary bypass (CPB), intraoperative injury to the conduction system and myocardium, postoperative metabolic abnormalities and electrolyte disturbances are known factors associated with increased risk of arrhythmias following surgery [4, 5]. The incidence and types of arrhythmia after cardiac surgery vary with age, the underlying lesion, the type of surgery, and local practice patterns. The overall incidence of early postoperative arrhythmia has been reported to be as high as 48% in children. [6]

After frequent premature ventricular contractions (PVCs), ectopic junctional tachycardia (JET) is the most common arrhythmia in patients undergoing congenital heart surgery. The operative and perioperative factors that may contribute to postoperative JET remain ill-defined [1]

This study aimed to assess the prevalence of early postoperative arrhythmias after cardiac operation in the pediatric population and to analyse possible risk factors.

Material and Methods

Following approval by the "Ethical Committee" of Ain-Shams University and the parents' consent, 30 patients of both sexes; who undergoing CPB for corrective surgery of congenital cardiac defects; were selected from the Cardiology Unit in the Pediatric department of Ain-Shams University hospitals. The females represented 60% (18 patients) while males were 40% (12 patients) of the study population. Their ages ranged from 4 to 144 months with an average of 51.11 (± 45.12) months. They had mean weights of 17 kg (± 10.61 kg) ranging from 3 to 33 kg.

This cross-sectional study was carried out between March 2013 and March 2014. All patients had preoperative sinus rhythm and normal preoperative magnesium and other electrolytes levels. All patients' records about type of surgery, intraoperative arrhythmias, cardiopulmonary bypass time, ischemic time, type of used inotropic drugs and the dose were taken before they were admitted to our specialised pediatric post-surgery intensive care unit (ICU)

Arrhythmias assessment

Arrhythmias were classified as supraventricular tachycardia (SVT), ectopic junctional tachycardia (JET), premature ventricular contractions (PVCs), complete heart block (CHB), junctional rhythm (JR) and ventricular tachycardia (VT). SVT was defined as retrograde P wave (AV reentrant) or 1:1 AV conduction. JET was defined as a narrow complex tachycardia with atrioventricular (AV) dissociation [7].

PVCs was diagnosed if there was premature QRS complex and prolongation of its duration for age with premature ventricular complex not preceded by premature atrial activity [8, 9]. CHB occurs when none of the atrial impulses is conducted to the ventricles. There is a complete loss of rhythm conduction from a working atrial pacemaker, thereby allowing the ventricular pacemaker to take over [10].

JR characterized by normal QRS morphology at a rate not exceeding the maximum junction escape rate for age (up to 3 years it is 50 to 80 beats/min and 40 to 60 beats/min over 3 years) and slower than the atrial escape rhythm (up to 3 years it is 80 to 100 beats/min up and 50 to 60 beats/min over 3 years) [7, 11]. VT was defined as series of 3 or more repetitive excitations originating from one of both ventricles. The QRS complexes are different from the usual QRS morphology with a prolonged duration of corresponding age [7].

Standard electrocardiograms (ECGs) were registered in all patients at the time of ICU admission, using (Cardiofax ECG-9620L) a real-time electrocardiograph with three channel recorder. Continuous ECG monitoring was performed during the entire ICU stay with (Drager Infinity Vista XL monitors). Before hospital discharge, a 12-lead ECG was routinely done. In case of arrhythmia in the postoperative course, the patient also had a 24 hr Holter recording before discharge.

A Vision™ Holter analysis system, manufactured by Spacelabs Burdick was used in this study. During analysis, the vision™ Holter analysis system uses a technique known as feature extraction to group the individual QRS complexes into forms based on their features. After the individual QRS complexes have been consolidated into forms, the forms are classified into one of the following categories: Normal (N), Ventricular (V), Paced (P), or Artifact (x). Identification of ventricular and supraventricular arrhythmia takes place. These are then classified as runs, couplets or isolated episodes. The interpretation of the Holter monitoring was then finalized after the cumulative analysis of the edited forms, arrhythmias and full disclosure of the recording

Operative details and inotropic drugs use

After recording the basic details like age and weight, a history of arrhythmia and related drugs was taken. Preoperative electrolytes levels were measured. Preoperative 12 leads electrocardiogram, and Preoperative echocardiography was done. General anaesthesia was performed in all patients. During the operation, the bypass and ischemic time were recorded. When necessary, the following inotropic drugs were used: Milrinone (0.3-0.75mcg\kg\min), Adrenaline (0.1- 0.5 mcg \kg\min), Dobutamine (5-20 mcg\kg\min), while weaning them from CPB. Post-operative stay in the intensive care unit (ICU stay) was also recorded.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS/Windows Version 16, SPSS Inc., and Chicago, IL, USA). Statistical significance was set at $P < 0.05$. The patients under study were classified into two groups according to the occurrence of postoperative arrhythmia: arrhythmic group (who get postoperative arrhythmia, and non-arrhythmic group (who did not get postoperative arrhythmia). The non-parametric data (qualitative) were expressed as frequency distribution: numbers and percentage of the total. Comparisons between the different non-parametric variables were analyzed using Chi-square test. While the parametric data (quantitative) were expressed as mean + SD. Student independent t-test was used to compare two parametric data.

Results

The current study documented that arrhythmias were found in 15 out of 30 patients who represented an overall incidence of 50%. Those were grouped as follow: 5 patients (33.33%) with Junctional ectopic tachycardia (JET), 4 patients (26.67%) with Junctional rhythm (JR), 2 patients (13.33%) with complete heart block (CHB), 2 patients (13.33%) with premature ventricular contractions (PVCs), 1patient (6.67%) got attack of ventricular tachycardia (VT) and another one (6.67%) got supraventricular tachycardia (SVT) (Table 1).

Table 1: Percentage of different types of early postoperative arrhythmias among study population

Type of arrhythmia	No.	%
Junctional ectopic tachycardia	5	33.33%
Junctional rhythm	4	26.67%
Complete heart block	2	13.33%
Premature ventricular contractions	2	13.33%
Ventricular tachycardia	1	6.67%
Supraventricular tachycardia	1	6.67%
Total	15	100.00%

Statistically significant differences between the arrhythmic and non-arrhythmic groups were found in relation to the age of operation (23 vs 33 months), weight (12 vs. 17 kg), ischemic time (74.5 vs. 54 min), cardiopulmonary bypass time (125.5 vs. 93.5 min), number of inotropic drugs used (1.6 vs. 1.16) and postoperative ICU stay (5.8 vs. 2.7 days), P < 0.05 (Table 2). It was noticed that younger the age, lower body weight, longer ischemic time and bypass time, and the more inotrope use, are all risk factors for the arrhythmic group and lead to increase the hospital stay. These risk factors lead to the increased possibility of postoperative arrhythmias.

Table 2: Comparison between the arrhythmic and non-arrhythmic groups

Variable	No arrhythmia		Arrhythmia		Independent t-test	
	Mean	SD	Mean	SD	t	P-value
AGE (months)	33.66	13.656	23.84	9.089	2.318	0.027
WEIGHT (kg)	17.00	12.21	12.13	6.44	2.768	0.010
ISCHEMIC TIME (min)	53.99	32.741	74.6	35.36	3.212	0.003
BYPASS TIME (min)	93.57	28.77	125.65	24.697	3.277	0.003
Inotrope no.	1.56	0.64	1.16	0.325	2.158	0.039
ICU stay (day)	2.7	0.9	5.8	1.2	4.648	0.000

Table 3 showed a significant relationship between the absence of inotropic support and absence of early postoperative arrhythmias. Inotropic support was not used in 5 patients, only one of them had arrhythmias (20%) while the other four patients (80%) did not experience any form of early postoperative arrhythmias (P-value 0.039). Adrenaline also was not used in 15 patients, of those four patients (26.6%) had arrhythmias while the other 11 patients (73.3%) did have any form of arrhythmia (P-value 0.028). However, the insignificant effect of the use of milrinone or dobutamine on the occurrence of early postoperative arrhythmias was detected (P value > 0.05), as nearly half the patients who used/did not use one of these drugs had arrhythmias.

Table 3: Comparison between arrhythmic and non-arrhythmic group as regard inotropic drugs use

		No arrhythmia		Arrhythmia		Chi-square test	
		No.	%	No.	%	X^2	P-value
Inotropic support	Used = 25	11	73.33%	14	93.33%	0.960	0.039
	Not used = 5	4	26.67%	1	6.67%		
Milrinon	Used = 4	2	12.50%	2	14.29%	0.156	0.693
	Not used = 26	14	87.50%	12	85.71%		
Adrenaline	Used = 15	4	26.67%	11	73.33%	4.800	0.028
	Not used = 15	11	73.33%	4	26.67%		
Dobutamine	Used = 19	9	60.0%	10	66.7%	0.000	1.000
	Not used = 11	6	40.0%	5	33.3%		

Moreover, adrenaline at doses below 0.1 mcg/kg/min was already running in 4 patients, three patients of them had no arrhythmias. Adrenaline at doses between 0.1-0.2 mcg/kg/min was used in 7 patients, six patients of them also had no arrhythmias. While adrenaline at doses > 0.2 mcg was used in 4 patients, all of them had arrhythmias (P-value 0.033). This means that there was a significant effect of increasing the dose of adrenaline more than 0.2 mcg/kg/min on increasing the risk of occurrence of arrhythmias. However, the insignificant effect of increasing the dose of dobutamine infusion on the increased occurrence of arrhythmias was found (P value > 0.05).

Table 4: Comparison between arrhythmic and non-arrhythmic group as regards dosage of inotropes used

		Arrhythmia		No arrhythmia		Total		P-value
		No.	%	No.	%	No.	%	
Dobutamine grade	5 < 10 ug	1	6.7%	4	26.67%	5	16.67%	
	10 < 20 ug	6	40.0%	5	33.33%	11	36.67%	0.235
	> 20 ug	3	20.0%	0	0.0%	3	10.00%	
Adrenaline grade	0.05 - < 0.1 ug	1	6.7%	3	20.00%	4	13.33%	
	0.1 - 0.2 ug	6	40.0%	1	6.67%	7	23.33%	0.033
	> 0.2	4	26.7%	0	0.0%	4	13.33%	

Discussion

Studies have been conducted on rhythm complications related to surgical repair of congenital heart disease, most have focused on rhythm disturbances as a late complication of a single diagnosis or procedure, whereas others have evaluated the risk of a single rhythm disturbance [12]

Current results demonstrated that arrhythmias were a frequent early complication after an open heart operation for congenital heart disease, despite the advances in surgical and CPB techniques as well as myocardial preservation. This high incidence, occurring in spite of all surgical technical improvements, can be explained by the performance of more complex surgical procedures in increasingly younger patients.

The current study found a peak prevalence of arrhythmias of 50 % which is near to previously

reported studies like Alp et al. (2014) 43.5%. Delaney et al. reported the prevalence of arrhythmias in their patients that necessitated intervention, 15% of their cohort. However, Grosse-Wortmann et al. (2010) reported peak prevalence of arrhythmias 79.1%, which was higher than all other reported studies. They explained this difference as being a result of Holter monitoring, which may reflect a more sensitive method of detection, especially of extrasystoles, instead of bedside monitoring [13-15].

Pathophysiologic causes for early postoperative arrhythmias are various, including direct surgical injuries like myocardial incision, results of cannulation, sutures close to the conduction system, and acute changes of the intracardiac pressure caused by volume and pressure overload [16].

Junctional ectopic tachycardia was the most frequent arrhythmia in the current study including 33.3% of the arrhythmic population, then Junctional rhythm 26.67%, complete heart block and premature ventricular contractions 13.3 % and ventricular tachycardia and supraventricular tachycardia 6.67% while in Alp et al. 2014 supraventricular extra-systole was the most common arrhythmia with 65%, ventricular extra-systole 24%, atrial fibrillation and supraventricular tachycardia 2.9% each, atrial flutter 1.9 % and Junctional rhythm 1%. After frequent PVCs, JET was the most common arrhythmia in patients undergoing congenital heart surgery [13].

The precise aetiology of JET is unknown, but it is believed to be the result of enhanced automaticity in the bundle of His, either in its right atrial or right ventricular portion, promoted by haemorrhage into the conduction tissues. It is postulated that disruption of conduction tissue, either by direct trauma or penetrating blood and interstitial inflammatory cells, may result in an irritable focus leading to JET [14].

In the present study, comparison between the arrhythmic and non-arrhythmic group of patients revealed a statistically significant difference as regard age at operation (23 vs. 33 months), ischemic time (74.5 vs. 54 min), cardiopulmonary bypass time (125.5 vs. 93.5 min), number of use of inotropic drugs (1.6 vs. 1.16) and postoperative ICU stay (5.8 vs. 2.7 days), $P < 0.05$. This came in agreement with Delaney et al. who showed a statistically significant difference between the mean values for the arrhythmic and non-arrhythmic groups about age at operation (22 vs 45 months), ischemic time (105 vs 44 min), cardiopulmonary bypass time (189 vs 109 min). Alp et al. reported that there was no association in their cohort to these risk factors, they explained that because they include neonates with simple procedures like PDA ligation and division [12, 13].

Pfammatter et al. reported that the incidence of early postoperative arrhythmia after cardiac operation in children was 11% of children with bypass time of < 50 minutes. This proportion rose to 33% for bypass time between 50 and 100 minutes and to 50% for bypass time > 100 minutes. They stated that the association between higher occurrence rate of arrhythmias and longer cardiopulmonary time might just reflect increasing complexity of the surgery [17].

Inotropic support is the mainstay of treatment of patients with low cardiac output that resulted either from a surgical intervention that included cardiopulmonary bypass or as a component of the underlying heart disease.

In this study, a strong relationship between the absence of inotropic support and absence of early postoperative arrhythmias was found. Inotropic support was not used in 5 patients, only one of them had arrhythmia (20%). This is consistent with what was mentioned by Smith et al. that patients, in whom inotropic support was not used, the experienced low incidence of early postoperative arrhythmias [18].

Statistically significant effect of the use of adrenaline; particularly if its dose was more than 0.2 ug/kg/min; on the occurrence of early postoperative arrhythmias was found. While the statistically insignificant effect of the use of either milrinone or dobutamine on the occurrence of early postoperative arrhythmias was found.

Milrinone acts independently of the adrenergic receptor through phosphodiesterase inhibition and therefore does not cause increased myocardial oxygen consumption or catecholamine stimulation to the heart [18].

In conclusion, early postoperative arrhythmias following surgery for congenital heart disease are relatively frequent in children: 50%. Junctional ectopic tachycardia and junctional rhythm were common types of arrhythmias. Lower age, lower body weight, longer CPB time, longer cross-clamp time, use of inotropic support; particularly adrenaline at a dose more than 0.2 mcg/kg/min, all are risk factors for postoperative arrhythmias.

Acknowledgements

We acknowledge all parents of the children in cardiology unit Faculty of Medicine, Ain Shams University where the study had been done for their cooperation during all the time of the study.

References

1. Delaney JW, Moltedo JM, Dziura JD, Kopf GS and Snyder CS. Early postoperative arrhythmias after pediatric cardiac surgery. The Journal of thoracic and cardiovascular surgery. 2006;131(6):1296-300. https://doi.org/10.1016/j.jtcvs.2006.02.010 PMid:16733160

2. Batra AS and Balaji S. Post operative temporary epicardial pacing: When, how and why? Annals of pediatric cardiology. 2008;1(2):120. https://doi.org/10.4103/0974-2069.43877 PMid:20300253 PMCid:PMC2840753

3. Krongrad E. Postoperative arrhythmias in patients with congenital heart disease. Chest. 1984;85:107-13. https://doi.org/10.1378/chest.85.1.107 PMid:6360568

4. Lan YT, Lee JC and Wetzel G. Postoperative arrhythmia. Current opinion in cardiology. 2003;18(2):73-8. https://doi.org/10.1097/00001573-200303000-00001

5. Hoffman TM, Wernovsky G, Wieand TS, Cohen MI, Jennings AC, Vetter VL, et al. The incidence of arrhythmias in a pediatric cardiac intensive care unit. Pediatric Cardiology. 2002;23(6):598-604. https://doi.org/10.1007/s00246-001-0079-y PMid:12530491

6. Valsangiacomo E, Schmid ER, Schupbach RW, Schmidlin D, Molinari L, Waldvogel K, et al. Early postoperative arrhythmias after cardiac operation in children. The Annals of thoracic surgery. 2002;74(3):792-6. https://doi.org/10.1016/S0003-4975(02)03786-4

7. Kim JJ, Collins KK and Miller-Hance WC. Arrhythmias: Diagnosis and Management. Anesthesia for Congenital Heart Disease. 2005:308-37.

8. Dick M. Clinical cardiac electrophysiology in the young: Springer Science & Business Media, 2006. https://doi.org/10.1007/0-387-29170-9

9. Scott O, Macartney FJ and Deverall PB. Sick sinus syndrome in children. Archives of disease in childhood. 1976;51(2):100-5. https://doi.org/10.1136/adc.51.2.100 PMid:1259455 PMCid:PMC1545887

10. Doniger SJ and Sharieff GQ. Pediatric dysrhythmias. Pediatric Clinics of North America. 2006;53(1):85-105. https://doi.org/10.1016/j.pcl.2005.10.004 PMid:16487786

11. Anderson RH, Baker EJ, Redington A, Rigby ML, Penny D and Wernovsky G. Paediatric Cardiology: Expert Consult-Online and Print: Elsevier Health Sciences, 2009.

12. Delaney J, Moltedo J, Dziura J, Kopf G and Snyder C. Early postoperative arrhythmias after pediatric cardiac surgery. The Journal of thoracic and cardiovascular surgery. 2006;131:1296 - 300. https://doi.org/10.1016/j.jtcvs.2006.02.010 PMid:16733160

13. Alp H, Baysal T and Karaarslan S. Supraventricular tachycardia due to blunt chest trauma in an adolescent. Ulusal travma ve acil cerrahi dergisi = Turkish journal of trauma & emergency surgery: TJTES. 2014;20(3):211-3. https://doi.org/10.5505/tjtes.2014.90337 PMid:24936844

14. Grosse-Wortmann L, Kreitz S, Grabitz R, Vazquez-Jimenez J, Messmer B, von Bernuth G, et al. Prevalence of and risk factors for perioperative arrhythmias in neonates and children after cardiopulmonary bypass: continuous holter monitoring before and for three days after surgery. Journal of Cardiothoracic Surgery. 2010;5(1):85. https://doi.org/10.1186/1749-8090-5-85 PMid:20955589 PMCid:PMC2974677

15. Grosse-Wortmann L, Kreitz S, Grabitz RG, Vazquez-Jimenez JF, Messmer BJ, von Bernuth G, et al. Prevalence of and risk factors for perioperative arrhythmias in neonates and children after cardiopulmonary bypass: continuous holter monitoring before and for three days after surgery. J Cardiothorac Surg. 2010;5(85):21. https://doi.org/10.1186/1749-8090-5-85

16. Heintz KM and Hollenberg SM. Perioperative cardiac issues: postoperative arrhythmias. Surgical Clinics of North America. 2005;85(6):1103-14. https://doi.org/10.1016/j.suc.2005.09.003 PMid:16326196

17. Pfammatter J, Wagner B, Berdat P, Bachmann D, Pavlovic M, Pfenninger J, et al. Procedural factors associated with early postoperative arrhythmias after repair of congenital heart defects. The Journal of thoracic and cardiovascular surgery. 2002;123:258-62. https://doi.org/10.1067/mtc.2002.119701 PMid:11828284

18. Smith AH, Owen J, Borgman KY, Fish FA and Kannankeril PJ. Relation of milrinone after surgery for congenital heart disease to significant postoperative tachyarrhythmias. The American journal of cardiology. 2011;108(11):1620-4. https://doi.org/10.1016/j.amjcard.2011.07.023 PMid:21890079 PMCid:PMC3941037

19. Abdelaziz O and Deraz S. Anticipation and management of junctional ectopic tachycardia in postoperative cardiac surgery: Single center experience with high incidence. Annals of pediatric cardiology. 2014;7(1):19. https://doi.org/10.4103/0974-2069.126543 PMid:24701080 PMCid:PMC3959055

20. Tharakan JA and Sukulal K. Post cardiac surgery junctional ectopic tachycardia: A 'Hit and Run'tachyarrhythmia as yet unchecked. Annals of pediatric cardiology. 2014;7(1):25. https://doi.org/10.4103/0974-2069.126545 PMid:24701081 PMCid:PMC3959056

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):945-947.
https://doi.org/10.3889/oamjms.2017.178
eISSN: 1857-9655
Clinical Science

Profile of Skin pH in Leukaemia's Children with Chemotherapy Treatments at Haji Adam Malik General Hospital, Medan

Radha Siregar*, Sri Purnama, Salia Lakswinar

Haji Adam Malik General Hospital, Dermatology and Venereology, Medan, Indonesia

Citation: Siregar R, Purnama S, Lakswinar S. Profile of Skin pH in Leukaemia's Children with Chemotherapy Treatments at Haji Adam Malik General Hospital, Medan. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):945-947. https://doi.org/10.3889/oamjms.2017.178

Keywords: Leukemia; Chemotherapy; Skin pH; Children; Skin barrier.

***Correspondence:** Radha Siregar. Haji Adam Malik General Hospital, Dermatology and Venereology, Medan, Indonesia. E-mail: dha_1302@yahoo.com

Received: 29-Jul-2017; **Revised:** 13-Sep-2017; **Accepted:** 21-Sep-2017; **Online first:** 05-Dec-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: One of the treatments for leukaemia is chemotherapy. Side effects and toxicity of this treatment can be seen on the skin, adnexal, and mucous membranes. They might increase potential hydrogen (pH) value on the skin surface, therefore, disrupting epidermal barrier defences.

AIM: To describe the pH of the skin in children with leukaemia who received chemotherapy.

SUBJECT AND METHOD: This study was an observational descriptive, cross-sectional study, conducted from March until December 2016 with 32 children with leukaemia who treated at Haji Adam Malik General Hospital, Medan as subjects. Skin pH was measured by a pH meter.

RESULT: We found mean skin pH in ALL (6.28 ± 0.58), CML (5.9 ± 0) and AML (6.5 ± 0.50). The mean skin pH after 1-5 weeks of chemotherapy was 6.13 ± 0.49, at 6-10 weeks (6.32 ± 0.51), and at 11-15 weeks (7.12 ± 0.36). The mean skin pH of patients with two drugs (5.98 ± 0.44), four drugs (6.28 ± 0.55), and six drugs (6.63 ± 0.56).

CONCLUSION: The highest mean of skin pH were obtained in AML group, 11-15 weeks length of chemotherapy and group with six drugs regimen.

Introduction

Leukaemia, commonly known as blood cancer or bone marrow cancer, is the uncontrolled growth of abnormal cells (neoplastic cells) as a result of normal cell mutation [1]. Acute Leukaemia in children accounts for 97% of all leukaemia in children.

According to the blood lineage affected, acute leukaemia can be divided into Acute Lymphoblastic Leukaemia (ALL) 82% and Acute Myeloblastic Leukaemia (AML) 18% [2]. ALL is cancer that frequently occurs in children. There are approximately 3000 new cases of ALL per year in the United States, 5000 cases in Europe, and roughly 2000 to 3000 cases in Indonesia [3].

Chemotherapy is a type of treatment that plays a role in killing neoplastic cells, but also indirectly affects normal cells [1]. Many of the side effects and toxicities of chemotherapy can be seen on the skin, adnexa, and mucous membranes of patients undergoing chemotherapy. In the skin, the side effects of chemotherapy include dry skin, allergic drug reaction, photosensitivity, hyperpigmentation, nail abnormalities, and hair abnormalities [4, 5].

In general, chemotherapy drugs can cause side effects on the skin by damaging the skin barrier [6]. The pH acidity of the skin surface is important for epidermal barrier defence function and the establishment and maintenance of epidermal integrity/ cohesion [7, 8]. The pH of the skin in children is reported to be the same as in adults that is around 4.0 - 6.0 [9].

Considering the side effects of chemotherapy on the skin and the important role of the skin barrier, it is essential to assess the effect of chemotherapy on skin pH. Until now, there is no data regarding the skin pH of children with leukaemia who undergo chemotherapy.

This research is a preliminary study to determine the effects of chemotherapy on skin pH.

Material and Methods

This is an observational descriptive study with a cross-sectional design. This research was conducted from March until April 2016 involving 32 leukemic children.

This research was conducted from March 2016 until all the required samples are acquired. The research was done in the Rindu B4 inpatient room, RSUP Haji Adam Malik, Medan. The target population of this research is leukaemia patients who had chemotherapy at RSUP Haji Adam Malik, Medan. The accessible population is the children with leukaemia who received chemotherapy at Rindu B4 inpatient room, RSUP Haji Adam Malik, Medan since March 2016. The sample of this study is the accessible population that meets inclusion and exclusion criteria. The inclusion criteria were informed consent signed by patient's parents. Patients with generalised skin conditions (e.g., ichthyosis, necrolysis epidermal toxic, atopic dermatitis, Netherton syndrome) were excluded.

After receiving permission from the subject's parents and the informed consent were signed, baseline data was recorded, and the patient's skin pH was measured using pH meter (Skincheck, Hanna Instruments, HI 99181N, USA).

The requirements before skin pH measurements are: (1) the subject's parents are asked to bathe the patient with soap until the skin is clean and it is not allowed to apply cosmetics or any topical preparations on the patient's lower arm. The measurement was performed at least 4 hours after the shower subject; (2) measurements were made at a stable room temperature with air conditioner (20°C) with a humidity of 40% and subjected to a minimum of 20 min; and (3) measurements are made in the volar area and if it is not possible, measurements can be made on the arm, upper leg or abdomen of the subject.

The collected data is then processed and then presented in the form of frequency distribution table and analysed descriptively based on several characteristics (duration of chemotherapy, types of leukaemia, number of chemotherapy drugs type).

Result

Most of the patients were male (62.5%), in 6-10 years old group (range 2-17 years old). 27 patients had ALL, and only one patient had CML. Most of them (62.5%) had been in chemo for 1-5 weeks (range 1-13 weeks). Based on protocol therapy that was given, samples were divided into two drugs group (Methotrexate, Vincristine), four drugs group (Methotrexate, Vincristine, Doxorubicin, L-Asparaginase), six drugs group (Methotrexate, Vincristine, Doxorubicin, L-Asparaginase, Mercaptopurine, Hydroxyurea). Most patients had four drugs regimen.

The mean skin pH in ALL patients is 6.28 ± 0.58. In CML and AML patients, the mean skin pH is 5.9 ± 0 and 6.5 ± 0.50, respectively (Table 1).

Table 1: Skin pH distribution based on types of leukaemia

Types of Leukemia		pH	pH
	Mean ± SD	Value	Value
ALL	6.28±0.58	5.3	7.4
CML	5.9±0	5.9	5.9
AML	6.5±0.50	6.1	7.2

The mean skin pH after 1-5 weeks of chemotherapy was 6.13 ± 0.49, at 6-10 weeks was 6.32 ± 0.51, and at 11-15 weeks was 7.12 ± 0.36. Based on these results, the longer the duration of chemotherapy the higher the pH of the skin (Table 2).

Table 2: Skin pH distribution based on chemotherapy phase

Phase of Chemotherapy		pH Minimum	pH Maximum
	Mean ±SD	Value	Value
1-5 weeks (n=20)	6.13±0.49	5.3	6.9
6-10 weeks (n=8)	6.32±0.51	5.6	7.1
11-15 weeks (n=4)	7.12±0.36	6.6	7.4

Based on the number of chemotherapy drugs type, the mean skin pH of patients with two types of drugs was 5.98 ± 0.44, four types of drugs were 6.28 ± 0.55, and six types of drugs was 6.63 ± 0.56. These results indicate that the more types of drugs used, the higher the pH of the skin. (Table 3)

Table 3: Skin pH distribution based on types of chemotherapy

Types of Chemotherapy		pH	pH
	Mean ±SD	Minimum Value	Maximum Value
2 drugs	5.98±0.44	5.3	6.8
4 drugs	6.28±0.55	5.6	7.1
6 drugs	6.63±0.56	5.6	7.4

Discussion

This study found that the average skin pH is increased in ALL (6.28 ± 0.58,) and AML (6.5 ± 0.50). Hoeger et al. in his study reported that normal skin pH in children aged > 4 weeks ranged from 5.0-5.5. The average skin pH in this study is higher than the normal pH range for children. In Indonesia, it has been reported that the normal skin pH range of children ages one month to 14 years is 4.76-5.067 [10].

The result of this study demonstrated that an increase in skin pH is directly proportional to the

duration of chemotherapy use. The skin pH after 1-5 weeks of chemotherapy is 6.13 ± 0.49, at 6-10 weeks is 6.32 ± 0.51, and at 11-15 weeks is 7.12 ± 0.36. This is the study conducted by Haedary et al. which found that most of the effects of chemotherapy on the skin depend on the dose and duration of chemotherapy [11].

The results showed that the average skin pH on the use of 2 types of drugs was 5.98 ± 0.44, on four types of drugs 6.28 ± 0.55, and on six types of drugs 6.63 ± 0.56. These results indicate that the more types of drugs used, the higher the pH of the skin. This is in line with the study by Wohlrab et al. which states that the nonspecific effects of chemotherapy drugs on skin epithelium and surrounding tissue can be observed in about 30% of cancer patients, regardless of the type of cancer. Factors that play a role in the emergence of such effects is regimens and combinations of chemotherapy used [12].

Webster et al. also revealed that the type, concentration, and dose of chemotherapy are the determining factors of skin toxicity on the course of administration of chemotherapy. Chemotherapy drugs and their metabolites will be excreted through the sweat glands. As a result, there are direct toxic effects in their accumulation on the stratum corneum, particularly in the plantar and palmar skin [13]. The effect of chemotherapy drugs on the skin barrier frequently occurs by interfering with the function of proliferation and differentiation of interfollicular keratinocytes and epidermal stem cells [14].

In conclusion, the potential of hydrogen on the skin of patients with ALL and AML was increased. In this study, we found higher pH in patients with longer duration of chemotherapy and subjects with a larger amount of drug types used. More research with longitudinal design, with bigger samples and a longer period of study, are needed to examine further about skin pH in leukemic children with chemotherapy.

References

1. Pumala, Susan E. Epidemiology of childhood acute myeloid leukaemia. Pediatric blood & cancer. 2013; 60(5):728-733. https://doi.org/10.1002/pbc.24464 PMid:23303597 PMCid:PMC3664189

2. Lazownsky P, lipton J, Fish J. Lanzkowsky's Manual of Pediatric Hematology and Oncology 6th Edition. United states. Academic press. 2016; 6:36.

3. Pakpahan S. Karakteristik Anak Yang Menderita Leukemia Akut Rawat Inap di RSUP H. Adam Malik Medan Tahun 2011-2012 [thesis]. Medan; Universitas Sumatera Utara, 2014.

4. Sachs L. The control of hematopoiesis and leukemia: from basic biology to the clinic. Proceedings of the National Academy of Sciences. 1996; 93(10):4742-49. https://doi.org/10.1073/pnas.93.10.4742

5. Wiemels J. Perspectives on the causes of childhood leukemia. Chemico-biological interactions. 2012; 196(3):59-67. https://doi.org/10.1016/j.cbi.2012.01.007 PMid:22326931 PMCid:PMC3839796

6. Fabbrocini G. Chemotherapy and skin reactions. J Exp Clin Cancer. 2012; 31:50. https://doi.org/10.1186/1756-9966-31-50 PMid:22640460 PMCid:PMC3583303

7. Hachem, J-P. pH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. Journal of Investigative Dermatology. 2003; 121(2):345-353. https://doi.org/10.1046/j.1523-1747.2003.12365.x PMid:12880427

8. Luebberding KS. Skin physiology in men and women: in vivo evaluation of 300 people including TEWL, SK hydration, sebum content and skin surface pH. International journal of cosmetic Science. 2013; 35(5):477-83. https://doi.org/10.1111/ics.12068 PMid:23713991

9. Primadiarti P, Rahmadewi, Zulkarnain I. Peningkatan pH Kulit Dermatitis Atopi pada Anak. Artikel Asli: FK Universitas Airlangga, 2014.

10. Hoeger PH, Enzmann CC. Skin physiology of the neonate and young infant: a prospective study of functional skin parameters during early infancy. Pediatric dermatology. 2002; 19(3):256-62. https://doi.org/10.1046/j.1525-1470.2002.00082.x

11. Heidary N, Naik H, Burgin S. Chemotherapeutic agents and the skin: an update. Journal of the American Academy of Dermatology. 2008; 58(4):545-70. https://doi.org/10.1016/j.jaad.2008.01.001 PMid:18342708

12. Wohlrab J, Bangemann N, Kleine-Tebbe A, Thill M, Kümmel S, Grischke EM, Richter R, Seite S, Lüftner D. Barrier protective use of skin care to prevent chemotherapy-induced cutaneous symptoms and to foksmaintain quality of life in patients with breast cancer. Breast Cancer: Targets and Therapy. 2014; 6:115. PMid:25114589 PMCid:PMC4126578

13. Webster-Gandy JD, How C, Harrold K. Palmar Plantar Erythrodysesthesia (Ppe): A Literature Review with Commentary On Experience in a Cancer Centre. Eur J Oncol Nurs. 2007;11:238-246. https://doi.org/10.1016/j.ejon.2006.10.004 PMid:17350337

14. Mancuso S, Greggi S, Giannice R, Margariti PA, Salerno MG, De Dilectis A, Scambia G. Cutaneous side-effects of antilastic chemotherapy: an emerging problem. Journal of applied cosmetology. 1998;16:165-78.

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):948-954.
https://doi.org/10.3889/oamjms.2017.209
eISSN: 1857-9655
Clinical Science

A Comparison of the Quality of Sleep between Pre and Post-Surgery Cervical Herniated Nucleus Pulposus Patients Utilizing the Anterior Discectomy Method

Ridha Dharmajaya[1*], Dina Keumala Sari[2], Ratna Akbari Ganie

[1]Neurosurgery Department, Universitas Sumatera Utara, Indonesia; [2]Nutrition Department, Universitas Sumatera Utara, Indonesia; [3]Clinical Pathology Department, Universitas Sumatera Utara, Indonesia

Citation: Dharmajaya R, Sari DK, Ganie RA. A Comparison of the Quality of Sleep between Pre and Post-Surgery Cervical Herniated Nucleus Pulposus Patients Utilizing the Anterior Discectomy Method. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):948-954. https://doi.org/10.3889/oamjms.2017.209

Keywords: Quality of sleep; Cervical herniated nucleus pulposus; Anterior discectomy operation.

***Correspondence:** Ridha Dharmajaya. Universitas Sumatera Utara, Indonesia. E-mail: ridha@usu.ac.id

Received: 24-Aug-2017; **Revised:** 24-Sep-2017; **Accepted:** 29-Sep-2017; **Online first:** 10-Dec-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Herniated Nucleus Pulposus (HNP) is the prolapse of the intervertebral disk through a tear in the annulus fibrosus. This causes nerve root compression with clinical pain manifestation and affects the quality of sleep.

AIM: The aim of this study was find out the comparison in the quality of sleep between before (pre) and after (post) surgery cervical HNP patients.

METHODS: This study was a retrospective cohort study. Ninety patients were asked to complete the Pittsburgh Sleep Quality Index (PSQI) questionnaire. All data which has been computed were analysed with the McNemar test.

RESULT: The outcome reveals that from 90 patient`s cervical HNP, 81 (90%) were 40 years old age group and 66 (73.3%) of them were women. The result showed that 66 (73.3%) patients have a bad sleep quality before surgery. Surgery has increased the quality of sleep after surgery 66 (73.3%) patients had good sleep quality. There was a significant difference in the quality of sleep pre and post operation (p = 0.001).

CONCLUSION: There was a significant difference in the quality of sleep between pre and post operation cervical HNP patient utilising anterior discectomy methods.

Introduction

Herniated Nucleus Pulposus (HNP) is the prolapse of the intervertebral disk through a tear in the annulus fibrosus which causes nerve root compression [1-3]. Prevalence of HNP is 1-2% of world population, and generally, HNP can occur at all vertebrae level from cervical until lumbar spine, eighty percent of HNP is lumbar HNP, and 20% is cervical HNP [4-6]. Sixty percent of age group with cervical HNP are 90-40 years old, and its prevalence is more often in males than females [7].

The most common cause of a cervical herniated nucleus pulposus is gradual degeneration of the disc, attenuation (weakening) of the posterior annulus fibrosis, and subsequent protrusion of the nucleus pulposus into the spinal canal causing compression of the nerve root [8, 9]. The disc injury occurs suddenly because of an accident or trauma, another cause of cervical herniated nucleus pulposus is low vitamin D level [10, 11]. Higher prevalence of vitamin D level found in Indonesian women which most of them also had polymorphism of vitamin D receptor gene, TaqI and BsmI [12, 13].

Vitamin D deficiency associated to polymorphism of vitamin D receptor gene that the t allele of vitamin D receptor TaqI is associated with a high risk of degenerative disc disease and disc bulge developing, especially in individuals younger than 40 years [14, 15]. Most of the women had polymorphism of vitamin D receptor gene, TaqI and BsmI, related to vitamin D deficiency in urban and rural areas [15, 16]. Besides vitamin D deficiency, obesity also the cause of recurrent hernia nucleus pulposus. The previous study reported that obesity was a strong and

independent predictor of recurrent HNP after lumbar microdiscectomy. Surgeons should incorporate weight loss counselling into their preoperative discussions with patients [17].

Cervical HNP clinical manifestation is a radiated pain from the neck to the upper extremities (arm). Pain can be worse in several positions such as neck extension, ipsilateral rotation and lateral flexion. The feel of pain in the neck can make the quality of sleep worsen [17, 18]. Cervical HNP treated with conservative and surgery therapy, and in the early stage, conservative therapy can be done to decrease symptoms, but in the concluding stage, surgery therapy has to be carried out [19]. Surgery will be performed if the conservative therapy fails or if the patients have myelopathy signs and symptoms. Myelopathy signs and symptoms are paresthesia/paresis and urinated-defecated-sexual disorders [17,20].

The gold standard for diagnosed Cervical HNP is with Magnetic Resonance Imaging (MRI), especially T2 sequence [21]. It showed the position of the spinal cord, whether there is a compression of the surrounding tissue, bone and cartilage to the spinal cord or not [21, 22]. The gold standard in the majority of the studies confirming the presence of a herniated disc was cross-sectional imaging and or surgery. The gold standard in the diagnosis is surgery; however, when assessing the validity of subjective complaints or physical examination findings, use of cross-sectional imaging as a gold standard may be considered an acceptable substitute. The validity of surgery as a gold standard can be questioned, however, as findings at surgery can be subjective [21-23].

The treatment for a cervical herniated disc almost always begins with a trial of conservative treatment, which is frequently effective [24]. Surgical intervention is considered when a patient with a cervical herniated nucleus pulposus continues to have pain, weakness, and or numbness and has failed conservative modalities [24, 25]. The goal of surgery is to remove the compression from the spinal cord and or spinal nerves to improve a patient's pain and level of function [22]. The preferred surgical treatment is an anterior cervical discectomy and fusion.

Every disease with pain symptoms, mental disorders (anxiety or depression) can also cause sleep disorders [26]. During recovery, the patient needs some more sleep and energy but can be disturbed with uncomfortable symptoms like pain [26, 27]. The difficulty or lack of sleep during a patient`s post-operation will inhibit the healing process [27]. The time of sleep is a time for cell regeneration, if the post-operative patients cannot sleep, the cell can not regenerate completely [26-29]. There were many patients with cervical HNP rejected to do the operation because of misunderstanding about this problem [30].

Based on this problem, the researchers were interested in comparing the quality of sleep during the pre-operation and post-operation cervical HNP patient utilising the anterior discectomy methods. The aimed of this study also for better understand about risk factor of HNP and pain for better management.

Methods

The study method is an observational study with retrospective analyses at the Adenin Adenan Hospital, Medan City, North Sumatera Province, Indonesia. The inclusion criteria are post-operative, where the cervical HNP patient utilising anterior discectomy methods and the data were collected in August 2014 until December 2016. The exclusion criteria were, the patients with another disease which cause disability and organ failure, and also unreachable patients or not willing patients to be subject. Data were directly from the subjects who were interviewed with the quality of sleep questionnaire, Pittsburgh Sleep Quality Index (PSQI) [31]. The result of this study was based on the questionnaire scores, if the score is greater than five the result is bad, but if the score is lower than or equal to 5 the result is good.

Surgical technique

The author used anterior discectomy before total disc replacement (TDR) placement in patients with a herniated disc. The herniated disc material was removed anteriorly in conjunction with preparing the disc space for TDR placement in this study. Using this technique of performing an anterior-based complete discectomy, it may remove the herniated disc fragment and successfully decompress the canal contents without having to reenter the canal through a previously operated area while minimising the revision decompression risks, eliminating the offending herniation. A similar technique has been described for anterior lumbar interbody fusion. A combination of curette and punch Kerrison rongeur can be used to take down the posterior longitudinal ligament and allow direct visualisation and nerve hook access into the epidural space [32-34].

Sample Measurement and Examination

The Pittsburgh Sleep Quality Index (PSQI) was the instrument used to evaluate the quality of sleep in adults. The PSQI questionnaire consists of nine self-questions and five questions of sleep partner. The PSQI scoring 7 component quality of sleep, the range of scores was 0 (not difficult) until 3 (very difficult). Summarize total component score (range 0-21), if total score outcomes < 5, the result is

good and if total score outcomes > 5, the quality of sleep result is bad [31].

The data were collected by the researchers through direct interview utilising the PSQI questionnaire. Every patient was asked ten questions each, and their answers were used to measure the quality of sleep during pre-operative and post-operative utilising the anterior discectomy method. Each patient obtained two qualities of sleep data, pre- and post-operative data.

Body mass index (BMI) included height (to the nearest 0.5 cm), weight (to the nearest 0.1 kg) and calculated as kg/m^2. Categorized BMI was based on Asia Pacific, < 18.5 classified as underweight, 18.5-22.9 classified as normoweight, 23-24.9 classified as overweight/at risk, 25-29.9 classified as obese I, and >30 classified as obese II [35].

Statistic Analytic

Power calculation and estimation of sample size were based on the primary outcome measure of the trial. Data are presented as mean and one standard deviation (SD) or as number and frequency (%). In the tabulation of data, McNemar statistic test was used to determine the difference between the quality of sleep during pre-operative and post-operative cervical HNP utilising anterior discectomy methods. The variable was nominal and ordinal variable with the category scale. Categorical data will be presented by the percentage. Level of significance was set at 5%, if the p-value < 0.05 then it means that any meaningful proportion has to differentiate between two group data. This study used SPSS (version 11.5; SPSS Inc, Chicago, IL) for data analysis.

Research Appropriateness

This study has passed the tests from Health Research Ethical Committee Medical Faculty of North Sumatera University, Medan City, North Sumatera with the number 98/TGL/KEPK FK USU-RSUP HAM/2016. Before participation, the subjects had to fill the approval sheet after they have gotten the explanation about the principal study, such as the aim, the examination, the advantages and the disadvantages of the study.

Results

Descriptive data are presented in Table 1, one hundred and two patients with cervical HNP has performed surgery utilising the anterior discectomy methods from 2014 to 2016, twelve patients could not fulfil the inclusion criteria in this study, leaving ninety patients who formed the basis for this study as subjects. Mean of outpatient clinics each year were 30-40 patients, for three years data had been collected.

Table 1: Clinical data and outcome measures (n=90)

Variables	f (frequencies)	Percentage (%)
Gender		
Male	24	26.7
Female	66	73.3
Age (years)	53.97±11.04	
Age group		
< 40 years old	9	10
> 40 years old	81	90
Body mass index (kg/m^2)	25.8±3.9	
BMI classification		
• Underweight	-	-
• Normal	4	4.4
• Overweight	79	87.8
• Obese I	7	7.8
• Obese II	-	-
Main symptom		
• Axial pain only	36	40
• Axial pain < Radicular pain	39	43.3
• Axial pain = Radicular pain	15	16.7
Preoperative MRI findings		
• Protrusion	39	43.3
• Extrusion	48	53.3
• Stenosis	3	3.4

Data for age are expressed as mean±SD

In Table 1, the result of this study showed that two times higher prevalence of HNP found in female than male in this study. Almost of the study subjects were older than 40 years and higher percentage body mass index which the highest was overweight.

Table 2: Pre-post operative quality of sleep outcome

Parameters	Frequencies (f)	Percentage (%)
Quality of sleep (pre-operative)		
• Good	24	26.7
• Bad	66	73.3
Quality of sleep (post-operative)		
• Good	75	85
• Bad	15	15

Table 2 and Table 3 summarise the pre-operative quality of sleep outcome, 66 (73.3%) subjects have a bad quality of sleep, and 24 (26.7%) subjects have a good quality of sleep post-operative. Post-operative showed that higher percentage of good quality of sleep than bad quality of sleep with significant value (p < 0.005)

Table 3: Quality of sleep cross-tabulation with McNemar test

		Post Operative Quality of Sleep		TOTAL
		Good (> 50)	Bad (< 50)	
Pre Operative Quality of Sleep	Good (> 50)	21	3	24
	Bad (<50)	54	12	66
TOTAL		75	15	90
McNemar Test			0,001	

All these subjects showed compression, using Magnetic Resonance Imaging (MRI) T2 sequence of HNP patients showed compression of the spinal canal, for cervical it would compress the spinal cord.

Nucleus pulposus of the disc would come out through the wall of the disc, filled the spinal canal and compress the spinal cord (Fig. 1).

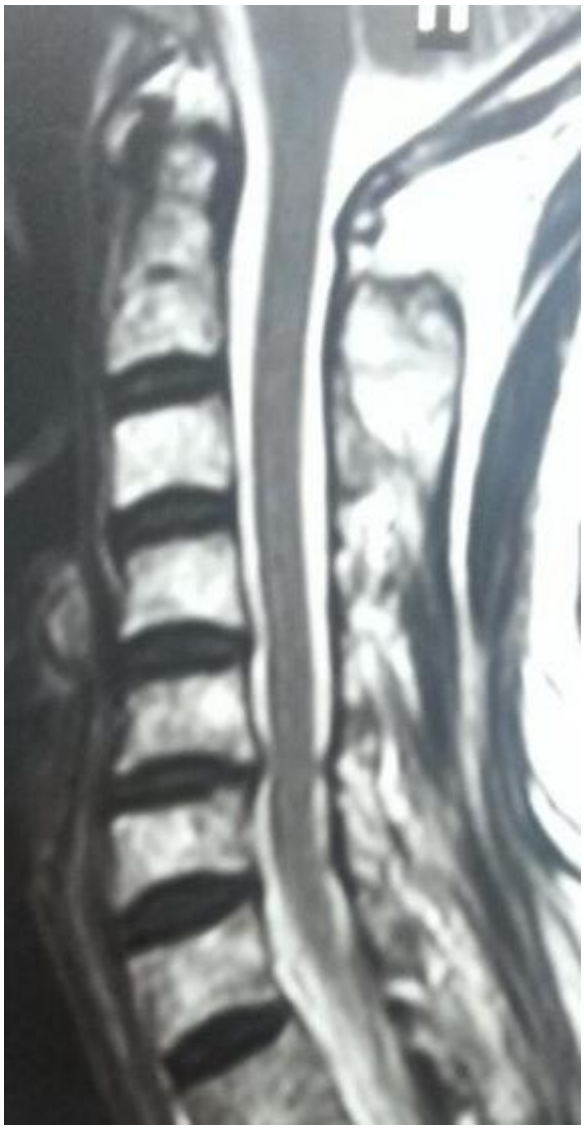


Figure 1: Magnetic Resonance Imaging T2 sequence of cervical spine, showed compression of spinal cord at C 5-6 level

Discussion

The study demonstrated that there is a different quality of sleep between before and after cervical HNP surgery utilising anterior discectomy methods, quality of sleep is important to be given careful consideration, as it affects the postoperative recovery substantially. Post-operative anterior discectomy methods offered the significantly better quality of sleep than pre-operative, which led to a significantly shorter stay in the hospital [36]. However, this effect was almost exclusively attributed to a significantly lower consumption of opioids postoperatively and sleeping pills [37-39]. As shown in this study, quality of sleep post-operative is a feature that influences the recovery.

Anterior cervical discectomy and fusion is a simple surgery procedure with low risk and short duration [40,41]. This procedure which was used in this study, good effect of intraoperative vertebral reduction and well-recovered function after the operation. After skin incision in a linear fashion at large line, platysma was open with sharp and blunt dissection. Retracted the neck muscle such as sternocleidomastoid, omohyoid, sternohyoid muscle and retracted trachea and thyroid to lateral and find the cervical spine bone. Recognised the level of the spine with needle marker and C-arm then starts discectomy (Fig. 2). Finished the discectomy and made fusion, using an autologous bone graft or titanium cage or polyetheretherketone (PEEK) [36]. This study used autologous bone graft taken from the hip of the patient.

In this study, women had a higher prevalence of HNP than men, probably due to vitamin D deficiency that will affect calcium serum in the circulation [12, 13]. Low vitamin D and calcium serum were the caused of osteoporosis, a metabolic bone disorder characterised by low bone mass and microarchitectural deterioration, with a subsequent increase in bone fragility and susceptibility to fracture [42]. The risk factor of vitamin D deficiency was a lifestyle, such as avoiding sunlight exposure and polymorphism of vitamin D receptor (VDR) gene was the cause of vitamin D deficiency [13, 15].

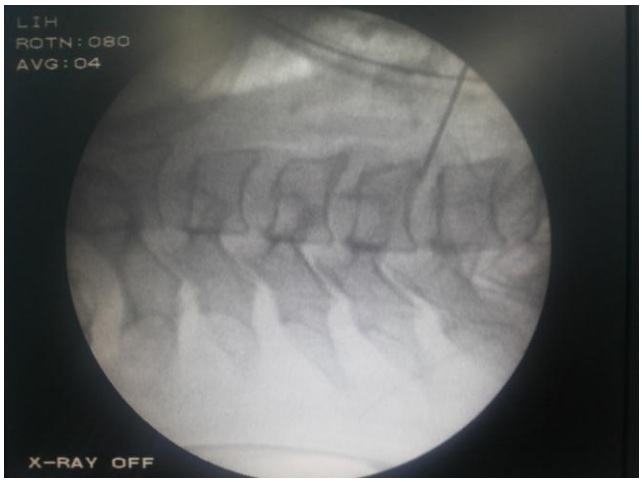


Figure 2: Radiological X-Ray view by C-Arm with needle marker

Vitamin D deficiency-insufficiency was found in healthy women with TaqI and BsmI single nucleotide polymorphisms in the VDR gene [15, 16]. Vitamin D deficiency and insufficiency are associated with increased risk of osteoporosis, poorer muscle function, low bone mass, and microarchitectural deterioration, with a subsequent increase in bone fragility and susceptibility to fracture [42]. The previous study showed that the TaqI polymorphism of vitamin D receptor gene is associated with the

development of degenerative disc disease in the lumbar spine with an odds ratio (OR) of 2.61 [14].

Aging had to correlate to HNP occurrence, in this study shows the result that the age group of the HNP patients was more than forty years older than the youngest or equal. It was appropriated with the other study results that more often HNP patients were between the age groups of 40 and 50 years old [43, 44]. This is because, the ageing process of the intervertebral disc, starts with the disc inflexibility, the lack of elasticity of the nucleus pulposus and disc degeneration [45]. Also, because of the repeated trauma on the intervertebral disc and tear of the annulus fibrosus [45-47].

The relative body weight gains the first day after surgery was an independent risk factor for bad quality of sleep in the present study [48]. But, in this study we did not asses the body weight gain, only body weight pre-operative. The relative body weight gain may be an effect of the increased release of stress hormones and inflammatory processes and thus may represent an increased stress response [49].

Degenerative disk disease can be particularly seen in overweight patients with greater BMI [49, 50]. Studies have demonstrated a probable correlation between obesity and lumbar spine related disorders, most likely secondary to increasing biomechanical stress in the lower thoracic and lumbar spine. Open spinal surgery in obese patients is associated with longer operative times, larger blood loss, and increased perioperative morbidity [51, 52].

Most studies have shown that postoperative pain perception can be affected by sleep disturbance producing a hyperalgesic state [37-39]. However, from this study particularly in the pre-operative period where the pain was the predominant cause of sleep disturbance, it is a pain which leads to sleep disturbance and that while a hyperalgesic state may be exacerbated by poor sleep, it is an initial pain that starts this process and consuming sleeping pills.

Previous studies showed that there were several factors contribute to disturbed sleep postoperatively [37-39, 53]. Use of opioids has been shown to change sleep architecture substantially in healthy subjects [38, 53]. The injury caused by surgery provokes a complex stress response involving the release of stress hormones, humoral mediators of the endocrine and metabolic system, and activation of the immune system [38, 39, 53].

Based on the results from this study, a conclusion can be drawn that there was a significantly different quality of sleep between the pre- and post-operation using anterior discectomy methods; that was due to the symptom of cervical HNP before surgery. The quality of sleep before surgery is bad and after surgery significantly changed for the better. Pain is the most common symptom of cervical HNP and one of the prevalence causes of sleep disorders in adults. Surgery is the definitive therapy for cervical HNP because it will decrease the symptoms and provide a better quality of sleep than before. This was appropriated with this study result that those patients who performed surgery will have better feelings of pain, functional pleasure and healing process than non-surgery therapy.

There are some methodological concerns in this study; the study was not powered to detect differences in other secondary outcome measures such as time operation, time of anaesthesia, and using sleeping pills. Also, this was not a randomised controlled trial. Therefore this study is seen as exploratory and hypothesis generating.

Recommendations were the doctors should do a follow-up with patients before and after surgery and patient with bad quality of sleep needs support from family and people around because cervical HNP can affect many aspects of life. There is a need for health workers and the society to be educated about cervical HNP to enhance early diagnosis.

References

1. Chung SK, Kim YE, Wang KC. Biomechanical effect of constraint in lumbar total disc replacement: a study with finite element analysis. Spine. 2009; 34:1281–6. https://doi.org/10.1097/BRS.0b013e3181a4ec2d PMid:19455003

2. Madhok R, Kanter A. Extreme-lateral, minimally invasive, a transpsoas approach for the treatment of far-lateral lumbar disc herniation. J Neurosurg Spine. 2010; 12: 347–50.

3. Borendal Wodlin N, Nilsson L, Kjølhede P. Health-related quality of life and postoperative recovery in fast track hysterectomy. Acta Obstet Gynecol Scand. 2011; 90:362–8. https://doi.org/10.1111/j.1600-0412.2010.01058.x PMid:21306322

4. DeLucca JF, Cortes DH, Jacobs NT, Vresilovic EJ, Duncan RD, Elliot DM. Human cartilage endplate permeability varies with degeneration and intervertebral disc site. Journal of Biomechanics. 2016; 49: 550–7. https://doi.org/10.1016/j.jbiomech.2016.01.007 PMid:26874969 PMCid:PMC4779374

5. Krutko AV, Baykov ES, Sadovoy MA. Reoperation after microdiscectomy of lumbar herniation: Case report. Int J of Surg Case Rep. 2016; 24: 119–23. https://doi.org/10.1016/j.ijscr.2016.04.043 PMid:27236580 PMCid:PMC4887591

6. Hammer C, Heller J, Kepler C. Epidemiology and pathophysiology of cervical disc herniation. Semin Spine Surg. 2016; 28(2): 64–7.

7. Jordan J, Konstantiniou K, O'Dowd J. Herniated lumbar disc. BMJ Clin Evid. 2009; 1118. PMid:19445754

8. Bowles RD, Setton LA. Biomaterials for intervertebral disc regeneration and repair. Biomaterial. 2017; 129: 154–67.

9. Hu B, Wang J, Wu X, Chen Y, Yuan W, Chen H. Interleukin-17 upregulates vascular endothelial growth factor by activating the JAK/STAT pathway in nucleus pulposus cells. Join Bone Spine. 2017; 84: 327–34.

10. Mayer JE, Iatridis JC, Chan D, Qureshi SA, Gottesman O, Hecht AC. Genetic polymorphisms associated with intervertebral disc degeneration. Spine J. 2013; 13: 299–317.

11. Boden SD. Spinescope. Semin Spine Surg. 2013; 25: 295–300.

12. Sari DK, Damanik HA, Lipoeto NI, Lubis Z. Occurrence of Vitamin D Deficiency among Woman in North Sumatera, Indonesia. Malaysian J Nutr. 2014;20:63–70.

13. Sari DK, Tala ZZ, Lestari S, Hutagalung S, Ganie RA. Vitamin D Supplementation in Women with Vitamin D Receptor Gene Polymorphisms: A Randomized Controlled Trial. Asian J Clin Nutr. 2017; 9(2):89–96.

14. Kenneth C, Danny C, Jaro K, Yiquin C, Jeffrey JT, Shea-Ping Y, et al. Association of the Taq I Allele in Vitamin D Receptor With Degenerative Disc Disease and Disc Bulge in a Chinese Population. Spine. 2006; 31 (10): 1143–48.

15. Sari DK, Tala ZZ, Lestari S, Hutagalung S, Ganie RA. Vitamin D receptor gene polymorphism among Indonesian women in North Sumatera. Asian J Clin Nutr. 2017; 9(1):44–50.

16. Sari DK, Tala ZZ, Lestari S, Hutagalung S, Ganie RA. Lifestyle differences in rural and urban areas affected the level of vitamin D in women with single nucleotide polymorphism in North Sumatera. Asian J Clin Nutr. 2017; 9(2):57–63.

17. Yeung JT, Johnson JI, Karim AS. Cervical disc herniation presenting with neck pain and contralateral symptoms. J of Med Case Reports. 2012; 6: 166. https://doi.org/10.1186/1752-1947-6-166 PMid:22741922 PMCid:PMC3411405

18. Mart M, Rozenberg S, Duplan B, et al. Quality of sleep in patients with chronic low back pain. Eur Spine J. 2008;839–44.

19. Pirris S, Dhall S, Mummaneni P, Dhall S, Kanter AS. Minimally invasive approach to extraforaminal disc herniations at the lumbosacral junction using an operating microscope: case series and review of the literature. Neurosurg Focus. 2008; 25:E10. https://doi.org/10.3171/FOC/2008/25/8/E10 PMid:18673039

20. Manchikanti L, Singh V, Calodney AK, Helm S 2nd, Deer TR, Benyamin RM et al. Percutaneous lumbar mechanical disc decompression utilizing Dekompressor®: an update of current evidence. Pain Physician. 2013; 16 (2Suppl): SE1–24.

21. Galluci M, Limbuci M, Paonessa A, Splendiani A. Degenerative disease of the spine. Neuroima Neuroimag Clin N Am. 2007; 17: 87–103. https://doi.org/10.1016/j.nic.2007.01.002 PMid:17493541

22. Kelekis AD, Filippiadis DK, Martin JB, Brountzos E. Standards of practice: quality assurance guidelines for percutaneous treatments of intervertebral discs. Cardiovasc Intervent Radiol. 2010; 33(5): 909–13. https://doi.org/10.1007/s00270-010-9952-5 PMid:20676639

23. Kelekis A, Filippiadis D. Percutaneous therapy versus surgery in chronic back pain: how important is imaging in decision-making? Imaging Med. 2013; 5(2): 187–96. https://doi.org/10.2217/iim.13.15

24. Nguyen C, Sanchez K, Roren A, Palazzo C, Falcou L, Drape J, et al. Anatomical specificities of the degenerated cervical spine: a narrative review of clinical implications, with special focus on targeted spinal injections. Annals of Physic and Rehab Med. 2016; 59: 276–81. https://doi.org/10.1016/j.rehab.2015.12.006 PMid:26904960

25. Chi JH, Tay B, Stahl D, Lee R. Complex Deformities of the Cervical Spine. Neurosurg Clin N Am. 2007; 18: 295–304. https://doi.org/10.1016/j.nec.2007.01.003 PMid:17556131

26. Freedman MK, Overton EA, Saulino MF, Horlding MY, Kornbluth ID. Interventions in chronic pain management.Arch Phys Med Rehabil. 2008; 89 Suppl 1: S41–6.

27. Roy-Byrnne PP, Davidson KW, Kessler RC, Asmundson GJG, Goodwin RD, Kubzansky L. Anxiety disorders and comorbid medical illness. Gen Hosp Psy. 2008; 30: 208–25. https://doi.org/10.1016/j.genhosppsych.2007.12.006 PMid:18433653

28. Zarrabian MM, Johnson M, Kriellaars D. Relationship Between Sleep, Pain, and Disability in Patients With Spinal Pathology. Arch of Physic Med and Rehab. 2014; 95:1504–9.

29. Durand G, Girodon J, Debiais F. Medical management of failed back surgery syndrome in Europe: Evaluation modalities and treatment proposals. Neurochirurgie. 2015; 61: S57–S65. https://doi.org/10.1016/j.neuchi.2015.01.001 PMid:25676909

30. Asmundson GJ, Katz J. Understanding the co-occurrence of anxiety disorders and chronic pain: state-of-the-art. Depress Anxiety. 2009; 26 (10): 888–901.

31. Mollayeva T, Thurairajah P, Burton K, Mollayeva S, Shapiro CM, Colantonio A. The Pittsburgh sleep quality index as a screening tool for sleep dysfunction in clinical and non-clinical samples: A systematic review and meta-analysis. Sleep Med Rev. 2016; 25: 52–73.

32. Salame K, Lidar Z. Minimally invasive approach to far lateral lumbar disc herniation: technique and clinical results. Acta Neurochir (Wien. 2010; 152: 663−8.

33. Cole JS 4th, Jackson TR. Minimally invasive lumbar discectomy in obese patients. Neurosurgery. 2007; 61:539–44. https://doi.org/10.1227/01.NEU.0000290900.23190.C9 PMid:17881966

34. Kim JM, Lee SH, Ahn Y, et al. Recurrence after successful percutaneous endoscopic lumbar discectomy. Minim Invasive Neurosurg. 2007; 50: 82–5. https://doi.org/10.1055/s-2007-982504 PMid:17674293

35. Gill T. Epidemiology and health impact of obesity: an Asia Pacific perspective. Asia Pac J Clin Nutr. 2006; 15 (Suppl): 3–14.

36. Lemcke J, Al-Zain F, Meier U, Suess O. Polyetheretherketone (PEEK) Spacers for Anterior Cervical Fusion: A Retrospective Comparative Effectiveness Clinical Trial. Open Orthop J. 2011; 5:348–53.

37. Dimsdale JE, Norman D, DeJardin D, Wallace MS. The effect of opioids on sleep architecture. J Clin Sleep Med. 2007; 3:33–6. PMid:17557450

38. Kjølhede P, Langström P, Nillson P, Wodlin NB, Nillson L. The Impact of Quality of Sleep on Recovery from Fast-Track Abdominal Hysterectomy. J Clin Sleep Med. 2012; 8(4):395–402.

39. Schuh-Hofer S, Wodarski R, Pfau DB, Caspani O, Magerl W, Kennedy JD. One night of total sleep deprivation promotes a state of generalized hyperalgesia: a surrogate pain model to study the relationship of insomnia and pain. Pain. 2013;154(9):1613–21. https://doi.org/10.1016/j.pain.2013.04.046 PMid:23707287

40. Glenn JS, Yaker J, Guyer RD, Ohnmeiss DD. Anterior discectomy and total disc replacement for three patients with multiple recurrent lumbar disc herniations. The Spine J. 2011;11:e1–e6. https://doi.org/10.1016/j.spinee.2011.07.030 PMid:21907631

41. Marcus JD, James AR, Härtl R. Minimally Invasive Surgical Treatment Options for Lumbar Disc Herniations and Stenosis. Semin Spine Surg. 2011; 23:20–6.

42. Sari DK, Tala ZZ, Lestari S, Hutagalung S, Ganie RA. Body mass index but not 25(OH)D serum is associated with bone mineral density among Indonesian women in North Sumatera: a cross sectional study. Asian J Clin Nutr. 2017; 9(1):37–43.

43. Amoretti N, Huwart L, Marcy PY, Foti P, Hauger O, Boileau P. CT- and fluoroscopy-guided percutaneous discectomy for lumbar radiculopathy related to disc herniation: a comparative prospective study comparing lateral to medial herniated discs. Skeletal Radiol. 2013; 42: 1:49–53. https://doi.org/10.1007/s00256-012-1422-5 PMid:22644540

44. Yar T. Spinal shrinkage as a measure of spinal loading in male Saudi University students and its relationship with body mass index. Saudi Med J. 2008; 29: 1453–7. PMid:18946572

45. Kaito T, Hosono N, Mukai Y, et al. Induction of early degeneration of the adjacent segment after posterior lumbar interbody fusion by excessive distraction of lumbar disc space. J Neurosurg Spine. 2010; 12:671–9. https://doi.org/10.3171/2009.12.SPINE08823 PMid:20515354

46. Botolin S, Puttlitz C, Baldini T, et al. Facet joint biomechanics at the treated and adjacent levels after total disc replacement. Spine. 2011; 36: E27–32. https://doi.org/10.1097/BRS.0b013e3181d2d071 PMid:20975623

47. Leahy M, Zigler JE, Ohnmeiss DD, et al. Comparison of results of total disc replacement in postdiscectomy patients versus patients with no previous lumbar surgery. Spine. 2008; 33:1690–3. https://doi.org/10.1097/BRS.0b013e31817bd2f0 PMid:18580740

48. Meredith DS, Huang RC, Nguyen J, Lyman S. Obesity increases the risk of recurrent herniated nucleus pulposus after lumbar microdiscectomy. The Spine J. 2010; 10:575–80.

49. Gilleard W, Smith T. Effect of obesity on posture and hip joint moments during a standing task, and trunk forward flexion motion. Int J Obes. 2007; 31:267–71. https://doi.org/10.1038/sj.ijo.0803430 PMid:16801923

50. Olsen MA, Nepple JJ, Riew KD, Lenke LG, Bridwell KH, Mayfield J, Fraser VJ, et al. Risk factors for surgical site infection following orthopaedic spinal operations. J Bone Joint Surg Am. 2008; 90:62–9. https://doi.org/10.2106/JBJS.F.01515 PMid:18171958

51. Park P, Upadhyaya C, Garton HJ, Foley KT. The impact of minimally invasive spine surgery on perioperative complications in overweight or obese patients. Neurosurgery. 2008; 62:693–9. https://doi.org/10.1227/01.neu.0000317318.33365.f1 PMid:18425016

52. Cole JS 4th, Jackson TR. Minimally invasive lumbar discectomy in obese patients. Neurosurgery. 2007; 61:539–44. https://doi.org/10.1227/01.NEU.0000290900.23190.C9 PMid:17881966

53. Chouchou F, Khoury S, Chauny JM, Denis R, Lavigne GJ. Postoperative sleep disruptions: a potential catalyst of acute pain? Sleep Med Rev. 2014; 18(3):273–82. https://doi.org/10.1016/j.smrv.2013.07.002 PMid:24074687

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):955-962.
https://doi.org/10.3889/oamjms.2017.193
eISSN: 1857-9655
Clinical Science

Comparative Study on Adding Pioglitazone or Sitagliptin to Patients with Type 2 Diabetes Mellitus Insufficiently Controlled With Metformin

Maryam Jameshorani[1], Saba Sayari[1], Narjes Kiahashemi[1*], Nima Motamed[2]

[1]Zanjan Metabolic Disease Research Center (ZMDR), Vali-e-asr Hospital, Zanjan University of Medical Science (ZUMS), Zanjan, Iran; [2]Department of Epidemiology, Zanjan University of Medical Science (ZUMS), Zanjan, Iran

Citation: Jameshorani M, Sayari S, Kiahashemi N, Motamed N. Comparative Study on Adding Pioglitazone or Sitagliptin to Patients with Type 2 Diabetes Mellitus Insufficiently Controlled With Metformin. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):955-962. https://doi.org/10.3889/oamjms.2017.193

Keywords: Metformin; Pioglitazone; Randomized trial; Sitagliptin; Type 2 diabetes

***Correspondence:** Narjes Kiahashemi, Vali-e-Asr Hospital, Zanjan University of Medical Science, Zanjan, Iran. Tel: +98-2433770801, Fax: +98-2433770751, E-mail: k.kiahashemi.1234@gmail.com

Received: 08-Oct-2017; **Revised:** 10-Oct-2017; **Accepted:** 11-Oct-2017; **Online first:** 12-Dec-2017

Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Diabetes mellitus is a progressive disorder that often requires combination therapy.

AIM: This study aimed to compare and study of add-on sitagliptin versus pioglitazone in patients with type 2 diabetes inadequately controlled with metformin.

METHODS: This 12-week, randomised, open-label and single centre study compared sitagliptin (100 mg daily, n = 80) and pioglitazone (30 mg daily, n = 80) in type 2 diabetic patients whose disease was not adequately controlled with metformin.

RESULTS: The mean change in HbA1c from baseline was -1.001 ± 0.83 with sitagliptin and -0.75 ± 1.20 with pioglitazone, and there were no significant difference between groups (P = 0.132). The mean change in fasting blood sugar (FBS) was -18.48 ± 33.32 mg/dl with sitagliptin and -20.53 ± 53.97 mg/dl with pioglitazone, and there were no significant difference between groups (P = 0.773). Sitagliptin caused 1.08 ± 2.39 kg decrease in weight, whereas pioglitazone caused 0.27 ± 2.42 kg increase in weight, with a between-group difference of 0.81 kg (P < 0.001). On the other hand, in sitagliptin group, there was greater improvement in lipid profile than pioglitazone group.

CONCLUSION: Sitagliptin and Pioglitazone demonstrated similar improvements in glycemic control in type 2 diabetes mellitus patients whose diabetes had been inadequately controlled with metformin. Nevertheless, sitagliptin was more effective than pioglitazone regarding lipid and body weight change.

Introduction

Due to the progressive decline in the function of pancreatic beta cells and chronic insulin resistance in patients with type 2 diabetes mellitus (T2DM), hyperglycemia increases over time in this group of patients [1, 2]. Several studies have demonstrated that hyperglycemia is one of the major risk factors in the development of microvascular complications in T2DM patients [3, 4]. On the other hand, clinical trials have shown that reduction in HbA1c can decrease the development of T2DM complications [5, 6]. For instance, every one percent HbA1c decrease is associated with Thirty- five percent decrease in risk of microvascular complications [7]. American Diabetes Association (ADA) recommended lowering the HbA1c to less than 7% in T2DM patients [8, 9]. Due to the complex nature and multiple metabolic defects of this disease, treatment with a single oral antihyperglycemic agent is not sufficient for reaching the desired goal, hence combination drug therapy is usually required to manage patients with T2DM [6, 8, 10, 11]. It should be noted that combination therapy with oral drugs requires the use of anti-hyperglycemic drugs with different complementary physiologic mechanisms to improve glycemic control [12].

Moreover, Beta-cell failure occurs long before T2DM is diagnosed and by this time, diabetic subjects have lost over 80% of their beta cell function. So, early treatment with anti-hyperglycemic drugs in diabetic patients can have positive effects on the preservation of residual pancreatic beta cells function [7, 8].

America Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) recommend metformin as a first line drug in the treatment of diabetic patients, because metformin is inexpensive, does not cause weight gain, has proven safety records and probably beneficial effects on the cardiovascular system. Sulphonylureas, thiazolidinediones and dipeptidyl peptidase 4 (DPP-4) inhibitors have also been recommended as an alternative treatment or in combination therapy with metformin in the ADA/EASD consensus [4, 13].

Metformin is a drug that can reduce Hb1Ac by increasing liver and peripheral tissue sensitivity to glucose, prevent hepatic gluconeogenesis and glycogenolysis. This reduction in Hb1Ac is about 1.2-3%, but metformin does not prevent beta cell failure, and after an initial decrease, HbA1c rises progressively [7, 14, 15].

Pioglitazone is one of the thiazolidinediones. Thiazolidinediones are Peroxisome Proliferator-Activated Receptor γ (PPAR- γ) agonists and are appropriate for use as monotherapy and in combination with metformin and/or a sulphonylurea in patients with T2DM [16,17]. Thiazolidinediones can decrease insulin resistance by increasing the sensitivity of muscle, liver, and adipose tissue to insulin. These drugs delay the progression of T2DM and can improve beta cell function and create a sustainable reduction in Hb1Ac [7, 18, 19].

Sitagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor that can affect the path of incretin hormones including Glucagon-Like Peptide 1 (GLP-1) and Glucose-dependent Insulin releasing Polypeptide (GIP). These hormones are secreted by the intestine endocrine cells in response to a meal. GLP-1 and GIP stimulate insulin secretion (in a glucose-dependent manner) and delay gastric emptying. Also, GLP-1 can affect pancreatic alpha cells and inhibit glucagon secretion. Within some minutes after the release of these hormones, GIP and GLP-1 undergo rapid metabolism to inactive metabolites by the enzyme DPP-4, hence sitagliptin by inhibiting this enzyme causes an increase in the duration of validity of hormones in the blood [2, 8, 10, 17].

As earlier mentioned, Metformin, Pioglitazone and sitagliptin each possess different but complementary mechanisms of action. Therefore, we can use these drugs together as a combination therapy [2, 12, 20].

The aim of the present study was to compare the efficacy of dual anti-hyperglycemic combination therapy in T2DM patients, to find the most efficient and effective method for treating these patients.

Materials and Methods

Study design

This open-label, single centre and randomised control trial (code: IRCT2015061619554N4) were done between January 2016 and January 2017 in vali-e-asr hospital under the approval of Zanjan Metabolic Disease Research Center (ZMDRC), Zanjan University of Medical Sciences.

This study was approved by the ethics committee of Zanjan University of Medical Sciences.

Patients

Eligible patients were men and women, 30-60 years of age with T2DM and inadequate glycemic control (HbA1c ≥8 and ≤9.5) while being actively treated with metformin. Their body mass index (BMI) was between 25-35.

Patients with an history of ketoacidosis, unstable or rapidly progressive diabetic retinopathy, nephropathy, neuropathy, impaired hepatic function (defined as plasma aminotransferase level three times higher than the upper limit of normal for age and sex), impaired renal function (defined as serum creatinine level higher than the upper limit of normal for age and sex), impaired digestive function (vomiting, diarrhea, dyspepsia), a serious cardiovascular disease with ejection fraction less than 35%, pregnant women, breastfeeding women and women planning for pregnancy were excluded. All patients provided written informed consent to participate in the study.

Treatment

One hundred and sixty (160) T2DM patients were randomly assigned in a 1:1 ratio to one of the following treatment groups by permuted block method using a central computer-based randomisation (Figure 1).

The first group: those treated with sitagliptin 100 mg/day in addition to their usual doses of metformin for 12 weeks.

The second group: those treated with pioglitazone 30 mg/day in addition to their usual doses of metformin for 12 weeks.

All the patients were trained on the diabetic regimen by the nutritionist. All the individuals were also advised to increase their physical activity. For example, 3 to 5 times per week and each time for 20 to 30 min walking briskly or cycling. Possible side effects of drugs were also explained to patients.

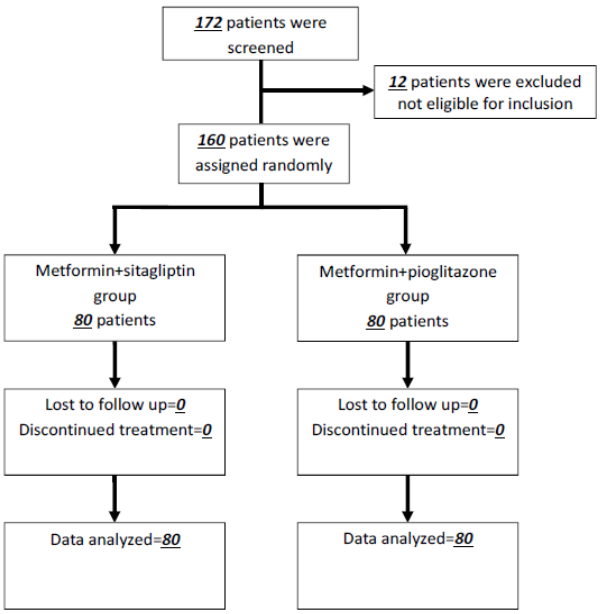


Figure 1: Study flowchart

Assessment

Before the study, all participants underwent an initial assessment that included FBS, Bs2hpp, HbA1c, Cholesterol, Triglyceride, HDL, BUN, Cr, AST and ALT in the laboratory of Vali-E-Asr Hospital. Blood pressure was measured using a manometer, weight by an analogue scale with an accuracy of 0.5 kg that was calibrated every day. Every month, the patients were followed regarding diet, physical activity, proper usage of drugs and side effects of drugs.

After three months FBS, BS2hpp, HbA1c, Chol, TG, HDL, BUN, Cr, AST and ALT were monitored in patients in the Vali-E-Asr Hospital central laboratory. Blood pressure and weight were also re-measured for each patient.

Statistical analysis

For collecting data, a questionnaire was designed in which demographic, anthropometric and the laboratory data were recorded. According to the results of the previous study that was reported, the mean change in HbA1c for sitagliptin and metformin groups were −0.86 (−1.02, −0.69) and −0.58 (−0.76, −0.40) respectively, the sample size was calculated with the power of 80% and two-sided type 1 error rate of 0.05 [13]. Therefore, At least 77 patients in each group were needed.

An intention-to-treat (ITT) analysis was performed for the 160 patients after randomisation, and statistical analysis was done using computer software SPSS version 18 (SPSS Inc, Chicago, IL, USA). The distribution of quantitative data was assessed using the Kolmogorov–Smirnov test. Treatment groups were compared using the Student's *t*-test for continuous variables. Comparison of only qualitative data (sex) was performed using Chi-square test. The differences in the changes of the HbA1c from 0–12 weeks were also determined using a multivariate analysis in which treatment groups were considered as predictor and age, sex, weight and lipid profile as covariates.

In this trial, results were presented as a mean ± standard deviation. A p value of < 0.05 was considered to be statistically significant.

Results

Demographic and baseline characters

One hundred and seventy-two patients whose diabetes had been inadequately controlled with metformin were screened for this trial. Twelve patients were excluded from the study because they did not meet the inclusion criteria. Therefore, 160 patients were randomly assigned to the metformin-sitagliptin or metformin-pioglitazone group. All of these patients completed the study (Figure 1). About 50% of patients were in the metformin plus sitagliptin group (Group 1) and 50% in the metformin plus pioglitazone group (Group 2). The mean age of Group 1 and Group 2 was 50.70 ± 7.85 and 55.12 ± 5.85, respectively. The percentage of female participants in the first group was 57.5% and in the second group was 71.3%.

Baseline variables in both groups are summarised in Table 1.

Table 1: Baseline parameters of the patients in each group

Variable	MEAN ± SD Group 1 (METFORMIN+SITAGLIPTIN)	Group 2 (METFORMIN+PIOGLITAZONE)	P value
Age (year)	50.70 ± 7.85	55.12 ± 5.85	<0.001
FBS (mg/dl)	171.09 ± 46.41	170.26 ± 56.14	0.919
BS2hpp (mg/dl)	276.80 ± 62.08	244.00 ± 77.73	0.004
HbA1c (%)	8.74 ± 0.60	8.63 ± 0.64	0.25
BUN (mg/dl)	10.30 ± 1.97	10.90 ± 3.25	0.16
Creatinin (mg/dl)	0.94 ± 0.16	0.95 ± 0.17	0.79
AST (U/L)	40.98 ± 37.52	27.81 ± 5.9	0.002
ALT (U/L)	40.89 ± 28.19	26.60 ± 6.42	<0.001
TG (mg/dl)	168.38 ± 80.76	176.74 ± 106.43	0.57
Chol (mg/dl)	177.00 ± 46.51	176.49 ± 35.98	0.93
HDL (mg/dl)	43.18 ± 8.38	43.95 ± 8.67	0.56
Weight (kg)	74.29 ± 13.62	73.01 ± 11.49	0.52
SBP (mmHg)	129.00 ± 18.18	121.81 ± 14.39	0.006
DBP (mmHg)	80.00 ± 9.27	69.88 ± 7.37	<0.001

FBS= fasting blood glucose; BS2hpp= two-hour blood glucose; HbA1c = glycosylated hemoglo- bin; BUN= blood urea nitrogen; AST = aspartate aminotransferase; ALT = alanine aminotransferase; TG = triglycerides; Chol= cholesterol; HDL = high-density lipoprotein; SBP= systolic blood pressure; DBP= diastolic blood pressure

HbA1C

Metformin-sitagliptin group (Group 1) and metformin-pioglitazone (Group 2) had similar baseline HbA1c level (Table 1). The mean HbA1c levels in the sitagliptin group ameliorated from 8.74 ± 0.60% to 7.74 ± 0.90% (p < 0.001) (Table 2). In the pioglitazone group, HbA1c improved from 8.63 ± 0.64% to 7.88 ± 1.2% (p < 0.001) (Table 3). The difference between the changes produced in the two groups was not

statistically significant (p = 0.132) (Table 4).

At the end, when the multivariate analysis to eliminate the confounding effects of other variables including age, sex, weight and lipid profile was conducted, no difference was detected between the two treatment groups in decreasing of HbA1c levels before and after treatment (β = 0.059, t = 0.360, P value = 0.719).

Table 2: The mean and standard deviation of variables in the study before and after treatment in the group treated with metformin and Sitagliptin (group 1)

Variable	MEAN ± SD		P value
	Before treatment	After treatment	
FBS (mg/dl)	171.09 ± 46.41	152.60 ± 42.33	<0.001
BS2hpp (mg/dl)	276.80 ± 62.08	228.29 ± 63.18	<0.001
HbA1c (%)	8.74 ± 0.60	7.74 ± 0.90	<0.001
BUN (mg/dl)	10.30 ± 1.97	10.18 ± 1.99	0.55
Creatinin (mg/dl)	0.94 ± 0.169	0.958 ± 0.15	0.48
AST (U/L)	40.98 ± 37.52	37.99 ± 23.55	0.78
ALT (U/L)	40.89 ± 28.19	38.14 ± 16.83	0.63
TG (mg/dl)	168.38 ± 80.76	133.98 ± 55.38	<0.001
Chol (mg/dl)	177.00 ± 46.51	153.31 ± 34.91	<0.001
HDL (mg/dl)	43.18 ± 8.38	46.35 ± 12.19	0.006
Weight (kg)	74.29 ± 13.62	73.2 ± 13.13	<0.001
SBP (mmHg)	129.00 ± 18.18	125.50 ± 13.20	0.025
DBP (mmHg)	80.00 ± 9.27	77.88 ± 7.41	0.012

FBS

The two treatment groups had comparable FBS at baseline (Table 1). The mean FBS levels in the sitagliptin group improved from 171.09 ± 46.41 to 152.60 ± 42.33 (p < 0.001) (Table 2). In the pioglitazone group, FBS improved from 170.26 ± 56.144 to 149.72 ± 46.66 (p < 0.001) (Table 3). The difference between the changes produced in the two groups was not statistically significant (p = 0.773) (Table 4).

BS2hpp

The mean changes in BS2hpp were −48.51 ± 57.13 mg/dl with sitagliptin and −31.51 ± 54.20 mg/dl with pioglitazone. The difference between the changes produced in the two groups was not statistically significant (P = 0.05) (Table 4).

Table 3: The mean and standard deviation of variables in the study before and after treatment in the group treated with metformin and pioglitazone (second group)

Variable	MEAN ± SD		P value
	Before treatment	After treatment	
FBS (mg/dl)	170.26 ± 56.144	149.72 ± 46.66	<0.001
BS2hpp (mg/dl)	244.00 ± 77.73	212.49 ± 66.56	<0.001
HbA1c (%)	8.63 ± 0.64	7.88 ± 1.20	<0.001
BUN (mg/dl)	10.90 ± 3.25	10.80 ± 2.67	0.54
Creatinin (mg/dl)	0.95 ± 0.17	1.005 ± 0.81	0.58
AST (U/L)	27.81 ± 5.90	27.60 ± 6.05	0.38
ALT (U/L)	26.60 ± 6.42	26.26 ± 6.80	0.231
TG (mg/dl)	176.74 ± 106.43	177.59 ± 86.79	0.937
Chol (mg/dl)	176.49 ± 35.98	171.50 ± 37.98	0.249
HDL (mg/dl)	43.95 ± 8.67	44.12 ± 8.28	0.823
Weight (kg)	73.01 ± 11.49	73.29 ± 11.50	0.314
SBP (mmHg)	121.81 ± 14.39	119.09 ± 14.01	0.06
DBP (mmHg)	69.88 ± 7.37	69.12 ± 7.78	0.397

Weight

A mean decrease in weight in Group 1 was 1.08 ± 2.39 kg from baseline after 12 weeks, and this change was statistically significant (p < 0.001) (Table 4). In contrast to this, subjects of Group 2 had a mean increase of 0.27 ± 2.42 kg in their weight from a baseline, which was not statistically significant (p = 0.314) (Table 4). The difference between the changes produced in the two groups was statistically significant (P < 0.001) (Table 4).

Lipid profile

The two treatment groups had comparable TG, chol and HDL at baseline (Table 1). The mean TG, chol and HDL levels in the sitagliptin group improved from 168.38 ± 80.76 to 133.98 ± 55.38 (p < 0.001), 177.00 ± 46.51 to 153.31 ± 34.91 (p < 0.001) and 43.18 ± 8.38 to 46.35 ± 12.19 (p = 0.006) (Table 2), respectively. In the pioglitazone group, TG, chol and HDL levels changed from 176.74 ± 106.43 to 177.59 ± 86.79 (p = 0.937), 176.49 ± 35.98 to 171.5 ± 37.98 (p = 0.249) and 43.95 ± 8.67 to 44.12 ± 8.28 (p = 0.823), respectively (Table 3). The difference between the changes produced in the two groups regarding these parameters was statistically significant (Table 4).

Table 4: The mean and standard deviation of changes in baseline variables after intervention in both groups

Variable	MEAN ± SD		P value
	Group	Group	
	metformin+sitagliptin	metformin+pioglitazone	
FBS (mg/dl)	-18.48 ± 33.32	-20.53 ± 53.97	0.773
BS2hpp (mg/dl)	-48.51 ± 57.13	-31.51 ± 54.20	0.05
HbA1c (%)	-1.001 ± 0.83	-0.75 ± 1.20	0.132
BUN (mg/dl)	-0.125 ± 1.88	-0.10 ± 1.47	0.92
Creatinin (mg/dl)	0.01 ± 0.13	0.055 ± 0.80	0.66
AST (U/L)	-2.99 ± 14.90	-0.21 ± 2.29	0.105
ALT (U/L)	-2.75 ± 13.02	-0.34 ± 2.50	0.106
TG (mg/dl)	-34.40 ± 52.97	0.85 ± 96.60	0.005
Chol (mg/dl)	-23.66 ± 39.33	-4.98 ± 38.39	0.003
HDL (mg/dl)	3.17 ± 9.96	0.17 ± 6.99	0.029
Weight (kg)	-1.08 ± 2.39	0.27 ± 2.42	<0.001
SBP (mmHg)	-3.50 ± 13.69	-2.72 ± 12.75	0.71
DBP (mmHg)	-2.12 ± 7.41	-0.75 ± 7.87	0.25

Other biochemical parameters

The change in blood pressure, liver function tests and kidney function tests at the end of the study from baseline was not significant between two groups (*P* > 0.05) (Table 4).

Adverse effects

In our study, there was no significant side effect such as hypoglycemia, oedema of the extremities, gastrointestinal symptoms, liver complications, kidney complications, cardiac complications and eye complications including macular oedema in both combination treatment groups. Also, both metformin-sitagliptin and metformin-pioglitazone were well tolerated.

Discussion

In this study, sitagliptin with pioglitazone in T2DM patients whose diabetes had been inadequately controlled with metformin was compared. This study aimed to evaluate which agent is preferable to an additional non-insulin antidiabetic drug for patients who have been uncontrolled with metformin.

The results demonstrated that although metformin-sitagliptin and metformin-pioglitazone had a great impact on the reduction of HbA1c, there was no significant difference between two groups (P value = 0.132). The rate of reduction of HbA1c in metformin-sitagliptin and metformin- pioglitazone groups was 1.001 ± 0.83 and 0.750 ± 1.20 respectively, and the difference between two groups was 0.26. Sung-Chen Liu in Taiwan achieved HbA1c reduction in metformin-sitagliptin group as 0.71 ± 0.12 and in the metformin-pioglitazone group as 0.94 ± 0.12. The difference between two groups in this study was 0.23, and there was no statistically significant difference between them (P-value = 0.17) [21]. As can be seen, the response to treatment in this study was slightly better than Sung-Chen Liu's study. Part of this difference depends on the mean age of the participants in the two studies. The mean age in our study was 52.91 ± 7.25 and was about 60 years in Sung-Chen Liu's study. Old age can reduce the response to treatment. Older patients usually have a longer duration of diabetes and insulin resistance in elderly patients can be more when compared to younger patients [22, 23]. All of these factors can reduce the response to treatment. Chawla et al. also could not find any statistically significant difference in HbA1c reduction between these two treatment groups (P-value = 0.203) [24].

Takihata et al. in a study that was done in Japan compared these two recent combination therapy in T2DM patients [13]. In contrast with our results, they reported that in Japanese with type 2 diabetes mellitus, sitagliptin is much more effective than pioglitazone. Although in their study, the difference between two groups was statistically significant (P value = 0.024), this result can be attributed to the lower body weight of the subjects in this study in comparison with our study (66.9 kg against 73.65 kg). Also, it can also be attributed to the fact that Japanese T2DM patients have lower levels of insulin secretion and insulin resistance than other races [25, 26].

According to our results, reduction in FBS was statistically significant after intervention in both groups, but no statistically significant difference was observed between treatment groups (P-value = 0.773). Chawla and Takihata, in two separate studies in line with the results of our study, did not report any significant difference in the FBS between the two-drug combination [13, 24]. In contrast, Sung-Chen Liu reported that the combination therapy, metformin-pioglitazone is more effective than metformin-sitagliptin with relation to the decreased FBS from baseline to endpoint [21]. Studies suggest that the impact of pioglitazone on blood sugar is by improving hepatic and peripheral insulin resistance [19, 24]. Based on this fact and because the population in Sung-Chen Liu's study were older than our study, it, therefore, implies their resistance to insulin may be higher. In this condition and due to the mechanism of pioglitazone function, this reason can be expected. There are few studies in which the effect of these two combination therapies on BS2hpp is compared. In this trial, we concluded that metformin-sitagliptin combination is more effective on BS2hpp. Sitagliptin can improve both fasting and postprandial hyperglycemia effectively [27, 28, 29], but pioglitazone improves mainly fasting hyperglycemia [30]. So with this description, our results are expected.

As shown from the trial, weight gain is a well-known consequence of pioglitazone treatment, while weight neutrality has been observed in sitagliptin studies both in monotherapy and combination therapy settings [31, 32]. Overweight is associated with insulin resistance so that weight loss can lead to improvements in insulin resistance and thus better response to treatment [33, 34]. This implies that weight neutrality of sitagliptin may offer a great advantage in T2DM management.

It can also be seen that metformin-sitagliptin in comparison with metformin-pioglitazone resulted in a better improvement in lipid profile status. In treatment with sitagliptin, cholesterol and triglyceride showed more reduction and HDL showed more increase. In contrast with the results from this study, Takihata [13] and Chawla [24] could not find any significant difference between two treatment groups regarding lipid profile. Sung-Chen Liu reported the better effect of metformin- sitagliptin on triglyceride levels, but in this study, pioglitazone was more effective in increasing HDL levels [21]. Although to date, there is not enough evidence about the certain mechanisms that improve the lipid profile in patients treated with Sitagliptin [24, 35], part of the improvement in lipid profile in our study can be attributed to weight loss that has occurred in sitagliptin recipients.

Takihata et el stated that during the study period, there was a statistically significant increase in serum creatinine level in both groups [13]. Hajime Meada et al. studied 1332 T2DM patients. Twenty percent of all these patients were treated with sitagliptin alone, 36% were treated in combination with one other drug, 31% were treated in combination with two other drugs, and 12% were treated in combination with 3 or more drugs. Eventually, they reported that the creatinine level was significantly increased after the intervention [36]. Our results showed that in both groups of patients, creatinine levels increased after

treatment. Although this finding was not statistically significant, it can be attributed to the smaller population and shorter period of treatment. Therefore, it seems that assessment of renal function in patients with these conditions is necessary.

Also, the results from this study showed that sitagliptin in comparison with pioglitazone is slightly better in the reduction of systolic and diastolic blood pressure, but there was no statistically significant difference between two groups in our study regarding systolic and diastolic blood pressure reduction. Mechanisms by which sitagliptin causes a reduction in blood pressure are GLP-1 receptor-mediated endothelial vasodilatation by nitric oxide stimulatory effect, the endothelium-independent vasodilatory effect of GLP-1 and increased excretion of sodium in urine that is done by proximal tubule [37].

Also in this study, there was no significant side effect such as hypoglycemia, oedema of the extremities, gastrointestinal symptoms, liver complications, kidney complications, cardiac complications and eye complications including macular oedema in both combination treatment groups. In Takihata's study, complications such as hypoglycemia (3.4% in the Sitagliptin group and 3.5% in the pioglitazone group) and gastrointestinal complications (5.2% in the Sitagliptin group and 1.8% in the pioglitazone group) were reported [13]. Some of these differences are due to the duration of treatment and antidiabetic drugs that patients were consuming before enrolling in the study. In our study, all patients were treated with metformin, and then sitagliptin or pioglitazone was added to the treatment, but in Takihata's study, patients were treated with metformin, Sulphonylurea or both and then pioglitazone or sitagliptin were added to the treatment. Non-occurrence of hypoglycemia in our study can be attributed to treatment with only metformin. In Chawla's study in line with our study, the patients were treated with metformin, and after enrolling in the study, pioglitazone or sitagliptin was added to their treatment. They did not report any significant side effects [24].

Furthermore in this study, unlike other studies, all patients were selected from those who were treated with only metformin. This kind of selection could help us to modify the effects of previous treatments in patients as a confounding variable.

This study has various limitations. In our study, participants were randomly assigned to treatment groups, but the mean age in two groups was not the same so that metformin-pioglitazone treatment group had higher age than the metformin-sitagliptin group. But in the multivariate model, age was considered as a covariate variable, hence the effects of these two drug regimen were compared by eliminating age. Another limitation of this study was the patients follow up period. Perhaps the time for follow up was not enough for some outcomes such as weight loss. Also, the period of treatment was too short to evaluate long-term glycemic control. On the other hand, in this study, the effects of drugs on insulin resistance was not evaluated. Insulin resistance is an important factor that can determine the outcomes and the rate of response to treatments in T2DM patients.

Finally, it can be concluded that both drug combinations were effective in reducing the levels of HbA1C, fasting blood glucose and blood glucose two hours after a meal and no significant difference was observed between the two treatment groups in improving the outcomes.

It is recommended that further long-term randomised control trials and multi-central studies with larger sample size should be carried out. In this way, we can provide a reference for choosing the best options as dual combination therapy in patients with type 2 diabetes and inadequate glycemic control.

Acknowledgment

We appreciate all Vallie-Asr Hospital personnel. All authors acknowledge Dr Saba Sayari for writing and final editing this paper.

References

1. Wysham C, Blevins T, Arakaki R, Colon G, Garcia P, Atisso C, et al. Efficacy and safety of dulaglutide added onto pioglitazone and metformin versus exenatide in type 2 diabetes in a randomized controlled trial (AWARD-1). Diabetes care. 2014;37(8):2159-67. https://doi.org/10.2337/dc13-2760 PMid:24879836

2. Raz I, Chen Y, Wu M, Hussain S, Kaufman KD, Amatruda JM, et al. Efficacy and safety of sitagliptin added to ongoing metformin therapy in patients with type 2 diabetes. Current medical research and opinion. 2008;24(2):537-50. https://doi.org/10.1185/030079908X260925 PMid:18194595

3. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. Bmj. 2000; 321(7258):405-12. https://doi.org/10.1136/bmj.321.7258.405 PMid:10938048 PMCid:PMC27454

4. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes care. 2015;38(1):140-9. https://doi.org/10.2337/dc14-2441 PMid:25538310

5. Chen YW, Wang JS, Sheu WH, Lin SY, Lee IT, Song YM, et al. Hemoglobin glycation index as a useful predictor of therapeutic responses to dipeptidyl peptidase-4 inhibitors in patients with type 2 diabetes. PloS one. 2017;12(2):e0171753. https://doi.org/10.1371/journal.pone.0171753 PMid:28182722 PMCid:PMC5300176

6. Cahn A, Cefalu WT. Clinical considerations for use of initial combination therapy in type 2 diabetes. Diabetes Care. 2016;39(Supplement 2):S137-45. https://doi.org/10.2337/dcS15-3007 PMid:27440826 PMCid:PMC5023033

7. Abdul-Ghani MA, Puckett C, Triplitt C, Maggs D, Adams J, Cersosimo E, et al. Initial combination therapy with metformin, pioglitazone and exenatide is more effective than sequential add-on therapy in subjects with new-onset diabetes. Results from the Efficacy and Durability of Initial Combination Therapy for Type 2 Diabetes (EDICT): a randomized trial. Diabetes, Obesity and Metabolism. 2015;17(3):268-75. https://doi.org/10.1111/dom.12417 PMid:25425451 PMCid:PMC5577982

8. Derosa G, Maffioli P, Salvadeo SA, Ferrari I, Ragonesi PD, Querci F, et al. Effects of sitagliptin or metformin added to pioglitazone monotherapy in poorly controlled type 2 diabetes mellitus patients. Metabolism. 2010;59(6):887-95. https://doi.org/10.1016/j.metabol.2009.10.007 PMid:20015525

9. Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, et al. Medical Management of Hyperglycemia in Type 2 Diabetes: A Consensus Algorithm for the and Adjustment of Therapy Initiation. Clinical diabetes. 2009; 27(1): 4-16. https://doi.org/10.2337/diaclin.27.1.4

10. Goldstein BJ, Feinglos MN, Lunceford JK, Johnson J, Williams-Herman DE. Effect of Initial Combination Therapy With Sitagliptin, a Dipeptidyl Peptidase-4 Inhibitor, and Metformin on Glycemic Control in Patients With Type 2 Diabetes. Diabetes care. 2007; 30:1979–1987. https://doi.org/10.2337/dc07-0627 PMid:17485570

11. Jabbour SA, Hardy E, Sugg J, Parikh S, Study 10 Group. Dapagliflozin is effective as add-on therapy to sitagliptin with or without metformin: a 24-week, multicenter, randomized, double-blind, placebo-controlled study. Diabetes Care. 2014;37(3):740-50. https://doi.org/10.2337/dc13-0467 PMid:24144654

12. Fonseca V, Staels B, Morgan JD, Shentu Y, Golm GT, Johnson-Levonas AO, et al. Efficacy and safety of sitagliptin added to ongoing metformin and pioglitazone combination therapy in a randomized, placebo-controlled, 26-week trial in patients with type 2 diabetes. Journal of Diabetes and its Complications. 2013;27(2):177-83. https://doi.org/10.1016/j.jdiacomp.2012.09.007 PMid:23116881

13. Takihata M, Nakamura A, Tajima K, Inazumi T, Komatsu Y, Tamura H, et al. Comparative study of sitagliptin with pioglitazone in Japanese type 2 diabetic patients: the COMPASS randomized controlled trial. Diabetes, Obesity and Metabolism. 2013;15(5):455-62. https://doi.org/10.1111/dom.12055 PMid:23279373

14. Einhorn D, Rendell M, Rosenzweig J, Egan JW, Mathisen AL, Schneider RL, Pioglitazone 027 Study Group. Pioglitazone hydrochloride in combination with metformin in the treatment of type 2 diabetes mellitus: a randomized, placebo-controlled study. Clinical therapeutics. 2000;22(12):1395-409. https://doi.org/10.1016/S0149-2918(00)83039-8

15. Thomas I, Gregg B. Metformin; a review of its history and future: from lilac to longevity. Pediatric Diabetes. 2017;18(1):10-6. https://doi.org/10.1111/pedi.12473 PMid:28052534

16. Kovacs CS, Seshiah V, Swallow R, Jones R, Rattunde H, Woerle HJ, et al. Empagliflozin improves glycaemic and weight control as add-on therapy to pioglitazone or pioglitazone plus metformin in patients with type 2 diabetes: a 24-week, randomized, placebo-controlled trial. Diabetes, Obesity and Metabolism. 2014;16(2):147-58. https://doi.org/10.1111/dom.12188 PMid:23906415

17. Dobs AS, Goldstein BJ, Aschner P, Horton ES, Umpierrez GE, Duran L, et al. Efficacy and safety of sitagliptin added to ongoing metformin and rosiglitazone combination therapy in a randomized placebo-controlled 54-week trial in patients with type 2 diabetes. Journal of diabetes. 2013;5(1):68-79. https://doi.org/10.1111/j.1753-0407.2012.00223.x PMid:22742523

18. Abdul-Ghani M, Migahid O, Megahed A, Adams J, Triplitt C, DeFronzo RA, et al. Combination Therapy With Exenatide Plus Pioglitazone Versus Basal/Bolus Insulin in Poorly Controlled Patients With Type 2 Diabetes on Sulfonylurea Plus Metformin: The Qatar Study. Diabetes Care. 2017:dc161738. https://doi.org/10.2337/dc16-1738

19. Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, et al. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. New England Journal of Medicine. 2006;355(23):2427-43. https://doi.org/10.1056/NEJMoa066224 PMid:17145742

20. Ceriello A, Johns D, Widel M, Eckland DJ, Gilmore KJ, Tan MH. Comparison of effect of pioglitazone with metformin or sulfonylurea (monotherapy and combination therapy) on postload glycemia and composite insulin sensitivity index during an oral glucose tolerance test in patients with type 2 diabetes. Diabetes Care. 2005;28(2):266-72. https://doi.org/10.2337/diacare.28.2.266 PMid:15677777

21. Liu SC, Chien KL, Wang CH, Chen WC, Leung CH. Efficacy and safety of adding pioglitazone or sitagliptin to patients with type 2 diabetes insufficiently controlled with metformin and a sulfonylurea. Endocrine Practice. 2013;19(6):980-8. https://doi.org/10.4158/EP13148.OR PMid:23807528

22. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science. 2003;300(5622):1140-2. https://doi.org/10.1126/science.1082889 PMid:12750520 PMCid:PMC3004429

23. Scheen AJ. Diabetes mellitus in the elderly: insulin resistance and/or impaired insulin secretion? Diabetes & metabolism. 2005;31:5S27-34.

24. Chawla S, Kaushik N, Singh NP, Ghosh RK, Saxena A. Effect of addition of either sitagliptin or pioglitazone in patients with uncontrolled type 2 diabetes mellitus on metformin: A randomized controlled trial. Journal of Pharmacology and Pharmacotherapeutics. 2013;4(1):27. https://doi.org/10.4103/0976-500X.107656 PMid:23662021 PMCid:PMC3643339

25. Welch S, Gebhart SS, Bergman RN, Phillips LS. Minimal model analysis of intravenous glucose tolerance test-derived insulin sensitivity in diabetic subjects. The Journal of Clinical Endocrinology & Metabolism. 1990;71(6):1508-18. https://doi.org/10.1210/jcem-71-6-1508 PMid:2229309

26. Taniguchi A, Nakai Y, Fukushima M, Kawamura H, Imura H, Nagata I, Tokuyama K. Pathogenic factors responsible for glucose intolerance in patients with NIDDM. Diabetes. 1992;41(12):1540-6. https://doi.org/10.2337/diab.41.12.1540 PMid:1446794

27. Aschner P, Kipnes MS, Lunceford JK, Sanchez M, Mickel C, Williams-Herman DE. Effect of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes. Diabetes care. 2006;29(12):2632-7. https://doi.org/10.2337/dc06-0703 PMid:17130196

28. Watanabe K, Kobayashi K, Takemoto M, Ishibashi R, Yamaga M, Kawamura H, Fujimoto M, Ishikawa T, Onishi S, Okabe E, He P. Sitagliptin improves postprandial hyperglycemia by inhibiting glucagon secretion in Werner syndrome with diabetes. Diabetes care. 2013;36(8):e119. https://doi.org/10.2337/dc13-0709 PMid:23881973 PMCid:PMC3714499

29. Iwamoto Y, Tajima N, Kadowaki T, Nonaka K, Taniguchi T, Nishii M, Arjona Ferreira JC, Amatruda JM. Efficacy and safety of sitagliptin monotherapy compared with voglibose in Japanese patients with type 2 diabetes: a randomized, double-blind trial. Diabetes, Obesity and Metabolism. 2010;12(7):613-22. https://doi.org/10.1111/j.1463-1326.2010.01197.x PMid:20590736

30. Kawamori R, Kadowaki T, Onji M, Seino Y, Akanuma Y, PRACTICAL Study Group. Hepatic safety profile and glycemic control of pioglitazone in more than 20,000 patients with type 2 diabetes mellitus: postmarketing surveillance study in Japan. Diabetes research and clinical practice. 2007;76(2):229-35. https://doi.org/10.1016/j.diabres.2006.08.017 PMid:17109986

31. Waugh J, Keating GM, Plosker GL, Easthope S, Robinson DM. Pioglitazone: A Review of its Use in Type 2 Diabetes Mellitus. Drugs. 2006; 66 (1): 85-109. https://doi.org/10.2165/00003495-200666010-00005 PMid:16398569

32. Dhillon S. Sitagliptin. Drugs. 2010 Mar 1;70(4):489-512. https://doi.org/10.2165/11203790-000000000-00000

PMid:20205490

33. Derosa G, Carbone A, Franzetti I, Querci F, Fogari E, Bianchi L, Bonaventura A, Romano D, Cicero AF, Maffioli P. Effects of a combination of sitagliptin plus metformin vs metformin monotherapy on glycemic control, β-cell function and insulin resistance in type 2 diabetic patients. Diabetes research and clinical practice. 2012;98(1):51-60. https://doi.org/10.1016/j.diabres.2012.05.022 PMid:22682949

34. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature. 2006;444(7121):840-6. https://doi.org/10.1038/nature05482 PMid:17167471

35. Monami M, Vitale V, Ambrosio ML, Bartoli N, Toffanello G, Ragghianti B, Monami F, Marchionni N, Mannucci E. Effects on lipid profile of dipeptidyl peptidase 4 inhibitors, pioglitazone, acarbose, and sulfonylureas: meta-analysis of placebo-controlled trials. Advances in therapy. 2012;29(9):736-46. https://doi.org/10.1007/s12325-012-0045-5 PMid:22923161

36. Maeda H, Kubota A, Tanaka Y, Terauchi Y, Matsuba I, ASSET-K Study group. The safety, efficacy and predictors for HbA1c reduction of sitagliptin in the treatment of Japanese type 2 diabetes. Diabetes research and clinical practice. 2012;95(1):e20-2. https://doi.org/10.1016/j.diabres.2011.10.011 PMid:22055835

37. Hussain M, Atif MA, Tunio AG, Ali B, Akhtar L, Serwar G. Effect of sitagliptin on glycemic control, body weight, blood pressure and serum lipid profile in type 2 diabetic hyperlipidemic patients. Journal of Ayub Medical College Abbottabad. 2016;28(2):369-72. PMid:28718546

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):963-966.
https://doi.org/10.3889/oamjms.2017.221
eISSN: 1857-9655
Case Report

PET Scan Misses Cutaneous Melanoma Metastasis with Significant Tumour Size and Tumour Thickness

Georgi Tchernev[1,2*], Liubomira Victor Popova[1]

[1]Department of Dermatology, Venereology and Dermatologic surgery, Medical Institute of Ministry of Interior (MVR-Sofia), General Skobelev 79, 1606, Sofia, Bulgaria; [2]Onkoderma - Policlinic for Dermatology and Dermatologic Surgery, Sofia, Bulgaria

Citation: Tchernev G, Popova LV. PET Scan Misses Cutaneous Melanoma Metastasis with Significant Tumour Size and Tumour Thickness. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):963-966. https://doi.org/10.3889/oamjms.2017.221

Keywords: melanoma; metastases; pet scan; wrong interpretation; surgery.

***Correspondence:** Georgi Tchernev. Medical Institute of the Ministry of Interior, Department of Dermatology, Venereology and Dermatologic Surgery, Sofia, Bulgaria; Onkoderma - Policlinic for Dermatology and Dermatologic Surgery, Sofia, Bulgaria. E-mail: georgi_tchernev@yahoo.de

Received: 23-Oct-2017; **Revised:** 28-Oct-2017; **Accepted:** 29-Oct-2017; **Online first:** 02-Sep-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Although PET-scan is an advanced, innovative and widely used method for monitoring patients with different types of cancer diseases, it is important to note that its application in patients with cutaneous melanoma is limited and should be reconsidered.

CASE REPORT: To affirm this new statement, we are presenting a case from our clinical practice of a patient with melanoma of the interdigital space (with resected in sano primary melanoma and performed complete lymphadenectomy) that showed locoregional and systemic progression in two months post operation. The PET scan performed within the second hospitalization (and before the second operation) did not detect the presence of any cutaneous metastases, which were clinically and histologically verified after the second operative procedure.

CONCLUSIONS: This data suggests that shortly more reliable and sensitive imaging methods for monitoring patients with cutaneous melanoma should be found. Having in mind that our patient has been operated twice in the area of the primary lesion (as the surgical wound underwent secondary healing), theoretically, the abundant cicatrization could have led to reduced glucose uptake in the surrounded cancerous tissue. Monitoring of a larger number of patients with locoregional metastases and surgical interventions in different locations would shed light on the observations shared by us.

Introduction

Positron emission tomography (PET) is a non-invasive nuclear imaging technique with uncomparable ability to assess functional and biochemical processes in tissues [1]. Due to its high sensitivity and specificity, it has a wide application in oncology for cancer diagnosis, staging, restaging, detection of the extent of local and regional disease spread, as well as in the monitoring of response to cancer treatment [2]. An important feature of PET is its ability to detect the earliest stages of many diseases due to its possibility to assess the biochemical and functional processes in tissues. This great advantage of PET explains the importance of the method to the clinical oncology practice [3]. It is important to emphasise that this characteristic significantly differs it from other high sensitivity methods like MRI (magnetic resonance imaging) and CT (computer tomography), which detect only anatomical or functional changes that have already occurred [4].

Specifically, about melanoma malignum, FDG-PET takes an important place in its detection and imaging [5].

Case report

A 58-year-old male patient presented to the department of dermatologic surgery because of the dark lesion on his right foot. Clinical examination revealed a dark brown to black pigmented macula, located in the interdigital space between the first and

the second finger of the right foot, with uneven distribution of colour and irregular borders. The lesion was removed by surgical excision under local anaesthesia, with 0.5 cm field of safety margins in all directions. Also, a lymph node dissection under general anaesthesia was performed with retroperitoneal entrance toward the iliac and femoral vessels. Intraoperatively were established dark coloured packages of enlarged lymph nodes with a firm texture and a diameter of 2.5 cm. Enlarged lymph nodes were observed in the obturator whole, with the same characteristic. A lymph node was found in the pelvis immediately adjacent to the v. iliaca external, infiltrated the vein wall. Radical lymph dissection was performed in a femoral, obturator and paraphiliac area.

Histological examination of the cutaneous lesion revealed moderately atypical cells with vesiculous nuclei, suspicious for melanoma, with tumour thickness 2 mm (Breslow).

Histological examination of the dissected lymph nodes verified total and non-total metastasis from melanoma, some with capsular infiltration, some of them without.

The patient was diagnosed in stage IIIC and referred for a PET-scan in 2 months. Two months later the patient presented to the department with two pigmented lesions with uneven borders in the same location (Fig. 1a).

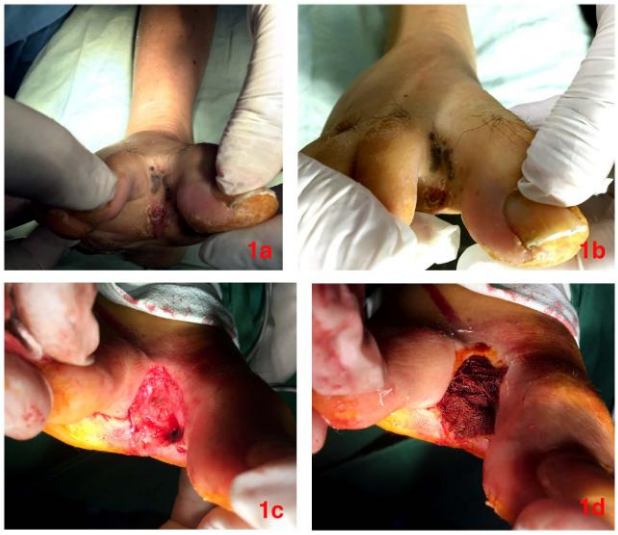


Figure 1: A, B) Clinical manifestation of interdigital malignant melanoma cutaneous metastases; C) Postoperative results after the second excision of a primary tumour, followed again with secondary healing; D) Surgical excision of the interdigital cutaneous metastases under local anaesthesia. Disinfection intraoperatively with povidone-iodine solution 10%

Preoperatively, PET-scan examination was performed, and it detected one right prevertebral lymph node (L3), measured 10mm with SUVmax 3.2; three enlarged paraaortic lymph nodes at the level of the bifurcation, measured up to 13 x 6 mm with SUVmax up to 4.9; and distal right parailiac lymph nodes, measured up to 17 x 10 mm with SUVmax 10.0. Infiltrative, probably inflammatory parenchymal changes of the right lung in the interlobe and paracardial with SUVmax up to 2.6 were also observed. However, no cutaneous metastases were detected on the PET-scan.

The cutaneous lesions were removed by surgical excisions, and the histological examination verified nodular melanoma malignum 1cm in diameter, with no ulceration; mitoses 1-2; Clark II-III; Breslow – 2.

By the clinical results, a restaging is performed in stage IV. The patient was referred for therapy with Pembrolizumab (KEYTRUDA) after hospitalisation in the oncology department.

Discussion

Nowadays, malignant melanoma is showing a tendency to increase in incidence – since 1970 it has approximately doubled with an estimated 68,720 new diagnosed in 2009 [6]. Early stages of melanoma are effectively treated with surgical excision [7]. However, by the time of diagnosis 15% of patients are presented with metastases or locally advanced tumor process [8].

FDG-PET has a wide application in oncology because of its significant role in cancer diagnosis, staging, restaging and therapeutic response monitoring in the most common cancers [9]. FDG (fluorodeoxyglucose) is an analog of glucose used as a radiotracer in order to detect tumor cells which are distinguished to have elevated glucose uptake [10]. It is a well-known fact that tumor cells have high metabolic needs of glucose, lipids and amino acids. PET imaging is based exactly on this distinctive feature of tumors, which explains the high sensitivity and specificity of the method when it refers to cancer diagnosis, staging and therapeutic response monitoring [11]. Glucose, as well as oxygen, growth factors and nutrients are critical factors that ensure the progress of cancer cells into solid tumors by activating their specific metabolic pathaways [12]. Microenvironment, especially hypoxia takes important place in the evolution of tumors [13]. In hypoxic conditions of 100-150 μm distance between the tumor cells and the nearest blood vessel, their ability to endure profound hypoxia is condition for their progress [14]. An adapting mechanism of cancerous cells is the ability to rely on anaerobic glucose metabolism, which ensures the major needs of energy for tumour growing [15].

Nowadays, PET takes a significant place in

oncology where it is widely used for cancer diagnostic, staging and therapy response monitoring [16]. Soon, in 1998, the limitations of PET were overcome with the development of the first combined PET/CT, firstly installed in University of Pittsburgh Medical Center [17]. This imaging technique provides an assessment of both biochemical and anatomic characteristics of tissues and is a significant advance in the evaluation of primary tumours, metastases, staging, therapy response monitoring and post-treatment recidives [18].

FDG-PET has a large application in evaluation of the extent of local and regional disease spread [19, 20]. Results of a meta-analysis evaluating the ability of PET in staging and restaging of cutaneous melanoma confirmed its usefulness in the detection of distant metastases. However, not so desirable results were observed in the evaluation accuracy of regional metastases as it does not detect microscopic disease [21]. Another report of Crippa et al. affirms reliable sensitivity and specificity in detection of lymph node metastases in patients with malignant melanoma. The presented results are showing that FDG-PET detected 100% of metastases ≥10 mm, 83% of metastases 6-10 mm, and 23% of metastases ≤ 5 mm. It is important to emphasise that the FDG-PET had high sensitivity (> or = 93%) only for metastases with more than 50% lymph node involvement or with capsular infiltration. However, this imaging method is not sensitive enough to detect subclinical microscopic disease [22]. The fact that PET sensitivity for melanoma lymph node metastases is dependent on tumour volume is also reported in data presented by Wagner et al. [23]

However, despite the successful results of PET in cancer diagnosis and evaluation, it has some important limitations that should not be neglected: (1) no quantitative system in assessing changes in FDG-metabolism in the therapeutic response. In the USA the development of such system is based on PET Response Criteria in Solid Tumors (PERCIST) [24]; (2) False-positive results. False positive results are possible because of increased FDG uptake in some normal body areas, such as lymphoid tissue and brown adipose tissue [25]. Another reason for false positive results may be the increased accumulation of FDG in some benign processes as inflammation or infection [26]; (3) low sensitivity. Low sensitivity may be observed in hypocellular cancers such as desmoplastic or mucinous tumours, as well as in micrometastases in breast cancer and melanoma [27]. Also, PET presents low sensitivity in well-differentiated low-grade tumours, which have lower glucose uptake, such as carcinoid tumours, renal cell carcinoma, bronchoalveolar-cell carcinoma and most prostate cancers [28].

We present a case of a patient with a malignant melanoma where two satellite cutaneous metastases measured 2 x 0.7 cm were not detected via PET-scan examination. With the presented case we want to report the unreliable results of PET-imaging when it refers to cutaneous melanoma metastases.

References

1. Verboom P, van Tinteren H, Hoekstra OS, et al. Cost-effectiveness of FDG-PET in staging non-small cell lung cancer: the PLUS study. Eur J Nucl Med Mol Imaging. 2003;30(11):1444–9. https://doi.org/10.1007/s00259-003-1199-9 PMid:14579081

2. Rohren EM, Turkington TG, Coleman RE. Clinical applications of PET in oncology. Radiology. 2004;231(2):305–32. https://doi.org/10.1148/radiol.2312021185 PMid:15044750

3. Peter Oehr,Hans-Jürgen Biersack, R. Edward Coleman. PET and PET-CT in Oncology. Springer Science & Business Media, 6.12.2012

4. McGeer PL, Kamo H, Harrop R, McGeer EG, Martin WR, Pate BD, Li DK. Comparison of PET, MRI, and CT with pathology in a proven case of Alzheimer's disease. Neurology. 1986;36(12):1569-74. https://doi.org/10.1212/WNL.36.12.1569 PMid:3491344

5. Gellén E, Sántha O, Janka E, Juhász I, Péter Z, Erdei I, Lukács R, Fedinecz N, Galuska, L, Remenyik É, Emri G. Diagnostic accuracy of 18F-FDG-PET/CT in early and late stages of high-risk cutaneous malignant melanoma. J Eur Acad Dermatol Venereol, 2015; 29: 1938–1944. https://doi.org/10.1111/jdv.13084 PMid:25753249

6. Mun G-H. Management of Malignant Melanoma. Archives of Plastic Surgery. 2012;39(5):565-574. https://doi.org/10.5999/aps.2012.39.5.565 PMid:23094257 PMCid:PMC3474418

7. Kimbrough CW, McMasters KM, Davis EG. Principles of Surgical Treatment of Malignant Melanoma. 2014; 94(5):973-88.

8. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin. 2009;59(4):225–49. https://doi.org/10.3322/caac.20006 PMid:19474385

9. Fletcher JW, Djulbegovic B, Soares HP, Siegel BA, Lowe VJ, Lyman GH, Coleman RE, Wahl R, Paschold JC, Avril N, Einhorn LH, Suh WW, Samson D, Delbeke D, Gorman M, Shields AF. Recommendations on the use of 18F-FDG PET in oncology. J Nucl Med. 2008;49(3):480-508. https://doi.org/10.2967/jnumed.107.047787 PMid:18287273

10. Khan N, Islam MM, Mahmood S, Hossain GA, Chakraborty RK. 18F-fluorodeoxyglucose uptake in the tumor. Mymensingh Med J. 2011;20(2):332-42. PMid:21522112

11. Zhu A, Lee D, Shim H. Metabolic PET Imaging in Cancer Detection and Therapy Response. Seminars in oncology. 2011;38(1):55-69. https://doi.org/10.1053/j.seminoncol.2010.11.012 PMid:21362516 PMCid:PMC3075495

12. Casciari JJ, Sotirchos SV, Sutherland RM. Variations in tumor cell growth rates and metabolism with oxygen concentration, glucoseconcentration, and extracellular pH. J Cell Physiol. 1992;151(2):386-94. https://doi.org/10.1002/jcp.1041510220 PMid:1572910

13. Xiao-Feng Li,Yang Du,Yuanyuan Ma, Gregory C, Postel A, Civelek C. 18F-Fluorodeoxyglucose Uptake and Tumor Hypoxia: Revisit 18F-Fluorodeoxyglucose in Oncology Application. Transl Oncol. 2014; 7(2): 240–247. https://doi.org/10.1016/j.tranon.2014.02.010 PMid:24699008 PMCid:PMC4101348

14. Fenton BM, Paoni SF, Beauchamp BK, Ding I. Zonal image

analysis of tumour vascular perfusion, hypoxia, and necrosis. British Journal of Cancer. 2002;86(11):1831-1836. https://doi.org/10.1038/sj.bjc.6600343 PMid:12087474 PMCid:PMC2375413

15. Kim Y, Lin Q, Glazer PM, Yun Z. Hypoxic Tumor Microenvironment and Cancer Cell Differentiation. Current molecular medicine. 2009;9(4):425-434. https://doi.org/10.2174/156652409788167113 PMid:19519400 PMCid:PMC2824923

16. Sai KK, Zachar Z, Bingham PM, Mintz A. Metabolic PET Imaging in Oncology. American Journal of Roentgenology. 2017:1-7. https://doi.org/10.2214/AJR.17.18112

17. Townsend DW, Beyer T. A combined PET/CT scanner: the path to true image fusion. Br J Radiol. 2002;75 (Spec No):S24-30. https://doi.org/10.1259/bjr.75.suppl_9.750024 PMid:12519732

18. Blodgett TM, Meltzer CC, Townsend DW. PET/CT: form and function. Radiology. 2007;242(2):360–85. https://doi.org/10.1148/radiol.2422051113 PMid:17255408

19. Mansour AA, Kelley MC, Hatmaker AR, Holt GE, Schwartz HS. Verification of musculoskeletal FDG-PET-CT findings performed for melanoma staging. Annals of surgical oncology. 2010;17(4):1144-51. https://doi.org/10.1245/s10434-009-0843-4 PMid:19967460

20. Strobel K, Dummer R, Husarik DB, Perez Lago M, Hany TF, Steinert HC. High-risk melanoma: accuracy of FDG PET/CT with added CT morphologic information for detection of metastases. Radiology. 2007;244(2):566–74. https://doi.org/10.1148/radiol.2442061099 PMid:17641374

21. Jimenez-Requena F, Delgado-Bolton RC, Fernandez-Perez C, et al. Meta-analysis of the performance of 18F-FDG PET in cutaneous melanoma. Eur J Nucl Med Mol Imaging. 2010;37(2):284–300. https://doi.org/10.1007/s00259-009-1224-8 PMid:19727717

22. Crippa F, Leutner M, Belli F, et al. Which kinds of lymph node metastases can FDG PET detect? A clinical study in melanoma. J Nucl Med. 2000;41(9):1491–4. PMid:10994727

23. Wagner JD, Schauwecker DS, Davidson D, Wenck S, Jung SH, Hutchins G. FDG-PET sensitivity for melanoma lymph node metastases is dependent on tumor volume. J Surg Oncol. 2001;77(4):237–42. https://doi.org/10.1002/jso.1102 PMid:11473371

24. Juweid ME, Cheson BD. Positron-emission tomography and assessment of cancer therapy. N Engl J Med. 2006;354(5):496–507. https://doi.org/10.1056/NEJMra050276 PMid:16452561

25. Chang JM, Lee HJ, Goo JM, et al. False Positive and False Negative FDG-PET Scans in Various Thoracic Diseases. Korean Journal of Radiology. 2006;7(1):57-69. https://doi.org/10.3348/kjr.2006.7.1.57 PMid:16549957 PMCid:PMC2667579

26. Long NM, Smith CS. Causes and imaging features of false positives and false negatives on 18F-PET/CT in oncologic imaging. Insights into Imaging. 2011;2(6):679-698. https://doi.org/10.1007/s13244-010-0062-3 PMid:22347986 PMCid:PMC3259390

27. Challapalli A, Aboagye EO. Positron Emission Tomography Imaging of Tumor Cell Metabolism and Application to Therapy Response Monitoring. Frontiers in Oncology. 2016;6:44. https://doi.org/10.3389/fonc.2016.00044 PMid:26973812 PMCid:PMC4770188

28. Tatci E, Ozmen O, Gokcek A, et al. 18F-FDG PET/CT rarely provides additional information other than primary tumor detection in patients with pulmonary carcinoid tumors. Annals of Thoracic Medicine. 2014;9(4):227-231. https://doi.org/10.4103/1817-1737.140134 PMid:25276242 PMCid:PMC4166070

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):967-969.
https://doi.org/10.3889/oamjms.2017.216
eISSN: 1857-9655
Case Report

Cerebral Venous Thrombosis in a Patient with Iron Deficiency Anemia and Thrombocytopenia: A Case Report

Walaa A. Kamel[1,2*], Jasem Y. Al-Hashel[1,3], K. John Alexander[1], Fathi Massoud[1], Fatima Al Shawaf[4], Ibtisam E. Al Huwaidi[5]

[1]Neurology Department, Ibn Sina Hospital, Kuwait; [2]Beni - Suef University, Egypt; [3]Kuwait University, Kuwait; [4]Mubarak Al-Kabeer Hospital, Kuwait; [5]Geriatric Health Services, MOH, Kuwait

Citation: Kamel WA, Al-Hashel JY, Alexander JK, Massoud F, Al Shawaf F, Al Huwaidi IE.. Cerebral Venous Thrombosis in a Patient with Iron Deficiency Anemia and Thrombocytopenia: A Case Report. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):967-969. https://doi.org/10.3889/oamjms.2017.216

Keywords: Cvt; Anemia; Thrombocytopenia; Case; Report.

***Correspondence:** Walaa Kamel. Neurology Department, Ibn Sina Hospital, Kuwait. E-mail: Walaaneuro@yahoo.com

Received: 11-Sep-2017; **Revised:** 10-Oct-2017; **Accepted:** 12-Oct-2017; **Online first:** 28-Nov-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: To report a potential association of thrombosis, thrombocytopenia with iron deficiency anaemia.

CASE REPORT: A 43-year-old female experienced an episode of a headache, with bilateral papilledema by neurological examination, magnetic resonance venography (MRV) brain showed cerebrovenous thrombosis (CVT), iron deficiency anaemia and thrombocytopenia by blood investigations, that was treated with iron supplementations and anticoagulation.

CONCLUSION: In this patient, cerebrovenous thrombosis (CVT) was discovered in a patient with thrombocytopenia and iron deficiency anaemia and treated with iron supplements and anticoagulation, we concluded that thrombocytopenia is not a protective factor against thrombosis especially with iron deficiency anaemia.

Introduction

Cerebral venous thrombosis (CVT) presents acutely to the emergency department usually with signs and symptoms of raised intracranial tension, namely headache, vomiting and blurred vision, altered sensorium, seizures and focal deficits and if the patient is not treated in time can result in permanent deficits, coma and even death [1]. However sometimes cerebral venous thrombosis can manifest in an indolent fashion as seen in our patient whose only complaint was a persistent headache.

Case Report

A 43-year-old female presented with the intermittent bitemporal headache of a throbbing nature mild to moderate in intensity associated with nausea, vomiting, and blurring of vision of 1-month duration, which was not responding to analgesics. She was diabetic, hypertensive dyslipidemic and obese. She also complained of menorrhagia of 3 years duration. On admission, she was afebrile, and her vitals were normal. Neurological examination revealed bilateral papilledema, no evidence of meningism or any focal neurological deficit. Routine blood investigation showed microcytic hypochromic anemia with hemoglobin value of 7.5 g/L (normal range: 12 - 16), RBC's value of 4.56 (normal range: 4.2 - 5.4), MCV is 55.6 (normal range: 82 - 97), low platelet count of 63,000/cumm (normal range 140,000-440,000), serum iron concentrate 3.3 mcmol/L (normal range: 9 - 30), transferrin 3.2 g/L (normal range: 2 - 3.6), iron saturation 4% (normal range: 20 - 40%), heptaglobin is 2.26 g/L (normal range: 0.30 - 2). Coagulation profile was normal including antithrombin III, factor V (laden), and activated protein C resistance, antiphospholipid antibodies, fibrinogen, protein C, protein S, and homocysteine. Serum vitamin B12 and folate were normal. Screening for vasculitis including rheumatoid factor, lupus anticoagulant, and Protein

immunofixation electrophoresis was all normal. Upper gastrointestinal and lower digestive endoscopies, endovaginal sonography, thoracoabdominal and pelvic computed tomography were performed and did not detect any malignant disease or source of active bleeding. MRI brain and MR venography revealed right transverse and sigmoid sinus, Right internal jugular vein thrombosis (Fig. 1). A lumbar puncture study showed the high opening pressure of 34 cm H_2O with no cells, normal glucose and protein.

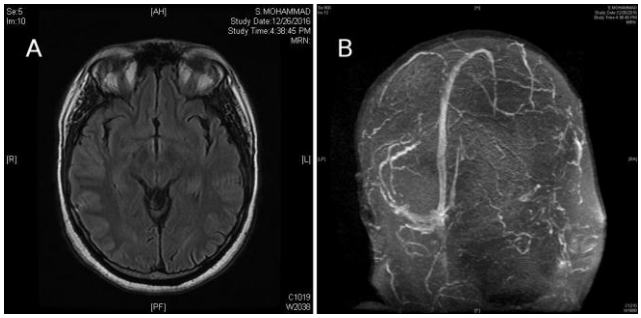


Figure 1: A) Normal MRI brain Axial FLAIR; B) MRV showing absent flow in the right transverse, sigmoid, and right internal jugular vein suggestive of cerebral venous thrombosis

The patient was treated as per protocol with low molecular weight (LMW) heparin, and this was later switched to Tablet Warfarin. Since she was found to be anaemic oral iron supplementation was also started. Daily haemogram was monitored, and it was observed that the platelet count rapidly increased and corrected to 200,000/cumm within one week. The patient's headache and blurring of vision gradually improved. The patient was discharged with gradual improvement of haemoglobin value of 8.2 g/dL, and the platelet count rose to 336,000/cumm. The cause of anaemia was presumed to be the prolonged history of menorrhagia this patient had after ruling out all other causes of the anaemia.

Discussion

Iron deficiency anaemia usually manifests as fatigue, exertional dyspnea and malaise. Neurological manifestations are not so common [2]. Our patient presented with symptoms of raised intracranial tension and who on the investigation was found to have CVT. Iron deficiency anaemia (IDA) is rarely recognized as a significant risk factor for stroke [3]. There are case reports of the association of iron deficiency and cerebral venous thrombosis with pseudotumor cerebri presentation which has been more often reported in children but only a few reports in the adult population [4 - 8]. Also, our patient had thrombocytopaenia which one would have expected to cause a bleeding tendency but paradoxically could have contributed to the development of the venous thrombosis as explained below.

Some mechanisms have been proposed to explain the association between Iron deficiency anaemia and thrombosis. Firstly iron is considered to be a regulator of thrombopoiesis, and normal iron levels are required to prevent thrombocytosis by inhibiting thrombopoiesis and consequent hypercoagulable state [9]. It was postulated that iron either directly or indirectly inhibits the rise in platelets above steady state by inhibitory mechanisms; but also is required for the production or synthesis of one or more essential platelet components. By this, they postulated that when an iron deficiency occurs, it affects the inhibitory compartment first, and thrombopoiesis is stimulated leading to thrombocytosis; but if the iron depletion is more severe, the essential component is affected leading to thrombocytopenia. However, the level of iron deficiency at which the switch occurs is yet to be established [10].

Another mechanism by which iron deficiency may contribute to a hypercoagulable state is by affecting flow patterns within the vessels [11]. The microcytosis resulting from iron deficiency causes reduced red cell deformability and increased viscosity, which contributes to thrombosis in a negative–pressure environment, as is found in veins. Akin et al. have suggested that the decrease in antioxidant defence in iron deficiency anaemia may cause increased oxidative stress which in return may result in a tendency toward platelet aggregation [12]. Thus the abnormal platelet count and function observed in iron deficiency anaemia could act synergistically to promote thrombus formation especially in the setting of an underlying atherosclerotic disease, all these conditions lead to a turbulent blood flow, causing platelets to come more frequently in contact with endothelial lining [11].

Unlike children, in which the major cause of IDA is insufficient dietary iron intake, chronic blood loss is the most common cause of IDA in adults especially in women with menstrual irregularities as was seen in our patient who suffered from menorrhagia. The resolution of severe symptomatic anaemia along with thrombocytopenia following iron supplementation strengthens the hypothesis that iron therapy plays an important role in improving thrombocytopenia associated with IDA [13].

The association of menorrhagia, IDA, and thrombocytopenia that was noticed in our patient was also rarely reported in the literature, and the resolution of thrombocytopenia with iron supplements will occur provided other causes of thrombocytopenia are excluded such as acute hemorrhage, hemolysis, trauma, folate and vitamin B12 deficiency and idiopathic thrombocytopenic purpura [13]. Akoi et al. evaluated the effects of iron therapy on platelet function among women with menorrhagia. They found

iron deficiency anaemia in women caused arachidonic acid-induced platelet dysfunction causing increased menstrual blood loss which can be reversed through iron repletion [14]. In a study conducted by Jens et al. to identify venous thromboembolism risk factors in patients with thrombocytopenia, (platelet count <100 x10^9/L) it was concluded that the risk of venous thrombosis in patients with thrombocytopenia was the same as in patients with normal platelet counts [15]. Therefore it can be inferred that low platelet count does not give any protection against thromboembolism. Similarly, it has also been observed that certain subpopulations of patients with Idiopathic thrombocytopenic purpura (ITP) are at significantly higher risk of thrombotic complications [16-17].

In conclusion, this case report illustrates that CVT can occur in the setting of anaemia and thrombocytopenia. Correction of iron deficiency rapidly restored the platelet count and reversal of the hypercoagulable state which may have contributed to the rapid recovery of the patient albeit in the presence of adequate anticoagulation. Hence this case illustrates that iron deficiency anaemia and thrombocytopenia can be considered as independent risk factors for CVT. Whether the menorrhagia is an additional risk factor for a hypercoagulable state is open to debate and needs to be studied.

References

1. Iurlaro S, Beghi E, Massetto N, Guccione A, Autunno M, Colombo B, Di Monda T, Gionco M, Cortelli P, Perini F, D'Onofrio F, Agostoni E. Does a headache represent a clinical marker in early diagnosis of cerebral venous thrombosis? A prospective multicentric study. Neurol Sci. 2004;25:S298-S2. https://doi.org/10.1007/s10072-004-0317-1 PMid:15549568

2. ai-Hao Huang,Je-Jen Su,Po-Hsien Lin.Iro deficiency anemia-A rare etiology of sinus thrombosis I adults. Acta Neurologica Taiwanica. 2010;19:2.

3. Sébire G, Tabarki B, Saunders DE, Leroy I, Liesner R, Saint-Martin C, Husson B, Williams AN, Wade A, Kirkham FJ. Cerebral venous sinus thrombosis in children: risk factors, presentation, diagnosis and outcome. Brain. 2005;128,477-489. https://doi.org/10.1093/brain/awh412 PMid:15699061

4. Aoki N, Sakai T. Cerebral sinus thrombosis in patients with severe iron deficiency anaemia due to myoma uteri. Acta Neurochir (Wien). 1989; 97: 131–134. https://doi.org/10.1007/BF01772824

5. Ho BL,Huang P,Khor GT,Lin RT.Stimultaneous thrombosis of cerebral artery and venous sinus. Acta NeurolTaiwan. 2008;17:112-116. PMid:18686652

6. Kinoshita Y, Taniura S, Shishido H, Nojima T, Kamitani H, Watanebe T . Cerebral venous sinus thrombosis associated with iron deficiency: two case reports. Neurol Med Chir (Tokyo). 2006; 46:589–593. https://doi.org/10.2176/nmc.46.589

7. Balci K,Utku U, Asil T, Buyukkoyuncu N. Deep cerebral vein thrombosis associated with iron deficiency anaemia in adults, Journal of Clinical Neuroscience. 2006; 14(2):181–184. https://doi.org/10.1016/j.jocn.2005.09.020 PMid:17161294

8. Ogata T, Kamouchi M, Kitazono T et al., Cerebral venous thrombosis associated with iron deficiency anemia. Journal of Stroke and Cerebrovascular Diseases. 2008; 17(6):426– 428. https://doi.org/10.1016/j.jstrokecerebrovasdis.2008.04.008 PMid:18984440

9. Beguin Y. Erythropoietin and platelet production. Haematologica.1999; 84(6):541–547. PMid:10366799

10. Gupta M, Joseph G. Severe thrombocytopenia associated with iron deficiency. Hospital Physician. 2001; 37: 49-54.

11. Hartfield DS, Lowry NJ, Keene DJ, Yager JY. Iron deficiency: a cause of stroke in infants and children. Pediatric Neurology.1997; 16(1):50–53. https://doi.org/10.1016/S0887-8994(96)00290-1

12. Akins PT, Glenn S, Nemeth PM, Derdeyn CP. Carotid artery thrombus associated with severe iron –deficiency anemia and thrombocytosis. Stroke. 1996;27:1002-1005. https://doi.org/10.1161/01.STR.27.5.1002 PMid:8623090

13. Ibrahim R, Khan A, Shahzad Raza MK, Dabas R, Haynes E, Gandhi A, Majumder OL, Zaman M. Triad of iron deficiency anemia, severe thrombocytopenia and menorrhagia—a case report and literature review. Clinical medicine insights. Case reports. 2012;5:23. https://doi.org/10.4137/CCRep.S9329 PMid:22493563 PMCid:PMC3320130

14. Akoi N, Sakai T. Cerebral sinus thrombosis in patients with severe iron deficiency anemia due to myoma uteri. Acta Neurochir (Wien). 1989; 97:131-134. https://doi.org/10.1007/BF01772824

15. Baelum JK, Moe EE, Nybo M, Vinholt PJ. Venous Thromboembolism in Patients With Thrombocytopenia: Risk Factors, Treatment, and Outcome. Clinical and Applied Thrombosis/Hemostasis. 2017;23(4):345-50. https://doi.org/10.1177/1076029615613158 PMid:26512063

16. Mc Crae K. Immune thrombocytopenia with pulmonary embolism and deep vein thrombosis. The Haematologist. .2011; 8-6.

17. Ruggeri M, Tosetto A, Palandri F, et al. Thrombotic risk in patients with primary immune thrombocytopenia is only mildly increased and explained by personal and treatment-related risk factors. J Tgromb Haemost. 2014;12:1266-1273. https://doi.org/10.1111/jth.12636 PMid:24942752

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):970-973.
https://doi.org/10.3889/oamjms.2017.222
eISSN: 1857-9655
Case Report

Primary Solitary Melanoma of the Lymphatic Nodes Or a Single Metastasis of Unknown Melanoma: Do We Need a New Staging System?

Georgi Tchernev[1,2*], Anastasiya Chokoeva[3], Lyubomira Victor Popova[1]

[1]Department of Dermatology, Venereology and Dermatologic Surgery, Medical Institute of Ministry of Interior (MVR-Sofia), General Skobelev 79, 1606, Sofia, Bulgaria; [2]Onkoderma - Policlinic for Dermatology and Dermatologic Surgery, Sofia, Bulgaria; [3]Medical University Plovdiv, Dermatology and Venereology, Plovdiv 4000, Bulgaria

Citation: Tchernev G, Chokoeva A, Popova LV. Primary Solitary Melanoma of the Lymphatic Nodes Or a Single Metastasis of Unknown Melanoma: Do We Need a New Staging System? Open Access Maced J Med Sci. 2017 Dec 15; 5(7):970-973. https://doi.org/10.3889/oamjms.2017.222

Keywords: Melanoma; Lymph node; Surgical; Therapy; Oncology.

***Correspondence:** Georgi Tchernev. Medical Institute of the Ministry of Interior, Department of Dermatology, Venereology and Dermatologic Surgery, Sofia, Bulgaria; Onkoderma - Policlinic for Dermatology and Dermatologic Surgery, Sofia, Bulgaria. E-mail: georgi_tchernev@yahoo.de

Received: 18-Sep-2017; **Revised:** 29-Oct-2017; **Accepted:** 30-Oct-2017; **Online first:** 04-Dec-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Malignant melanoma is a disease which has a cutaneous origin in 90% of the patients, but in rare cases, it could be discovered as secondary deposits with unknown primary site. Metastatic Malignant Melanoma occurs without a primary site in about 3% of all melanoma patients, and it could be divided into two main groups: metastatic lymph nodes' involvement or non-lymph nodes disease. The lack of unified classification and staging system, provided by AJCC (2009), as well as the lack for curtain diagnostic and therapeutic protocol, prompt us to raise the question what is the right way to precede in cases of metastasis of the lymph nodes, without evidence of a primary tumour?

CASE REPORT: We report a case of 67-years- old woman who presented in the dermatology clinic after a surgical removal of an enlarged lymph node in her left femoral area, verified histologically as a metastasis of melanoma. After a diagnostic refinement in the clinic, the diagnosis of metastasis of malignant melanoma was confirmed by histology revision. We use the presented case to create for the first time in the world literature a novel stereotype of thinking, which is also followed by a stereotype of clinical behaviour – gentle to the patient, but providing a certain amount of security and satisfaction for the medical staff.

CONCLUSION: The affection of a single lymph node in the absence of a primary tumour should not automatically lead to the conclusion that it is a single metastasis, but rather a primary melanoma of the lymph nodes, in cases of a negative PET scan, for example. In these cases, the measuring of the tumour thickness should guide the further therapeutic behaviour and determine the approach.

Introduction

Metastatic melanoma with unknown primary site (MUP) is defined as the progressive stage of disease, with no evidence of a primary tumour [1]. Although with rare incidence, metastatic melanoma with unknown primary site could occur in the lymph nodes, the skin or the inner organs, like lung, brain or liver metastasis, occupying 2-6% of all melanoma cases [1, 2]. According to results from a retrospective review for a period of ten years, made by Massachusetts General Hospital (MGH) and the Dana-Farber Cancer Institute (DFCI) the epidemiology of MUP shows higher prevalence of the cases, affecting the lymph nodes (35 patients), compared to those, affecting the skin (12 patients) or viscera (23 patients) [2]. Although the aetiology of MUP is not fully understood, an unrecognised or misdiagnosed primary melanoma seems to be the most logical explanation for its occurrence [1, 3]. Regarding the fact that such primary lesion is usually not found, the strong immunological potential of melanoma itself, which could lead to incomplete or complete remission of the primary lesion by the host's immune response has been implicated as a possible explanation of this phenomenon [3]. Another explanation for the possible reason for MUP is based on the theory that a prior lesion had been removed without a straight-laced histology or it is linked to nevus cell aggregates (NCA) in the lymph nodules [4, 5] It has been also hypothesized that ectopic melanocytes, located in

lymph nodes or inner organs could undergo malignant transformation in the same way as the cutaneous cells, in response to oncogenic stimuli or genetic predisposition [1, 6]. Hence, it has been established that relatives of patients with all kinds of cancers with unknown origin are at increased risk of developing the same, or other malignancies, including lung, pancreatic and colon cancers [6]. This in term confirms the pleomorphic nature and oncogenesis of these types of cancers, which remain in top 5 reasons for dead among cancer's patient around the world [1]. Most of the cases have been diagnosed at stage III, followed by stage IV disease, while the most common primary site of metastatic affection is the lymph nodes, followed by lungs and skin [1]. Staging classification resembles this in cutaneous melanoma, where limited cutaneous or nodal metastasis are classified in stage III, in contrast to those, disseminated in various body sites – in stage IV [1, 2].

Although, there is no significant differentiation in staging between MUP and melanoma with known origin, according to American Join Committee of Cancers' recommendations from 2009, there are some differences in the biological behaviour, as well as in the prognostic rate and genetic signature of these two sides of the same coin [1]. MUP patients have a better prognosis than those with metastatic melanoma with a known primary and better survival rate compared to non-lymph nodes MUP in general [1, 3]. Herein, we present a case of metastatic melanoma in the lymph node, without evidence of primary site tumour, as we focus the attention on some critical points in the diagnostic and therapeutic behaviour, which are often challenging.

Case report

We present a 67-years-old Caucasian female patient who came to the dermatology clinic for a diagnostic clarification and follow up. A surgical removal of an atypical and enlarged lymph node in the left femoral area was done a month ago, according to her medical history (Fig. 1a). Detailed medical history was obtained, but the patient did not report any precipitating event for the swelling. Postoperatively, the histological evaluation confirmed: metastases in the lymph node from Melanoma (Fig. 1b, c, d). Comorbidities include colon irritable, stomach polyps, arterial hypertension, endometrial hyperplasia, uterine fibroids. No surgical history was reported before the lymph node removal. The patient was recommended for a diagnostic refinement which was done in a month after the surgical treatment in an internal disease clinic. All necessary examinations for confirmation of the diagnosis of MUP according to the oncology protocols [7] were done. The patient underwent thorough examinations, including detailed medical history, physical examinations and consultations with different medical specialists, as well as positron emission tomography (PET scan), biochemical and haematological tests and histology revision. Physical examination with otorhinolaryngology specialist showed up two enlarged lymph nodes in her neck: one at each side: measuring 2/1 cm, painless, firm on palpation and non-fluctuant. The overlying skin was described as intact with no redness.

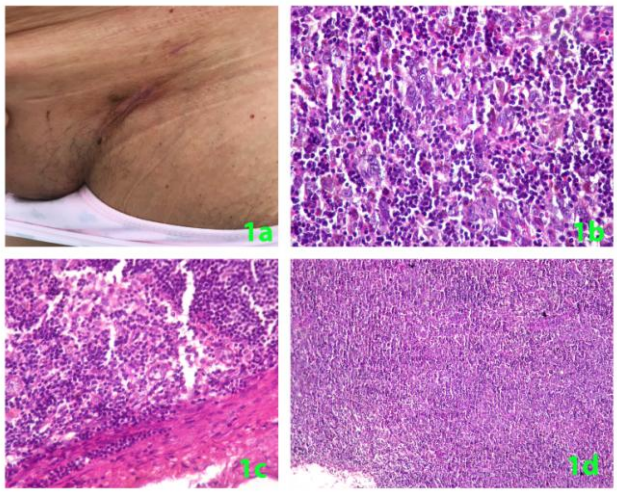


Figure 1: a) Patient with melanoma of the lymph node, treated surgically; b, c, d) Tumour cells infiltrating the lymphatic node

PET scan performed with oral and i.v. contrast did not show any abnormalities. No primary site tumour evidence or metastatic lesion was seen. Physical examination with gastroenterologist, gynecologist and ophthalmologists were done. The genital examination did not detect any primary site lesion. Both gastroscopies of gullet, stomach and duodenum and colonoscopy did not reveal any abnormalities which could be related to a primary site tumour. Blood tests established high levels of S-protein and LDH, considered as markers for melanoma. Second histology revision confirmed metastases of malignant melanoma as the previous one. The patient was referred for immunohistochemical tests, which revealed a positive staining for Melan A and HMB 45.

Discussion

Metastatic melanoma of unknown origin is a diagnosis of exclusion. A wide range of diagnostic procedures should be performed to exclude primary cutaneous, lymph node or visceral organ affection [1,

2]. Except for the ordinary diagnostic procedure with clinical examination, blood tests, biochemistry and imaging diagnosis in order to exclude metastatic spread in melanoma with cutaneous or mucosal origin, a multidisciplinary approach is required to exclude any primary involvement in cases of metastatic disease, including otorhinolaryngological examination; ophthalmological examination, gynecological examination for women and urological for men [8]. Although imaging diagnostic procedures are also considered helpful, some studies show that although better than CT scan in lymph node staging, the false-positive rate is high in PET-positive lymph nodes measuring less than 1 cm in diameter [9]. Although F-18 FDG PET/CT is found as a useful method in staging cutaneous and non-cutaneous melanoma, an extremely low detection rate is reported in locating the primary carcinoma of metastatic melanoma and axillary metastasis in patients with cancer of unknown primary site [10]. Differentiation in genetic signature of primary and metastatic melanoma with unknown origin has also been provided, with a higher rate of mutations in BRAF and NRAS genes in MUP patients, as well as more common mutations in TERT-promoter [1]. Although promising, these discoveries could not be implicated as routine diagnostic procedures, because of the high price of the method and its limitations.

The lack of unified classification and staging system, provided by AJCC (2009), as well as the lack for certain diagnostic and therapeutic protocol, prompt us to raise the question what is the right way to proceed in cases of metastasis of the lymph nodes, without evidence of a primary tumour? Furthermore, the lack of unified including criteria and staging in all of the studies on that issue often leads to contradictory results regarding the best diagnostic approach and prognosis rate, which additionally confuse the clinicians and patients themselves.

We use the presented case to create for the first time in the world literature a stereotype of thinking, which is also followed by a stereotype of clinical behaviour – gentle to the patient but providing a certain amount of security and satisfaction for the medical staff. The important point is to pay accurate attention to these data in future guidelines, which are not insignificant.

The affection of a single lymph node in the absence of a primary tumour should not automatically lead to the conclusion that it is a single metastasis, but rather a primary melanoma of the lymph nodes, in cases of a negative PET scan, for example. In these cases, the measuring of the tumour thickness should guide the further therapeutic behaviour and determine the approach. Herein, we suggest the staging of MUP to be similar to stage I and II of cutaneous melanoma but strictly referred to the primary melanoma of the lymph nodes.

In this way, a lymphadenectomy should be recommended for tumours with thickness over 1 mm, or less than 1 mm, but with additional risk factors such as increased number of mitosis, age under 40 years, vessels invasion, and so on. This group should also include cases of melanomas that have been regressed and subsequent they could not be detected after the metastatic spread. But we should not forget that the mucosal melanomas can be amelanotic and composed by up to a hundred malignant cells, which would be undetectable on PET scan also.

Despite all the mentioned variants, the presence of several affected lymph nodes should be interpreted as a sure marker for metastatic disease, weather, the affection of a single lymph node is more indicative for a primary lymph node melanoma, which would require more soft approach in cases with smaller tumour thickness, or at least in some acceptable limits with which clinicians should comply with. Therefore, the former statement that MUP of single lymph nodes has similarities to Stage III disease and should be treated with aggressive surgical management [11] is no longer needed.

References

1. Heppt MV, Tietze JK, Reinholz M, Rahimi F, Jung A, Kirchner T, Ruzicka T, Flaig MJ, Berking C. Disease kinetics but not disease burden is relevant for survival in melanoma of unknown primary tumour. Discov Med. 2015; 20(110):231-7. PMid:26562476

2. Katz KA, Jonasch E, Hodi FS, Soiffer R, Kiwitkiwiski K, Sober AJ, Haluska FG. Melanoma of unknown primary: the experience of Massachusetts General Hospital and Dana- Farber Cancer Institute. Melanoma Res. 2005; 15(1):77-82. https://doi.org/10.1097/00008390-200502000-00013 PMid:15714125

3.Kamposioras K, Pentheroudakis G, Pavlidis D. Malignant Melanoma of unknown primary site. To make the long story short. A systematic reviw of the literature. Crit Rev Oncol Hematol. 2011; 78(2):112-126. https://doi.org/10.1016/j.critrevonc.2010.04.007 PMid:20570171

4. De Waal AC, Aben KK, van Rossum MM, Kiemeney LA. Melanoma of unknown primary origin: A population-based study in the Netherlands. Eur J Cancer. 2013; 49(3):676–683. https://doi.org/10.1016/j.ejca.2012.09.005 PMid:23031553

5. Ridolfi L, Rosen P, Thaler H. Nevus cell aggregates associated with lymph nodes: Estimated frequency and clinical significance. Cancer. 1977; 39:164–171. https://doi.org/10.1002/1097-0142(197701)39:1<164::AID-CNCR2820390127>3.0.CO;2-T

6. Samadder NJ, Smith KR, Hanson H, Pimentel R, Wong J, Boucher K, Akerley W, Gilcrease G, Ulrich CM, Burt RW, Curtin K. Familial Risk in Patients With Carcinoma of Unknown Primary. JAMA Oncol. 2016; 2(3):340-6. https://doi.org/10.1001/jamaoncol.2015.4265 PMid:26863281

7. Giuliano AE, Mosely SH, Morton DL. Clinical aspects of Unknown Primary Melanoma. Ann Surg. 1980; 191(1):98-104. https://doi.org/10.1097/00000658-198001000-00018 PMid:7352784 PMCid:PMC1344625

8. Christopoulos P, Doulias T, Koutelidakis I, Papaziogas B. Stage four malignant melanoma of unknown primary site in young man. BMJ Case report. 2012. https://doi.org/10.1136/bcr-2012-006283

9. Toba H, Kondo K, Otsuka H, Takizawa H, Kenzaki K, Sakiyama S, Tangoku A. Diagnosis of the presence of lymph node

metastasis and decision of operative indication using fluorodeoxyglucose-positron emission tomography and computed tomography in patients with primary lung cancer. J Med Invest. 2010; 57(3-4):305-13. https://doi.org/10.2152/jmi.57.305 PMid:20847531

10. Yu X, Li X, Song X, Dai D, Zhu L, Zhu Y, Wang J, Zhao H, Xu W. Advantages and disadvantages of F-18 fluorodeoxyglucose positron emission tomography/computed tomography in carcinoma of unknown primary. Oncol Lett. 2016; 12(5):3785-3792. https://doi.org/10.3892/ol.2016.5203

11. Cormier JN, Xing Y, Feng L, Huang X et.al. Metastatic Melanoma to lymph nodes in patients with unknown primary sites. Cancer. 2006; 106(9):2012-2020. https://doi.org/10.1002/cncr.21835 PMid:16568458

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):974-977.
https://doi.org/10.3889/oamjms.2017.196
eISSN: 1857-9655
Case Report

Spontaneous Anterior Lens Capsule Rupture Of a Patient with Alport Syndrome - A Case Report

Kalina Trifonova[1], George Jordanoff[1], Valentin Stoyanov[1], Kiril Slaveykov[2*]

[1]Trakijski Universitet Stara Zagora, Meditsinski fakultet, Otorhinolaryngology, Stara Zagora, Bulgaria; [2]Trakijski Universitet Stara Zagora, Meditsinski fakultet, General Medicine, Armeiska 11, Stara Zagora 6000, Bulgaria

Citation: Trifonova K, Jordanoff G, Stoyanov V, Slaveykov K. Spontaneous Anterior Lens Capsule Rupture Of a Patient with Alport Syndrome - A Case Report. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):974-977. https://doi.org/10.3889/oamjms.2017.219

Keywords: Alport syndrome; Genetic disease; Lens; Capsule rupture; Phacoaspiration

***Correspondence:** Kiril Slaveykov. Trakijski Universitet Stara Zagora Meditsinski fakultet, General Medicine, Armeiska 11, Stara Zagora 6000, Bulgaria. E-mail: kirilslaveykov@gmail.com

Received: 31-Aug-2017; **Revised:** 01-Oct-2017; **Accepted:** 16-Oct-2017; **Online first:** 11-Dec-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Alport syndrome is a progressive genetic disease which is characterised by glomerulonephritis, sensorineural deafness and ocular abnormalities.We aimed to present a clinical case of a patient with Alport syndrome with spontaneous anterior lens capsule rupture.

CASE REPORT: A 16-year-old male with histologically proven Alport syndrome was hospitalised in the Department of Ophthalmology, University Hospital „Prof. Stoyan Kirkovich“, Stara Zagora with low vision, pain, redness, high IOP and rupture of the anterior lenticular capsule of the right eye. Phacoaspiration was successfully performed (Millenium, Bausch& Lomb, Rochester, New York, USA) with the bimanual irrigation-aspiration system (Geuder AG, Heidelberg, Germany) with excellent visual results.

CONCLUSION: As the syndrome is quite rare and can lead to diagnostic difficulties for the ophthalmologist complications such as spontaneous or traumatic rupture of the capsule are not uncommon. In such cases, phacoaspiration is an efficient method for clear lens extraction after ruptures of the anterior lenticular capsule of young patients with Alport syndrome.

Introduction

Alport syndrome is a common cause of inherited kidney failure but often goes unrecognised [1]. It is a progressive hereditary disease, characterised by glomerulonephritis /hematuria and proteinuria, leading to renal failure, sensorineural deafness and ocular abnormalities [2]. Alport syndrome has a prevalence of 1/5000, and 85% of patients have the X-linked form, where affected males develop renal failure and usually have a high-tone sensorineural deafness by the age of 20 [3].

Autosomal dominant and autosomal recessive inheritance has also been reported. [4] The disease is the cause of about 2% of end-stage renal disease in Europe and the United States. Alport syndrome is clinically and genetically heterogeneous. It is related to mutations in the genes encoding one of three chains, α3, α4 α5 of type IV collagen, the main component of basement membranes, expressed in the glomerular basement membrane. COL4A5 mutations are associated with X-linked Alport syndrome, which represents 80 to 85% of cases and is more severe in boys than in girls [5]. This protein is probably common to the basement membranes of the glomerulus, cochlea, retina, lens capsule, and cornea. However, the alpha 3(IV) and 4(IV), as well as the alpha 5(IV) collagen chains, are usually absent from the affected basement membranes, because the abnormal alpha 5(IV) molecule interferes with the stability of all three. The loss of these collagen molecules from the affected basement membranes results in an abnormal ultrastructural appearance. Mutations in COL4A3 or COL4A4 are associated with autosomal Alport syndrome [3].

Ocular changes are uncommon and subtle in

young patients with Alport syndrome and suggest that the signs increase in frequency and severity with age [6]. The types of ocular defects mostly involve the lens, the retina and more rarely the cornea [7]. The typical ocular associations are a dot-and-fleck retinopathy which occurs in about 85% of affected adult males, anterior lenticonus which occurs in about 25%, and the rare posterior polymorphous corneal dystrophy. The retinopathy and anterior lenticonus are not usually demonstrated in childhood, but worsen with time so that the retinal lesion is often present at the onset of renal failure, and the anterior lenticonus, later [3]. Retinal abnormalities include a perimacular dot-and-fleck retinopathy and a peripheral fleck retinopathy, which might occur independently of each other; a 'dull macular reflex' or 'lozenge', when the perimacular flecks are confluent; and, rarely, a macular hole caused by retinal thinning [1].

Giant macular holes are one possible retinal complication of Alport syndrome. Their pathogenesis differs from idiopathic macular holes and may result from the combination of collagen type IV abnormalities in the basement membranes of both Bruch's membrane and the internal limiting membrane, along with anomalous vitreoretinal adhesion [8]. Additional ocular features described in X-linked Alport syndrome include other corneal dystrophies, microcornea, arcus, iris atrophy, cataracts, spontaneous lens rupture, spherophakia, posterior lenticonus, a poor macular reflex, fluorescein angiogram hyperfluorescence, electrooculogram and electroretinogram abnormalities, and retinal pigmentation [3]. Anterior lens capsule rupture is a rare complication in adolescence as we found only 3 reported cases in the literature [4, 9, 10].

The histopathologic findings of patients with Alport syndrome examined by transmission electron microscopy show that the thickness of the anterior lens capsules is decreased (4-13 micron) and that there are many vascular dehiscences localised at the inner part of the lens capsule. There are large numbers of capsular dehiscences containing fibrillar materials and vacuoles. The anterior capsules are fragile in this disease, forming the basis for the progressive lenticonus and anterior polar cataract [2, 11, 12, 13]. It can also explain the cases of spontaneous anterior lens capsule ruptures

We aimed to present a clinical case of a patient with Alport syndrome with spontaneous anterior lens capsule rupture.

Case report

A 16-year-old male patient checked in the Emergency Department of the University Hospital „Prof. Stoyan Kirkovich“ in the afternoon on the 15th of June 2015, with complaints of pain, redness and low vision of his right eye. He was urgently admitted to the Ophthalmology Department on the same evening. The patient did not report sustaining any injury to his eye or head and did not remember any such trauma in the recent past, though admitting to often rubbing his eyes. He was uncertain when the complaints had first appeared, but this was the first time this had happened. Up until this point, he had never been examined by an ophthalmologist. The examination provided us with the following findings:

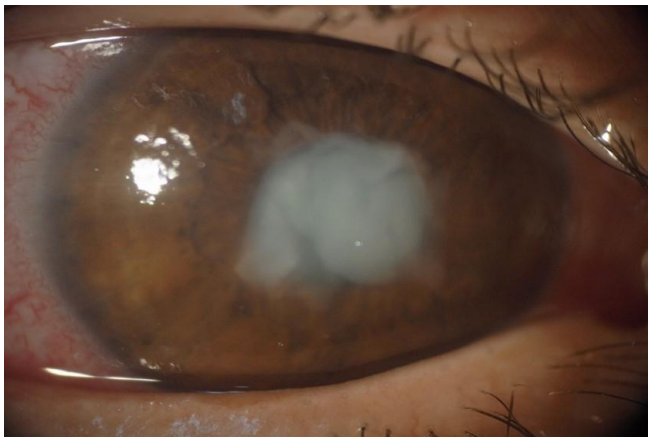


Figure 1: Anterior lens capsule rupture on diffuse light

Right eye: Vision was light perception and projection due to the opacified lens particles. Conjunctival congestive hyperemia and corneal subepithelial cystoid oedema were present, with decreased transparency. The pupil was narrow and did not respond to mydriatics and light; the anterior chamber was shallow. IOP was increased (49 mmHg) because of the lens-induced secondary glaucoma. The anterior capsule was ruptured with swollen and opacified lens particles (Fig. 1, 2). The posterior segment could not be examined.

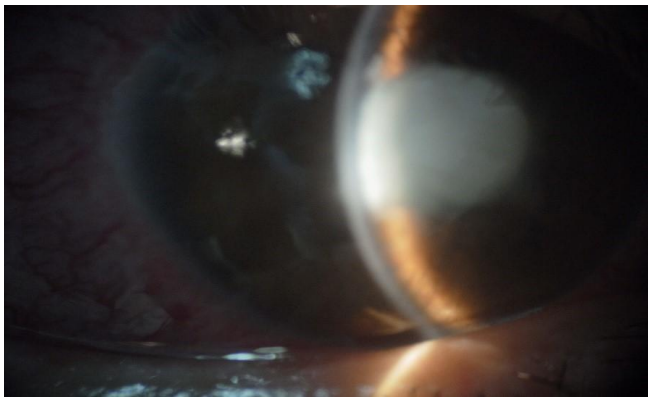


Figure 2: Anterior lens capsule rupture on slit examination

Left eye: Vision was low due to amblyopia and anterior polar cataract - 0,3/0,4 with +3,00 Dpt

Sph. /+8.00 Dpt from autorefractometer data/ (Fig. 3, 4). No other pathological findings were present.

The admitting ophthalmologist considered trauma or tumour of the right eye as a possible etiological factor at first. The performed CT - scan of the orbit showed no visible abnormalities. Before appointing additional examinations, the mother of the patient mentioned that he had been diagnosed with Alport syndrome in June 2014.

Figure 3: Anterior polar cataract on slit lamp examination of left eye

The mother had a complicated pregnancy with proteinuria and peripheral oedema. She had a C-section at 36 g.w. And the baby weighed 1700 g. He has been in an incubator for 1.5 months on O2 therapy. The patient has normal intellectual and physical development at the moment. The first symptoms of the disease started at age 7, with proteinuria, hematuria and hearing loss. He was followed for two months, and no further investigations were made. After a seven-year period, he admitted again for proteinuria, hematuria, hypoproteinemia and hypocalcemia in January 2014. During his stay sensorineural deafness was diagnosed by an ENT specialist. The mother refused a renal biopsy to be performed at this time, but she agreed on June the same year. Alport syndrome was confirmed histologically. According to the records, no ophthalmological problems were found at the time.

The only medication the patient takes is Enalapril for his hypertension and proteinuria.

His ten-year-old sister also has hematuria and proteinuria, while his grandfather on the mother side is on haemodialysis because of glomerulonephritis.

Urine test showed the following results: +++ protein, a large number of granulocytes, hyalin and erythrocytes, lots of leucocytes, ketone bodies (-), glucose (-), urobilinogen – not increased.

Blood test results showed: total protein - 49 g/l, albumin - 30 g/l, Urea - 6,5 mmol/l, creatinin -84, Na- 141 mmol/l, K – 4.0 mmol/l, Hgb -159, Ery – 5.29 $x10^{12}$/L, Leu – 8.5 10^9/L, Ptl -263 10^9/L.

The patient was prepared for operation by lowering his IOP with Azopt 3 x 1 drops, Cusimolol 2 x 1 drops, Acetazolamide 2 x 250 mg, Glycerine 30% 1000 mg 3 x 3.4 spoons and anti-inflammatory therapy with Uniclophen 3 x 1 and Dextobrin 5 x 1 drops. IOP dropped to 37 mmHg after the therapy.

The operation was performed under general anaesthesia with unsatisfactory mydriasis. Side port and main incision were made, and the lens particles in the anterior chamber were evacuated with the bimanual irrigation-aspiration system (Geuder AG, Heidelberg, Germany) followed by aspiration of those left in the capsular sac. Due to the insufficient visualisation through the undilated iris and lack of proper capsulorhexis extraction through the rupture was arduous. After the pieces of the anterior capsule were sufficiently visualised a foldable acrylic IOL was placed in the remaining capsular sac.

On the next day, the IOP was 11.5 mmHg. The visual acuity was still low 0.1 with IOL in place. The cornea was mildly edematous, with the fibrinous membrane in the pupil which was medically dilated. There were lens particles in 12 and 1 o'clock.

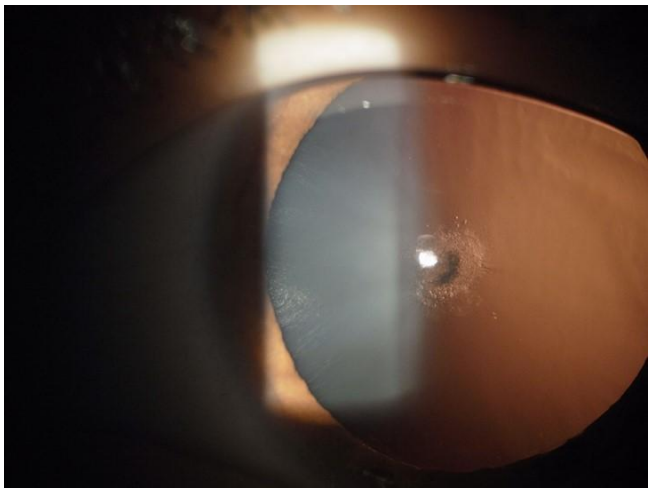


Figure 4: Anterior polar cataract on slit lamp examination of left eye on retroillumination

Potassium iodide 2 x 1, Oftaquix 5 x 1, Maxidex 5 x 1 and Yellox 3 x 1 drop were prescribed for home treatment. On the 10^{th}-day control examination, visual acuity had improved – 0.4/0.5 suggesting this was his dominant eye before the incident and the fibrinous membrane almost completely resorbed. At day 30 visual acuity was 0.8 with 0.5 D cyl 90° with no remnants of lens particles or the fibrinous membrane. There were no visible changes of the fundus.

Discussion

Taking good care of a patient with Alport syndrome needs the interprofessional collaboration of nephrologists, ENT doctors and ophthalmologists. A timely consultation with an ophthalmologist needs to be considered every time a patient is diagnosed with the disease to avoid the wrong diagnosis of unexpected complications.

As the anterior cataract and lenticonus are a frequent ocular complication of Alport syndrome, the surgeon must keep in mind the fragile anterior capsule when trying to perform phacoaspiration.

Phacoaspiration and implantation of IOL can be successfully performed in patients with Alport syndrome with spontaneous and traumatic rupture.

References

1. Savige J, Colville D, Opinion: Ocular features aid the diagnosis of Alport syndrome. Nat Rev Nephrol. 2009; 5(6):356-60. https://doi.org/10.1038/nrneph.2009.65 PMid:19474829

2. Choi Jh, Na Ks, Bae Sh, Roh Gh. Anterior lens capsule abnormalities in Alport syndrome. Korean J Ophthalmol. 2005; 19(1):84-9. https://doi.org/10.3341/kjo.2005.19.1.84 PMid:15929494

3. Colville DJ, Savige J, Alport syndrome. A review of the ocular manifestations. Ophthalmic Genet. 1997;18(4):161-73. https://doi.org/10.3109/13816819709041431 PMid:9457747

4. Olitsky SE, Waz WR, Wilson ME. Rupture of the anterior lens capsule in Alport syndrome. J AAPOS. 1999; 3(6):381-2. https://doi.org/10.1016/S1091-8531(99)70051-0

5. Heidet L, Gubler MC. [Alport syndrome: Hereditary nephropathy associated with mutations in genes coding for type IV collagen chains]. Nephrol Ther. 2016; 12(7):544-551. https://doi.org/10.1016/j.nephro.2016.09.001 PMid:27816395

6. Jacobs M, Jeffrey B, Kriss A, Taylor D, Sa G, Barratt TM. Ophthalmologic assessment of young patients with Alport syndrome. Ophthalmology.1992; 99(7):1039-44. https://doi.org/10.1016/S0161-6420(92)31853-6

7. Hentati N, Sellami D, Makni K, Kharrat M, Hachicha J, Hammadi A, Feki J. Ocular findings in Alport syndrome: 32 case studies. J Fr Ophtalmol. 2008;31(6 Pt 1):597-604. https://doi.org/10.1016/S0181-5512(08)75461-5

8. Mercé E, Korobelnik JF, Delyfer MN, Rougier MB. A new case of giant macular hole in a patient with Alport syndrome. J Fr Ophtalmol. 2012; 35(8):573-9. https://doi.org/10.1016/j.jfo.2011.12.007 PMid:22695059

9. Pelit A, Oto S, Yilmaz G, Akova YA. Spontaneous rupture of the anterior lens capsule combined with macular hole in a child with Alport's syndrome. J Pediatr Ophthalmol Strabismus. 2004;41(1):59-61. PMid:14974838

10. Wilson ME Jr, Trivedi RH, Biber JM, Golub R. Anterior capsule rupture and subsequent cataract formation in Alport syndrome. J Pediatr Ophthalmol Strabismus. 2004;41(1):59-61.

11. Streeten BW, Robinson MR, Wallace R, Jones DB. Lens capsule abnormalities in Alport's syndrome. Arch Ophthalmol. 1987; 105(12):1693-7. https://doi.org/10.1001/archopht.1987.01060120091033 PMid:3689194

12. Kato T, Watanabe Y, Nakayasu K, Kanai A, Yajima Y. The ultrastructure of the lens capsule abnormalities in Alport's syndrome. J Ophthalmol. 1998; 42(5):401-5. https://doi.org/10.1016/S0021-5155(98)00031-8

13. Citirik M, Batman C, Men G, Tuncel M, Zilelioglu O. Electron microscopic examination of the anterior lens capsule in a case of Alport's syndrome. Clin Exp Ophtalm. 2007; 90(5):367-70. https://doi.org/10.1111/j.1444-0938.2007.00134.x PMid:17697183

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):978-982.
https://doi.org/10.3889/oamjms.2017.189
eISSN: 1857-9655
Stomatology

Prevalence of Oral Mucosal Lesions and Their Association with Severity of Psoriasis among Psoriatic Patients Referred To Dermatology Clinic: A Cross-Sectional Study in Kashan/Iran

Rezvan Talaee[1,2], Zohreh Hajheydari[3*], Ahmad Yeganeh Moghaddam[4], Seyyed Alireza Moraveji[5], Bentolhoda Fatahi Ravandi[6]

[1]Department of Dermatology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran; [2]Autoimmune Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran; [3]Department of Dermatology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran; [4]Department of Otolaryngology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran; [5]Kashan University of Medical Sciences, Kashan, Iran; [6]Kashan University of Medical Sciences, Kashan, Iran

Citation: Talaee R, Hajheydari Z, Moghaddam AY, Seyyed Alireza Moraveji SA, Ravandi BF. Prevalence of Oral Mucosal Lesions and Their Association with Severity of Psoriasis among Psoriatic Patients Referred To Dermatology Clinic: A Cross-Sectional Study in Kashan/Iran. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):978-982. https://doi.org/10.3889/oamjms.2017.189

Keywords: Psoriasis; Oral lesions; Prevalence.

***Correspondence:** Zohreh Hajheydari. Department of Dermatology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran. E-mail: zhajheydari@yahoo.com

Received: 21-Jun-2017; **Revised:** 20-Sep-2017; **Accepted:** 23-Sep-2017; **Online first:** 24-Oct-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Psoriasis is a common inflammatory papulosquamous disease of the skin with unknown aetiology that may be associated with the abnormal T-cell function.

AIM: This study was aimed to determine the prevalence of oral mucosal lesions and their associations with severity of psoriasis in psoriatic patients attending the dermatologic clinic of Shahid Beheshti Teaching Hospital at Kashan, Iran.

METHODS: In this cross-sectional descriptive analytic study, all consecutive patients with psoriasis who referred to the dermatologic clinic at the Shahid Beheshti Hospital of Kashan University of Medical Sciences in Kashan City/ Iran were enrolled. All patients were examined for the presence of oral lesions, and the clinical and epidemiological features of the disease were recorded in a questionnaire for each patient. A biopsy was taken from atypical suspected patients with oral lesions by an expert dermatologist. Data were analysed using the Statistical Package for Social Science (Version 18). Descriptive statistics tests; Cross tabulation and Chi-square with Fisher's exact test were used.

RESULTS: Of total 177 psoriatic patients, 62 cases (35%) of patients were male, and 115 cases (65%) were female. Mean age of patients was 31.71 years. The oral lesion was seen in 83 cases (46.9%). Fissure tongue (28.2%) and geographic tongue (4.5%) were the most common oral lesions. The prevalence of oral lesions in patients with psoriasis significantly was associated with age, history of oral lesions, and with the onset of the oral lesions ($P < 0.05$). The most common form of psoriasis was chronic plaque form (Psoriasis Vulgaris) observed in 147 cases (83.1). The majority of patients (67.2%) were suffering from a mild form of the disease. Psoriasis Area and Severity Index (PASI) score of most patients was mild.

CONCLUSIONS: The prevalence of oral lesions such as Fissure tongue and geographic tongue is higher in psoriatic patients suggests further studies for approving the clinical importance of these apparently nonspecific lesions as possible predictors or markers of the severity of the cases suspected to Psoriasis disease.

Introduction

Psoriasis is a common chronic, genetically determined, scaly, an inflammatory papulosquamous immune-mediated disease of the skin with unknown aetiology, but it is known that there is a defect in the proliferation and differentiation of keratinocytes associated with inflammatory cell infiltration particularly consisting T-lymphocytes, macrophages and neutrophils [1-3]. Other contributing factors as environmental causative agents of the disease are included trauma, infections such as staphylococci, streptococci, HIV and candida species, medications, stresses, cigarette smoking and alcohol [4]. Although nail and joint involvements are well documented, the oral manifestations of the disease were still a subject of controversy [5-7].

Psoriasis is clinically classified into two main groups: pustular and non-pustular lesions. Non-

pustular psoriasis is included: Psoriasis Vulgaris (early and late onset) Guttate psoriasis Erythrodermic psoriasis, Palmoplantar psoriasis, Psoriatic arthritis (PsA), and Inverse psoriasis. Pustular psoriasis is composed of Generalized pustular psoriasis (von Zumbusch type), Impetigo herpetiformis, and Localized pustular psoriasis which included Palmoplantar pustular psoriasis (Barber type) and Acrodermatitis continua of Hallopeau [8].

The most common manifestations of the disease is a chronic desquamative plaque which involves the extensor surface of elbows, knees, and scalp [1, 8]. Additionally, nail involvement is usually seen in 20-25% of patients, and psoriatic arthritis occurs in 5-8% of the patients [1, 2, 9].

The prevalence of Psoriasis has been estimated approximately 2-3% around the world. It has two peaks of onset; first between 16 to 22 years old and then in late of 57 to 60 years and affects both sexes equally [10, 11]. Furthermore, Siblings and children of psoriatic parents are at increased risk of developing the disease [1].

Although mucosal involvements are rare, they are related to pustular, plaque-type and erythrodermic skin involvements. Also, various lesions have been described including white, yellowish, grey or translucent plaques, annular forms or diffuse areas of erythema and geographic tongue [12, 13]. Although there is controversy about the occurrence of psoriatic lesions on oral mucous membranes, the results of a recent review showed that the prevalence of fissured tongue was ranging from 9.8% to 47.5% and geographic tongue occurred between 5.6% and 18.1% of patients with psoriatic disease [2].

The results of other studies have suggested an increase in the prevalence of oral lesions specially fissured tongue (FT) and geographic tongue (GT) among psoriatic patients [13-16]. Furthermore, it has shown that patients with tongue lesions also had a nail and genital involvement and it seems GT (not FT), is more common in early onset psoriasis and maybe an indicator of disease severity [15]. Nonspecific tongue lesions have also reported frequently in psoriatic patients. Additionally, it has also been estimated that the presence of either GT or FT can be characterised as a sign for psoriasis and statistically there has been a relationship between the frequencies of each of these two types of lesions with the presence of cutaneous psoriasis [14, 16].

The purpose of this study was to identify the prevalence of oral lesions and to detect possible associations between psoriasis severity and oral lesions in patients with psoriasis who referred to the dermatologic clinic at the Shahid Beheshti teaching Hospital of Kashan City in Iran.

Material and Methods

In this cross-sectional descriptive analytic study, all consecutive patients with psoriasis who referred to the dermatologic clinic at the Shahid Beheshti Hospital of Kashan University of Medical Sciences in Kashan City/Iran were enrolled. This study was according to the Declaration of Helsinki and approved by ethic committee of research deputy of Kashan University of Medical Sciences, and the written consent forms were signed by all participants.

All recruited psoriatic patients were examined by a dermatologist for various oral lesions such as white, yellowish, gray or translucent plaques, annular forms, or diffuse areas of erythema, GT, FT and benign migratory glossitis (BMG) etc., time of onset and history of the lesions, and biopsy was taken in cases with atypical oral lesions.

A questionnaire including demographic characteristics, the clinical and epidemiological features of the disease was completed for each patient. Furthermore, the degree of erythema, thickness, scaling of and extension of involved area in different parts of the body of patients were calculated based on Psoriasis Area and Severity Index (PASI) score and recorded in the medical records of the patients by physician. Collected data were analysed using the Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Inc., Chicago, IL, USA). The association between studied variables was calculated using descriptive statistics tests such as Chi-squared test and Fisher's exact test, and $P < 0.05$ was considered statistically significant.

Results

In total, 177 psoriasis patients were enrolled, of these, 62 (35%) were men and 115 (65%) women. The mean age of the patients was 31.71 years, and most of the patients were in age group of 21-30 years. The mean duration of the disease was 7.54 years. Most patients were suffering from the mild form of the disease which observed in 119 (67.2%) of the patients. Other demographic characteristics of the patients have shown in Table 1. Of total 177 patients, 83 patients (46.9%) had oral lesions, and 94patients (53.1%) had no history of oral lesions. In 166 patients (93.8%), the onset of oral lesions was at the beginning of the disease and in 11 patients (6.2%) at the late phase of the disease.

The most common type of oral lesion was fissured tongue in 50 patients (28.4%). The prevalence of oral lesions in patients based on the distribution of gender has shown in Table 2 ($P = 0.215$).

Table 1: The prevalence of demographic characteristics among studied population

Variables	No.	%
Age (year)		
< 20	46	26
21-30	57	32.2
31-40	29	16.4
41-50	20	11.3
>50	25	14.1
Duration of the disease		
<1	34	19.2
1-5	55	31.1
5-10	46	26
>10	42	23.7
PASI score		
Mild	119	67.2
Moderate	26	14.7
Severe	32	18.1

In 167 patients (94.4%), joints were not involved, and 10 patients (5.6%) had joint involvement (P = 0.106). Furthermore, 133 patients (57.1%) had nail involvement (P = 0.898). Topical therapy was the most common type of treatment used in 131 patients (74%), whereas systemic treatment was the lowest type of therapeutic approach used in 7 patients (7.4%).

Table 2: The prevalence of oral lesions based on sex distribution in the studied population

Oral Lesion	Male (No.)	Female (No.%)	Total (No.%)
No Lesion	29 (30.9)	65 (69.1)	94 (52.1)
Fissure Tongue	23 (46)	27 (54)	50 (28.4)
Geographical Tongue	3 (37.5)	5 (62.5)	8 (5.4)
White Plaque	2 (33.3)	4 (66.7)	6 (3.4)
Yellow Plaque	1 (33.3)	2 (66.7)	3 (1.7)
White Plaque and Fissure Tongue	1 (33.3)	2 (66.7)	3 (1.7)
Geographical Tongue and Fissure Tongue	0 (0)	3 (100)	3 (1/7)
Erythematous Plaque	1 (50)	1 (50)	2 (1/1)
White Plaque and Geographical Tongue	0 (0)	2 (100)	2 (1/1)
Erythematous Plaque and Fissure Tongue	0 (0)	2 (100)	2 (1/1)
Fissure Tongue and Aphthous	1 (50)	1 (50)	2 (1/1)
Gray Plaque and Fissure Tongue	0 (0)	1 (100)	1 (0/6)
Geographical Tongue and Erythematous Plaque	1 (100)	0 (0)	1 (0/6)
Total	62 (35)	115 (65)	177 (100)

Chronic plaque-type psoriasis (Vulgaris) in 147 (83.1%) of the patients was the most common type of psoriasis disease among the studied population followed by Palmoplantar psoriasis(3.4%), Psoriatic nails (2.8%), Guttate psoriasis (1.7%), Inverse psoriasis (0.6%), Inverse psoriasis& Chronic plaque-type psoriasis (2.3%), Chronic plaque-type psoriasis& Psoriatic nails (2.3%), Chronic plaque-type psoriasis& Psoriatic arthritis (1.2%), Generalized pustular psoriasis (1.1%), Localized pustular psoriasis (0.6%), Chronic plaque-type psoriasis & Palmoplantar psoriasis(0.6%), and Chronic plaque-type psoriasis& Other form of Psoriasis(0.6%).

Oral lesions were observed in 83 (46.9%) of the patients, and chronic plaque-type psoriasis was the most common type of the psoriatic disease seen in 69 patients (83.1%) with oral lesions, and 78 (83%) of patients without oral lesions.

The prevalence of oral lesions among psoriatic patients based on PASI score has demonstrated in Table 3.

Table 3: The prevalence of oral lesions among psoriatic patients based on PASI Score

Oral Lesion	PASI Score			Total
	Mild	Moderate	Severe	
	No. %	No. %	No. %	No. %
No Lesion	63	14	16	93
	67.7%	15.1%	17.2%	100%
White Plaque	3	1	2	6
	50 %	16.7%	33.3%	100%
Yellow Plaque	2	0	1	3
	66.7%	0%	33.3%	100 %
Geographical Tongue	5	1	2	8
	62.5%	12.5%	25%	100%
Fissure Tongue	34	8	8	50
	68%	16%	16%	100%
Erythematous Plaque	2	0	0	2
	100%	0%	0%	100%
White Plaque and Geographical Tongue	2	0	0	2
	100%	0%	0%	100%
White Plaque and Fissure Tongue	3	0	0	3
	100 %	0%	0%	100%
Gray Plaque and Fissure Tongue	0	0	1	1
	0%	0%	100 %	100%
Geographical Tongue and Fissure Tongue	2	1	0	3
	75%	25%	0%	100 %
Geographical Tongue and Erythematous Plaque	0	1	0	1
	0%	100%	0%	100%
FissureTongue and Erythematous Plaque	1	0	1	2
	50%	0%	50%	100%
Erythematous Plaque and Aphthous	2	0	0	2
	100%	0%	0%	100 %
Total	119	26	32	177
	67.2%	14.7%	18.1%	100 %

Discussion

Although psoriasis is a common skin disorder, reviewing the literature reveals that the oral mucosa involvement among psoriatic patients is relatively rare, and remains a controversial subject [3, 5, 9, 17, 18]. The present study investigated the prevalence of oral lesions and also a probable association between severities of psoriasis in psoriatic patients. Our results showed no significant correlation between the prevalence of lesion in psoriatic patients and sex (P = 0.215), drug consumption, joint (P = 0.106) and nail involvement (P = 0.898), family history (P = 0.234), duration of the disease (P = 0.139) and PASI score (P = 0.927). Whereas, the prevalence of oral mucosa lesion in psoriatic patients had a significant correlation with age (P = 0.047), previous history of oral lesions, and the onset of the psoriasis (P < 0.05).

The prevalence of oral lesions in this study was 46.9% within the range reported by Azmi et al., and less than reported by Griffiths et al. and Pérez et al. [3, 6, 9]. We found no significant relationship between the presences of oral lesions in psoriatic patients with sex. The Similar result was reported by Taheri et al. [18]. Likewise, Taheri et al., the average age of our patients was 31.71, and most of the patients were in age group of 21-30 years, but this result was dissimilar with the result reported by Pérez et al. [6, 18].

Fissured tongue is a developmental anomaly of the tongue dorsum most often associated with

geographic tongue and is also increased in psoriasis [2, 19, 20]. The results of a literature review showed that the prevalence of fissured tongue was ranging from 9.8% to 47.5% and of the geographic tongue was between 5.6% and 18.1% [2]. Likewise, in our study, the most common type of oral lesions was FG (28.4%), GT geographic tongue (4.5%) and both lesions (1.7%) which showed these two lesions were the most frequent oral lesions in psoriatic patients. The 28.4% prevalence of FG in our study was less than reported by Danesh Pazhooh et al. (33%), and Hernandes-Pérez et al. (47.5%), but higher than the results of Zargari (8.2%) [6, 13-15]. These results were different from reported by the textbook of dermatology [21]. The difference in the prevalence of these lesions in our study and other studies can be indicated the higher prevalence of these lesions among psoriatic patients [15, 20].

Additionally, it has shown that psoriasis correlates with HLA CW6 whereas, GT is related to HLA B15, DR7 and FT are associated with HLA-DRB1 which probably indicated the higher prevalence of oral lesions in psoriatic patients could be related to the disease itself [9, 21].

Our study showed that chronic plaque-type psoriasis was the most common type of psoriasis (83%) in which oral lesions had the highest prevalence (83.1%). A similar study conducted by Germi et al. on psoriatic patients (FT: 22.6%, GT: 9.1%), revealed that FT and GT could be suggested as an oral manifestation of plaque-type psoriasis [21].

Another study by Tomb et al. also showed that there was a strong correlation between psoriasis and fissured geographic tongue [23]. Danesh Pazhooh et al. in their investigation reported that 32% of patients with psoriasis and GT had severe psoriasis, which can be indicated a correlation between this lesion and severity of the disease. Furthermore, we similarly found that nonspecific oral lesions were frequently observed in psoriatic patients [9, 13, 23].

Additionally, our study revealed that in 93.8% of the psoriatic patients, the onset of oral lesions was at the early stage of disease while in 6.2%, it occurred at the end of the disease and there was also a significant correlation between the prevalence of oral lesions in psoriatic patients and onset of disease.

Likewise, in Zargari et al. study, the Geographic tongue was observed in 7% of patients with early psoriasis and 1% of patients with late psoriasis. They concluded that the incidence of geographic tongue in early psoriasis might be indicated the severity of the disease [15]. Most of our patients with various oral lesions (67.6%) suffered from a mild form of psoriasis, and we found no significant relationship between the incidence of oral lesions and PASI Score. By our results, Azmi MG et al. stated, although the prevalence of FT and GT was relatively higher among patients with severe psoriasis assessed by PASI scores, the difference was not significant in less severe cases [3]. In the present study, joint involvement was observed in 6.5% of patients, in which, 2.4% had concomitant oral lesions. Our result showed no meaningful correlation between the presence of oral lesions in psoriasis and joint involvement. In contrary, Keshavarz et al. demonstrated facial involvement (55%) associated with geographic tongue (24%) and psoriatic arthritis (13%). The authors concluded that there is a significant relation between the facial lesion and increasing severity of the disease [24].

Nail involvement in this study was 24.9% and had no significant relationship with the presence of oral lesions in psoriatic patients. A similar result was reported by Zargar et al. (15).

The most common treatment was topical therapy (74%), and systemic therapy (4%) was the least therapeutic method used for treatment. We found no significant correlation between the prevalence of oral lesions in psoriatic patients and drug consumption. Also, Azmi MG et al. demonstrated that method of disease management such as medicated vs non-medicated for psoriasis couldn't influence the prevalence of FT and GT [3].

In conclusion, the prevalence of oral lesions such as Fissure tongue and geographic tongue is higher in psoriatic patients suggests further studies for approving the clinical importance of these apparently nonspecific lesions as possible predictors or markers of the severity of the cases suspected to Psoriasis disease. The HLA-typing study is recommended to approve the correlation between Fissure tongue and psoriasis. Also, further studies to identify the relationship between the presence of oral lesions in psoriatic patients with other variables such as nail and joint involvement, cigarette smoking and other systemic diseases are recommended.

References

1. Habif. Thomas P, Campbell JL, Chapman Jr MS, Dinulos JGH, Zug KA. Psoriasis and Other Papulosquamous Diseases In Skin Disease: Diagnosis and Treatment, 3rd Edition, Philadephia, Elsevier, 2011; P: 120.

2. Picciani BL, Domingos TA, Teixeira-Souza T, Santos VD, Gonzaga HF, Cardoso-Oliveira J, Gripp AC, Dias EP, Carneiro S. Geographic tongue and psoriasis: clinical, histopathological, immunohistochemical and genetic correlation-a literature review. Anais brasileiros de dermatologia. 2016;91(4):410-21. https://doi.org/10.1590/abd1806-4841.20164288 PMid:27579734 PMCid:PMC4999097

3. Darwazeh AM, Al-Aboosi MM, Bedair AA. Prevalence of oral mucosal lesions in psoriatic patients: A controlled study. Journal of Clinical and Experimental Dentistry. 2012;4(5):e286-e291. https://doi.org/10.4317/jced.50905 PMid:24455037 PMCid:PMC3892215

4. Di Meglio P, Villanova F, Nestle FO. Psoriasis. Cold Spring Harb Perspect Med. 2014;4(8). https://doi.org/10.1101/cshperspect.a015354 PMid:25085957

PMCid:PMC4109580

5. Costa SC, Hirota SK, Takahashi MD, Andrade H Jr, Migliari 2. DA. Oral lesions in 166 patients with cutaneous psoriasis: a controlled study. Med Oral Patol Oral Cir Buccal. 2009;8:e371-5.

6. Hernández-Pérez F, Jaimes-Avelda-ez A, Urquizo-Ruvalcaba 3. Mde L, Díaz-Barcelot M, Irigoyen-Camacho ME, Vega-Memije ME, et al. Prevalence of oral lesions in patients with psoriasis. Med Oral Patol Oral Cir Bucal. 2008; 13:e703-8. PMid:18978710

7. Shulman JD, Carpenter WM. Prevalence and risk factorsassociated with geographic tongue among US adults. Oral Dis. 2006; 12(4):381-6. https://doi.org/10.1111/j.1601-0825.2005.01208.x PMid:16792723

8. Sarac G, Koca TT, Baglan T. A brief summary of clinical types of psoriasis. Northern Clinics of Istanbul. 2016;3(1):79-82. PMid:28058392 PMCid:PMC5175084

9. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. Lancet 2007; 370:263-5. https://doi.org/10.1016/S0140-6736(07)61128-3

10. Dreyer LN, Brown GC. Oral Manifestations of Psoriasis, Clinical Presentation and Management. N Y State Dent J. 2012; 78(3):14-8. PMid:22803270

11. Marina EM, Botar-Jid C, Bolboaca SD, Roman II, Senila CS, Mihu CM, et al. Patterns of clinical nail appearances in patients with cutaneous psoriasis. Clujul Med. 2017;90(1):22-27. https://doi.org/10.15386/cjmed-679 PMid:28246493 PMCid:PMC5305083

12. Whan B. Kim, Dana Jerome, JensenYeung. Diagnosis and management of psoriasis. Canadian Family Physician. 2017; 63: 278-285.

13. Daneshpazhooh M, Mosleh H, Akhyani M and Etesami M. Tongue lesions in psoriasis: a controlled study. BMC Dermatology. 2004, 4:1. https://doi.org/10.1186/1471-5945-4-16 PMid:15527508 PMCid:PMC538751

14. Bruce AJ, Rogers RS, 3rd. Oral psoriasis. Dermatol Clin. 2003; 21:99-104. https://doi.org/10.1016/S0733-8635(02)00065-7

15. Zargari O. The prevalence and significance of fissured tongue and geographical tongue in psoriatic patients. 2006;31(2):192-5.

16. Singh S, Nivash S, Mann BK. Matched case-control study to examine association of psoriasis and migratory glossitis in India. Indian J Dermatol Venereol Leprol. 2013; 79:59-64. https://doi.org/10.4103/0378-6323.104670 PMid:23254730

17. Mattsson U, Warfvinge G, Jontell M.Oral psoriasis-a diagnostic dilemma: a report of two cases and a review of the literature.Oral Surg Oral Med Oral Pathol Oral Radiol. 2015;120(4):e183-9. https://doi.org/10.1016/j.oooo.2015.03.005 PMid:25944682

18. Perez FH, Avelda-ez AJ, Ruvalcaba MD, Barcelot MD, Camacho ME, Memije ME, Taylor AM. Prevalence of oral lesions in patients with psoriasis. Med Oral Patol Oral Cir Bucal. 2008;1(13):703-8.

19. Hussein AA, Darwazeh AM, Al-Jundi SH. Prevalence of oral lesions among Jordanian children. Saudi J Oral Sci. 2017; 4:12-7.

20. Sculty C, Hegarty A. The oral cavity and lips. In: BurnsT, Breathnach S, Cox N, Griffths C. Rook's Text book of Dermatology. 8th ed. Blackwell, 2010: P69.1-129.

21. Germi L, Giorgi VD, Bergamo F, Niccoli MC, Kokelj F, Simonacci M, et al. Psoriasis and oral lesions. Multicentric, observational and controlled study. Dermatology Online Journal January. 2012; 18 (1): 11.

22. Tomb R, Hajj H, Nehme E.Oral lesions in psoriasis.Annales de Dermatologieet de. 2010; 137 (11): 695-702.

23. Daneshpazhooh M, Mosleh H, Akhyani M, Etesami M. Tongue lesions in psoriasis: a controlled study.BMC Dermatology. 2004; 4(16):1-4. https://doi.org/10.1186/1471-5945-4-16

24. Keshavarz E, Roknsharifi S, Shirali Mohammadpour R, Roknsharifi M. Clinical features and severity of psoriasis: A comparison of facial and nonfacial involvement in Iran. Arch Iran Med. 2013;16:25-8. PMid:23273232

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):983-986.
https://doi.org/10.3889/oamjms.2017.201
eISSN: 1857-9655
Stomatology

Temporomandibular Disorders Treatment with Correction of Decreased Occlusal Vertical Dimension

Ljuben Guguvcevski[1], Nikola Gigovski[1], Aneta Mijoska[1*], Katerina Zlatanovska[2], Ana Arsova-Gigovska[3]

[1]Faculty for Dental Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]Faculty for Medical Sciences, University Goce Delchev, Shtip, Republic of Macedonia; [3]Dental Practice "Denta Nova", Skopje, Republic of Macedonia

Citation: Guguvcevski Lj, Gigovski N, Mijoska A, Zlatanovska K, Arsova-Gigovska A. Frequency of Symptoms of Temporomandibular Disorders among Prishtina Dental Students. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):983-986. https://doi.org/10.3889/oamjms.2017.201

Keywords: Occlusal vertical dimension; Temporomandibular disorder; Occlusal splint.

***Correspondence:** Aneta Mijoska. Faculty of Dental Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: amijoska@yahoo.com

Received: 16-Aug-2017; **Revised:** 20-Sep-2017; **Accepted:** 27-Sep-2017; **Online first:** 25-Oct-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: The term decreased occlusal vertical dimension refers to the reduced distance between two anatomical points while the teeth are in a state of occlusion. The development of this situation is about some parafunctional activities of the masticatory system.

AIM: To evaluate the value of decreased occlusal vertical dimension in cases with temporomandibular disorder and to follow up the influence of corrective treatment with occlusal splints and definitive prosthetic construction upon the elimination of clinical symptoms.

MATERIAL AND METHODS: Eight cases with decreased occlusal vertical dimension accompanied with temporomandibular disorders were treated with an occlusal splint, as part of reversible occlusal treatment. After reducing, or complete elimination of the symptoms related to problems of decreased occlusal vertical dimension, the definitive prosthetic therapy was performed.

RESULTS: The mean value of decreased occlusal vertical dimension in our patients is 8.5 mm, and the mean value of therapy time with an occlusal splint in these patients was 3.5 months.

CONCLUSION: Occlusal splint is a part of reversible occlusal therapy in cases with decreased occlusal vertical dimension. After reducing the symptoms related to decreased occlusal vertical dimension definitive prosthetic therapy can be done.

Introduction

Decreased occlusal vertical dimension (dOVD) is irregularity between upper and lower jaw about a vertical plane. dOVD has decreased the distance between two anatomical points. Those points are very often arbitrary checked dots on the top of the nose and the top of the mentum while the teeth are in a state of intercuspidation. In the development of this disorder has to pass the longer time in collaboration with some factors such as uncontrolled gnashing and tightening of the teeth, than psychological stress and finally trauma on jaw bones. Very often muscle hyperactivity as a result of emotional stress is a mean factor in the development of abrasion on occlusal teeth surfaces and normally consecutive presence of dOVD. All these factors together cause the pure development of decreased occlusal vertical dimension. This situation can be an etiologic factor in the development of temporomandibular disorders. The most frequent symptoms which accompanying temporomandibular disorders are in relation with middle range feeling of pain in the region of jaw joints, feeling of arthralgic pain, fast development of fatigue in masticatory muscles, and for some time there is evidence of clicking and popping in temporomandibular joints. There are difficulties in normal food chewing and presence of ear tinnitus. In cases with severe dOVD there is inflammation in mouth angles and presence of skin regard in the same region, around the mouth angles.

All these symptoms influence esthetic view of the patient's face. There is evidence of depression on the lower face third with the development of sulci around the mouth. In consideration with all these, mentioned above, we can say that our patient is unhappy with his/her esthetic appearance.

Ekberg et al. 1998 [1] suggest the use of occlusal splints in the treatment of temporomandibular disorders, and the authors point out that the treatment effects are not still completely understood.

With the use of occlusal splints, we can stabilise unstable occlusion which is the direct reason for temporomandibular disorders developing. Unstable occlusion in the intercuspal position may cause temporomandibular disorders, state Hagag et al. 2000 [2].

Unger 2001 [3] indicates that occlusal appliances are used for diagnosis and treatment of pain and dysfunction related to the mastication system, especially if the precise diagnosis could not be established because of objective reasons.

The development of temporomandibular disorders is in direct relation with individual physiological adaptability and in that moment when this adaptability is disturbed then the most sensitive structures in masticatory system present the first signs of damage, state Guguvcevski 2006 [4].

Wessel et al. 2006 [5] used occlusal stabilisation splints in the treatment of temporomandibular disorder follow by dOVD. According to the obtained results, authors conclude that this kind of splints has positive values in the treatment, but many subjects still had to click TMJs.

Savabi et al. 2007 [6] point out that immediate application of occlusal splints has no significant effect on the activity of masseter and temporal muscles.

Barao et al. 2011 [7] conclude that during the use of occlusal splints in patients with dOVD and temporomandibular disorders there is a significant increase in the muscle temperature in both masticatory muscles, masseter and temporal.

Considering the different views of the authors about the use of occlusal splints in dOVD treatment and decrease of temporomandibular disorder symptoms, the following aims are established in the study: (1) to evaluate the value of dOVD and its influence in developing temporomandibular disorders; and (2) After treatment with interocclusal splints and stabilisation of clinical signs and symptoms, to promote the definitive prosthetic treatment.

Materials and Methods

Eight patients (five male and three female) with dOVD and diagnosed temporomandibular disorders were evaluated. The mean age of our cases was 42 years. In establishing the diagnosis of this disorder, we used Research Diagnosis Criteria, which help us to verify the presence of temporomandibular disorder. Besides other characteristic signs and symptoms which follow temporomandibular disorder, there was evidence of dOVD in all examined patients. In every single patient, the value of mandible rest position was measured. For determination of rest position, the well known phonetic method was used.

Figure 1: The measurement of rest position with special gauges (left) and measured value of OVD. Evident presence of dOVD was noticed in examined patient (right)

After measurement of OVD, every patient gets an interocclusal splint made from colourless acrylic resigns. The purpose of this appliance was to correct the dOVD. This procedure obtains correction of distance between upper and lower jaw. Interocclusal splints were used during night and 3 to 4 hours during the day. The duration of its use was 2.5 to 5 months with a mean value of 3.5 months. Clinical examination during that period presents positive withdrawal of temporomandibular disorder with improving symptoms. We also noticed decreasing of pain, with increasing of comfort in the mouth and more comfortable chewing function. After this, the definitive prosthetic treatment was done. Fixed prosthodontic frameworks were done in five patients, while in other three patients there was an indication for prosthetic treatment with removable dentures.

Results

According to previously noticed anatomic landmarks, the value of rest position was measured in every patient, and after that, the distance between the same dots was also measured but now in a state of occlusal contact. The difference between these two measurements is exactly the value of OVD according to Millet et al. [8].

It's important to point out that there is a large distance between rest position and OVD. This fact explains there is a presence of dOVD in every examined patient. Table 1. present the value of rest position (RP) and OVD, and of course the difference between these two values obtained by the use of measurement gauge under standard conditions. The values of mandible rest position are obtained by the use of the well known phonetic method. Later with the same instrument, we measured the value of OVD

when the teeth are in a state of contact. The difference between these two measurements is presented in Table 1. The mean value of OVD in our study is 8.5 mm.

Table 1: The value of OVD measured in our cases

Number of patients	Value of rest position in mm (RP)	Value of OVD (in mm)	RP-OVD
1.	76	68	8
2.	73	64	9
3.	69	60	9
4.	78	70	8
5.	74	65	9
6.	77	67	10
7.	76	68	8
8.	73	66	7
Mean value	74.5	66	8.5

Discussion

Temporomandibular disorder is a disease with multicausal aetiology. Many external factors can be directly involved in the genesis of this disorder. Uncontrolled use of the masticatory systems, such as its overloading with gnashing of teeth is a factor for developing the dOVD. Latter, this situation leads to some problems such as muscle fatigue, feeling of pain in temporomandibular joints, difficulties in food consumption and naturally the development of some esthetic changes. In all cases with evident presence of dOVD and symptoms which are specific for this disorder the treatment had to be done, stated Guguvcevski [9]. These points are about changes of dOVD and that means return in normal jaw relation and increasing of dOVD. Increasing and correction of dOVD are optimally done with occlusal splints (OS). Li et al. [10] stated that occlusal splints caused positive remodelling of the periodontal tissue and condylar cartilage. The remodelling led to the acceptance of this situation, so the prosthetic framework is adapted to the previously corrected OVD. Botelho et al. [11] have similar findings, and the authors proposed OS to be used as complementary or additionally therapy in temporomandibular disorder treatment. Nilsson [12] point out that temporomandibular disorder accompanied with orofacial pain and dOVD are followed with different factors such as tooth clenching and grinding, then enhanced psycho-social stress and trauma to jaws which may be important etiologic factors. Signs and symptoms of dOVD are a common cause for the use of different intraoral appliances in efforts to solve unusual jaw function. Chang et al. [13] made investigations on effects produced by the use of OS in cases with temporomandibular disorders and presence of pain in temporomandibular joints, and the authors stated that occlusal splints could be very useful tool in the treatment of patients with pains and sounds of popping in temporomandibular joints. With the use of OS in the treatment of dOVD there is an immediate adaptation of joint's mechanoreceptors, stated Naito et al. [14].

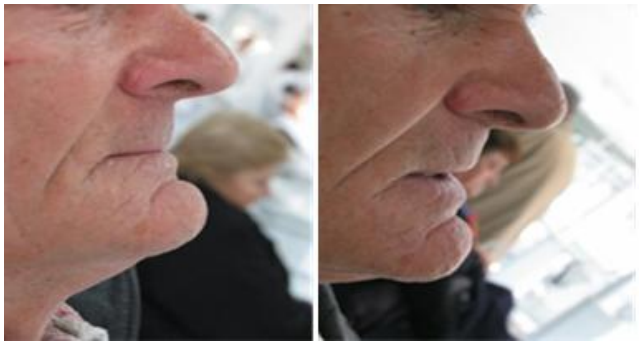


Figure 2: Profile view of the patient with dOVD (left) and the same patient with corrected dOVD (right)

Every patient got OS for correction of OVD. Increasing of previously dOVD was done according to functional and esthetic criteria. The mean value of increased OVD is 8.5 mm, and this means that for this amount is decreased the difference between rest position and OVD. The period of adaptation to corrected vertical relation was for 3.5 months (from 2.5 to 5 months), and during this time all of our patients had to use OS during the night according to previously determined the regime of uses. A daily use of the OS was in the period from 3 to 4 hours. During this period three visits were done - the first visit after the first week, the second visit after three weeks of using the OS and finally, the third visit was done after two months. Afte that, the reduction of symptoms compared with the very beginning of the treatment with OS was evaluated.

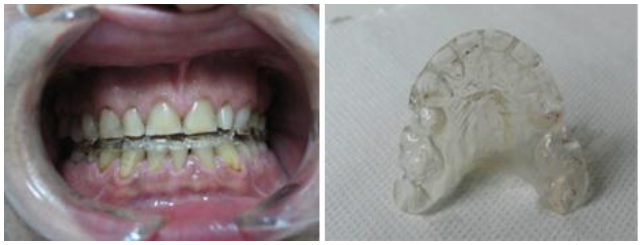


Figure 3: Patient with dOVD and with all accompanying symptoms. OS incorporated in patient's mouth (left) and outside view of OS for correction of dOVD

Wassell et al. [15] described the success of 80% in patients with temporomandibular disorders treated with OS. The authors got the efficiency in treatment after five months uses of OS. In our study, the percent of efficiency was 75%, and that means the therapy in six patients was positive, while in two other patients there was no reduction of symptoms of temporomandibular problems.

After complete reduction of the signs and symptoms which were present at the beginning of the treatment from which suffer our patients the definitive prosthetic treatment was done. The kind of the prosthetic treatment was in relation with the indication of prosthetic therapy of every single patient. The need

of the use of OS in dOVD treatment was also stated by Cutbirth [16]. In five patients the prosthetic treatment was done with fixed constructions, while in three cases the treatment was done with corresponding removable dentures. It's important to notice that prosthetic constructions, fixed or removable, must be fabricated after previously corrected OVD with the use of OS.

Corrected OVD with OS as an interphase in treatment which gives positive effects on masticatory muscle activity, state Abekura et al. [17] and Stumbaun et al. [18]. The use of OS minimises the uncontrolled habit of clenching and bruxing with teeth, and there is a complete reduction of the symptomatology which is in relation with this habit.

Authors as Millet, Chandu [19] and Torii [20] suggest that in cases where the dOVD has to be changed, it has to be done with the use of OS. This approach gives us the chances of additional corrections in increasing of OVD and following the positive effects in uses of OS. This approach reduced most of the signs and symptoms of temporomandibular disorders. It's that the definitive prosthetic treatment can be done after complete reduction of all signs and symptoms of dOVD.

In conclusion, after a clinical investigation of patients with dOVD and after the treatment following facts can be concluded: 1. dOVD is one of the reasons for the development of temporomandibular disorders; and 2. Definitive prosthetic treatment can be done after correction of the dOVD with the use of OS.

References

1. Ekberg EC, Vallon D, Nilner M. Occlusal appliance therapy in patients with temporomandibular disorders. A double-blind controlled study in a short-term perspective. Acta Odontol Scand. 1998; 56(2):122-8. https://doi.org/10.1080/00016359850136102 PMid:9669465

2. Hagag G, Yoshida K, Miura H. Occlusion, prosthodontic treatment, and temporomandibular disorders: a review. J Med Dent Sci. 2000; 47(1):61-6. PMid:12162528

3. Unger F. The management of temporomandibular joint disorders. The role of occlusal splints.Rev Stomatol Chir Maxillofac. 2001; 102(1):47-54. PMid:11345625

4. Guguvčevski Lj. Conservative Approach to the Temporomandibular Joint Hypermobility Treatment. (Plenary thesis). 11th Congress of BaSS, Book of Abstracts, Sarajevo, 2006: 27 p.

5. Wassell RW, Adams N, Kelly PJ. The treatment of temporomandibular disorders with stabilising splints in general dental practice: one-year follow-up. J Am Dent Assoc. 2006; 137(8):1089-98. https://doi.org/10.14219/jada.archive.2006.0347 PMid:16873324

6. Savabi O, Nejatidanesh F, Khosravi S. Effect of occlusal splints on the electromyographic activities of masseter and temporal muscles during maximum clenching. Quintessence Int. 2007; 38(2):e129-32. PMid:17510715

7. Barão VA, Gallo AK, Zuim PR, Garcia AR, Assunção WG. Effect of occlusal splint treatment on the temperature of different muscles in patients with TMD. J Prosthodont Res. 2011; 55(1):19-23. https://doi.org/10.1016/j.jpor.2010.06.001 PMid:20591761

8. Millet C, Leterme A, Jeannin C, Jaudoin P. Vertical dimension in the treatment of the edentulous patient. Rev Stomatol Chir Maxillofac. 2010;111(5-6):315-30. PMid:21192343

9. Guguvčevski Lj. Derangements of the TMJ Disc. Apolonia. 2010; 12(24):47-54.

10. Li Y, Zhang Z, Wu S, Qiao Y. A novel experimental design model for increasing occlusal vertical dimension. J Craniofac Surg. 2010; 21(2):450-7. https://doi.org/10.1097/SCS.0b013e3181cfe986 PMid:20489449

11. Botelho AL, Silva BC, Gentil FH, Sforza C, da Silva MA. The immediate effect of the resilient splint evaluated using surface electromyography in patients with TMD. Cranio. 2010; 28(4):266-73. https://doi.org/10.1179/crn.2010.034 PMid:21032981

12. Nilsson H. Resilient appliance therapy of temporomandibular disorders. Subdiagnoses, sense of coherence and treatment outcome. Swed Dent J Suppl. 2010; 206:9-88.

13. Chang SW, Chuang CY, Li JR, Lin CY, Chiu CT. Treatment effects of maxillary flat occlusal splints for the painful clicking of the temporomandibular joint. Kaohsiung J Med Sci. 2010; 26(6):299-307. https://doi.org/10.1016/S1607-551X(10)70043-7

14. Naito S, Ishida T, Kokai S, Fujita K, Shibata M, Yabushita T, Ono T. Functional adaptability of temporomandibular joint mechanoreceptors after an increase in the occlusal vertical dimension in rats. The Angle Orthodontist. 2011;81(3):453-9. https://doi.org/10.2319/082010-489.1 PMid:21261493

15. Wassell RW, Adams N, Kelly PJ. Treatment of temporomandibular disorders by stabilising splints in general dental practice: results after initial treatment. Br Dent J. 2004; 197(1):35-41. https://doi.org/10.1038/sj.bdj.4811420 PMid:15243608

16. Cutbirth ST. Increasing vertical dimension: considerations and steps in the reconstruction of the severely worn dentition. Pract Proced Aesthet Dent. 2008; 20(10):619-26. PMid:19274959

17. Abekura H, Yokomura M, Sadamori S, Hamada T. The initial effects of occlusal splint vertical thickness on the nocturnal EMG activities of masticatory muscles in subjects with a bruxism habit. Int J Prosthodont. 2008; 21(2):116-20. PMid:18546763

18. Stumbaum M, Konec D, Schweiger J, Gernet W. Reconstruction of the vertical jaw relation using CAD/CAM. Int J Comput Dent. 2010; 13(1):9-25. PMid:20481288

19. Chandu A, Suvinen TI, Reade PC, Borromeo GL. The effect of an interocclusal appliance on bite force and masseter electromyography in asymptomatic subjects and patients with temporomandibular pain and dysfunction. J Oral Rehabil. 2004; 31(6):530-7. https://doi.org/10.1111/j.1365-2842.2004.01377.x PMid:15189309

20. Torii K, Chiwata I. A case report of the symptom-relieving action of an anterior flat plane bite plate for temporomandibular disorder. Open Dent J. 2010; 4:218-22. https://doi.org/10.2174/1874210601004010218 PMid:21243070 PMCid:PMC3020490

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):987-993.
https://doi.org/10.3889/oamjms.2017.180
eISSN: 1857-9655
Stomatology

Testing the Effect of Aggressive Beverage on the Damage of Enamel Structure

Mitar Lutovac[1], Olga V. Popova[2], Gordana Macanovic[3], Radoman Kristina[4], Bojana Lutovac[5], Sonja Ketin[6*], Rade Biocanin[7]

[1]University Nikola Tesla, Belgrade, Serbia; [2]Southern Federal University, Russia; [3]International University Brchko, Brchko, Bosnia and Herzegovina; [4]High Medical School, Podgorica, Montenegro; [5]FIMEK, Novi Sad, Serbia; [6]University of Novi Sad, Square Dositeja Obradovica 6, 21000, Novi Sad, Serbia; [7]Institute of Biomedical Sciences, Faculty of Pharmacy and Health in Travnik, Travnik, Serbia

Citation: Lutovac M, Popova OV, Macanovic G, Kristina R, Lutovac B, Ketin S, Biocanin R. Testing the Effect of Aggressive Beverage on the Damage of Enamel Structure. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):987-993. https://doi.org/10.3889/oamjms.2017.180

Keywords: Nanomorphology; enamel surface,AFM procedure; roughness parameters.

***Correspondence:** Sonja Ketin. University of Novi Sad, Square Dositeja Obradovica 6, 21000, Novi Sad, Serbia. E-mail: ketin.sonja@gmail.com

Received: 26-May-2017; **Revised:** 09-Sep-2017; **Accepted:** 22-Sep-2017; **Online first:** 01-Dec-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Dental erosion is a common problem in modern societies, owing to the increased consumption of acid drinks such as soft drinks, sports drinks, fruit juice. Examining the enamel surface with the Atomic Force Microscopy (AFM) enables more precise registering and defining the changes of enamel surface structure and microhardness. This method can be used to compare the efficiency of application of different preventive and therapy materials and medicaments in dentistry. The chronic regular consumption of low pH cola drinks encouraged the erosion of the teeth. The loss of anatomy and sensitivity are direct results of acid cola dissolving coronal tooth material. Under the influence of coca cola, a change of crystal structure and nanomorphology on enamel surface occurs.

AIM: This paper reflects dental damage from abusive cola drinking, and the clinical presentation can be explained from data presented in this thesis.

MATERIAL AND METHODS: The trial was conducted on a total of 40 extracted teeth which were divided into two groups treated with the solution of coca cola during 5 minutes, and then prepared and tested with a standard AFM procedure, type SPM-5200. Quantitative analysis was performed by comparing the roughness parameters (Ra) of the treated and non-treated sample.

RESULTS: Based on the test of a hypothesis of the existence of differences between the treated and untreated sample, with an application of a t-test, it is shown that there are statistically highly significant differences between Ra of the treated sample with a 5-minute treatment of coca cola and Ra of the same sample without the treatment.

CONCLUSION: Use of AFM enables successful monitoring of changes on enamel surface as well as the interpretation of the ultrastructural configuration of the crystal stage and the damage created under the influence of different external factors.

Introduction

In the last three decades, the consumption of “Sugary” or “Sugar-sweetened Beverages” (SSBs) including carbonated “soda” soft drink, sports and energy beverages, fruit drinks, and sweetened bottled waters have increased dramatically. SSB is consumed by 66% of children and 77% of adolescent’s daily. Including adults and adolescents, males have been found to consume more SSBs than females. Older females consume the lowest (42 calories/day) amount, with the highest consumption rates (70%) by male adolescents, 12-19 years old (273 calories/day). Consumption of SSBs has also been associated with poor dietary habits, weight gain, obesity, and type 2 diabetes, predominately in adults, but also recognised in children and adolescents. Continuously sipping soda creates an acid bath for teeth. This softened area is ideal for bacteria to enter. As well, the sugar content of the soda is converted to acid by the bacteria on the teeth. There is no question that erosion causes significant damage to dental enamel, particularly among young people.

Dental erosion is defined as the irreversible loss of dental structure due to a chemical process and without the involvement of microorganisms. This process is the result of the action of acids whose pH is lower than 4.5.

Enamel is the hardest substance in the body, and it protects the crowns of the teeth. However, it is susceptible to demineralisation from acids. Acids are produced when certain bacteria colonise the tooth surface and metabolise carbohydrates. If this process continues, it may eventually lead to the development

of carious lesions in the enamel and dentin. Another source of acid is dietary. Many foods and beverages contain acids that also can lead to demineralisation of the enamel.

Tooth enamel is made of a billion crystals of carbonised hydroxyapatite that are packed in individual prisms winding from enamel-dentin border toward the tooth surface.

Enamel prisms are formed by the complex interaction of ectodermal and ectomesenchymal tissues that coordinate the action of ameloblasts (cells responsible for their synthesis).

Each enamel prism is a product of a single ameloblast and stretches uninterruptedly from the enamel-dentin border to tooth surface.

Enamel surface is not flat. It has a wavy structure because at places where Retzius' striae end such striae overlap in the form of steps, with the appearance of shallow grooves referred to as *perikymata*.

Although enamel has pronounced hardness, it is also fragile at the same time and similar to glass, so that for these reasons it could appear to be susceptible to breaking. Despite that, enamel can take loads higher than 1000 N several times during the day. The overall enamel microstructure is formed in such a way to adjust to such loads. This is also contributed by the support of elastic dentin and the structures such as enamel tufts at the dentin-enamel junction.

Besides the fact that it represents the hardest biological tissue, tooth enamel may be quickly damaged under the influence of various factors. Enamel damage can be divided into two large groups, i.e. infective and non-infective [1, 2].

Infective damage or tooth caries occurs as a consequence of demineralisation caused by the bacteria organised in a special ecological formation: oral biofilm – dental plaque. In certain conditions, the so-called cariogenic bacteria (specific species of streptococci) dominate on the tooth surface. They can create organic acids but can survive in acidic conditions. They suppress neutral or useful bacteria. For acidogenic biofilms to form and exert a cariogenic effect, the presence of sugar is necessary. Sugars originate from food and can be obvious such as those from candies, refined buns, snacks, beverages, or hidden, like for example in juices.

Beverages that decrease pH in the oral cavity and on the tooth surface, thus potentially leading to dental erosions, include fruit juices, soft drinks, sports beverages, other fizzy drinks, as well as various pickled vegetables (due to the content of acetic acid). Ideally, the pH of saliva lies within the range of 5.5–6.5; a pH of 5.5 is accepted as the threshold level for the development of dental caries.

When wear between enamel surfaces occurs at low pH, stress cracks are generated and propagate within the enamel, releasing particles. This particulate debris becomes trapped between the contacting surfaces, causing the two-bodyabrading system to transform into ahighwear, three-body abrasion system. This transformation does not appear to happen in low-pH media because the opposing surfaces have a smoother appearance; in fact, it appears that erosion modulates attrition to the extent that wear is reduced by an apparent polishing effect on the contacting surfaces. Degradation of enamel is a complex phenomenon, but erosion appears to be the predominating factor at low pH levels.

Table 1: Beverage characteristics

Group	Composition	pH (s.d.)	TA (s.d.)
Coca-Cola Classic (Coke)	Carbonated water, High fructose corn syrup, Caramel colour, Phosphoric acid, Natural flavours, Caffeine	2.49 (.006)	9.57 (1.87)
Diet Coke	Carbonated water, Caramel colour, Aspartame, Phosphoric acid, Potassium benzoate, Natural flavours, Citric acid, Caffeine	3.16 (.015)	9.11 (1.63)
Red Bull	Water, Sucrose, Glucose, Sodium citrate, Taurine Glucuronolactone, Caffeine, Inositol, Niacinamide, Calcium-Pantothenate, Pyridoxine HCL, Vitamin B12	3.32 (.006)	28.99 (4.17)
Tap Water (Water)	Water, various minerals	7.55 (.010)	----

pH: potential (power) of hydrogen, TA: titratable acidity, s.d.: standard deviation

Excessive consumption of drinks with an acid pH tends to cause the demineralisation of the dental enamel, though this effect may be reversible given the saliva's ability to remineralise 12-13 the teeth. Individuals consuming citric fruits more than twice a day have a 37 times greater risk of developing lesions through erosion than those who do not. Similar risks appear to occur with the consumption of apple vinegar (10 times higher), sports drinks (4 times higher) or sodas (4 times higher), when consumed every day. The advancing loss of dental structure through erosion could be as much as around 1 μm per day.

Signs of soda pop erosion:

- Broad shiny concavities on smooth surface enamel
- Glazed appearance
- Wide buccal concavities in mandibular premolars and molars
- Concavities with an enamel cuff at the free gingival margin
- Deep shiny concavities occlusally in premolars and molars
- Restorations that 'rise' above the occlusal surface
- Sealants that 'rise' above the occlusal surface
- Thin maxillary central incisors

- Increased incisal translucency in maxillary central incisors
- Surface characteristics missing
- Loss of surface detail in the primary dentition

In dentistry, the development of which has been greatly influenced both by the knowledge of and observing the biological and mechanical properties of hard (mineralised) tissues, the research with using this precise technique has started only in recent years. The results of the analyses of oral cavity tissue introduced the dental science into nano-era [3-6]. Only with the development of AFM technologies, it was possible to observe more subtle surface changes of enamel. Also, AFM studies start to be more and more used in researches in dentistry too, that observe the surface changes such as the dental plaque and mineralised and coloured deposits, surface properties of different materials and morphological and mechanical changes on mineralised tissues [7-9]. In recent years AFM has been more and more used to investigate erosions and early stadiums of demineralisation on enamel surface – the first results show that this is a very convenient tool. For the investigations to be comparable as much data as possible should be collected about the enamel nanomorphology in different biological and pathological conditions [10-13].

We chose to use the average roughness (Ra) of our samples as a measure because it has been most commonly used in previous studies, so it was easier to make a proper comparison of our results [14].

By using special research nanoprobes, it is possible nowadays to measure resolutions and movements at the level of nanometers and picomolar [15]. This also enables precise registering of physical properties of healthy – unchanged enamel, dentin and cement of the tooth root, as well as carrying out of the analyses of chemical processes and biological transformations that until now were impossible to perform [3, 4, 15, 16].

This study aimed to investigate dental damage from abusive cola drinking, and the clinical presentation can be explained from data presented in this thesis.

Material and Methods

The study was carried out on a total of 40 extracted teeth divided into two groups from which samples were taken (3 mm x 2 mm x 2 mm in size) and which were treated with the solution of coca cola and then prepared and tested with the standard procedure using AFM of JSPM-5200 type. One group of samples was not treated, while the other was treated for 5 minutes, with the analysis of enamel nanostructure performed after the treatment. Every sample was fixed to the microscope holder by using cyanoacrylate adhesive.

Images were made with the very slowed down scanning of the surface of every 25.0 μm^2, and with 0.1 Hz scanning frequency with 256 lines per sample, to avoid damaging of the probe.

Surface roughness was measured by average roughness (Ra) automatically on WinSPM software.

The average roughness is defined as an average distance of the centerline when they are observed as if they are local minimums and local maximums. This roughness is defined by the following formula :

$$R_a = \frac{1}{L}\int_0^L \left| f(s) - Z_0 \right| ds$$

where Z_0 is the centerline of the profile of the length L:

$$Z_0 = \frac{1}{L}\int_0^L f(s)ds$$

Figure 1 presents an image of the profile with absolute values about the centreline. The distance of their average height from the height of the centreline profile is defined as average roughness.

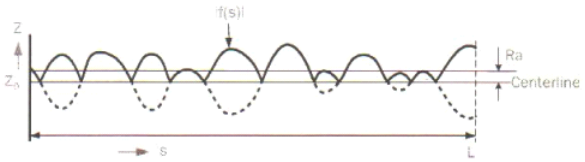


Figure 1: Average roughness

Competence at the level of AFM nanometry can be observed in the same way as in conventional measurements because the average roughness (Ra) at one measurement point is the arithmetic mean of local maximums and minimums of enamel surface calculated for that measurement point. All our measurement points have a surface of 5 x 5 μm, i.e. 256 x 256 or 512 x 512 pixels.

Results

Enamel surface morphology has been described both regarding quality and quantity. Quantitative analysis was based on a comparison of Ra roughness parameter between different samples and on statistical analysis of obtained values.

In the initial assumption of the study, we intended to start analysing the effect of coca cola on the enamel surface during a 60-second interval.

Preliminary tests have not identified any significant difference between the treated and non-treated samples. Therefore, a study with a 5-minute treatment was undertaken.

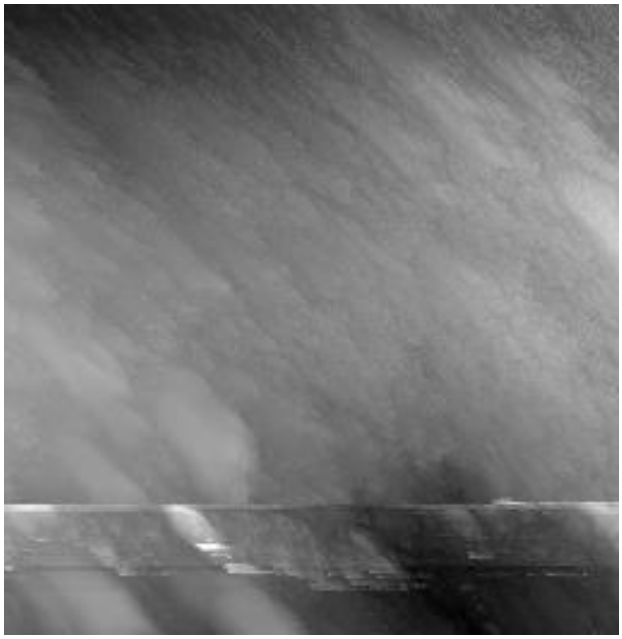


Figure 2: Untreated control surface

Figures 2 and three present untreated samples and samples that were treated with coca cola for 5 minutes.

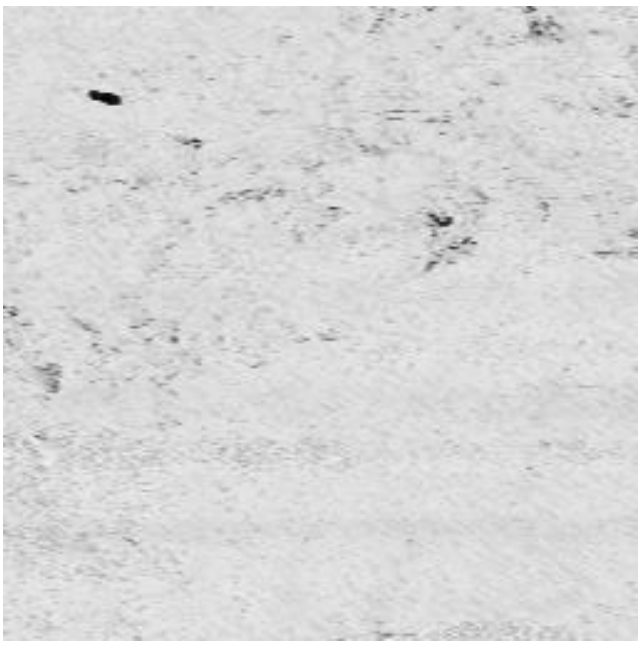


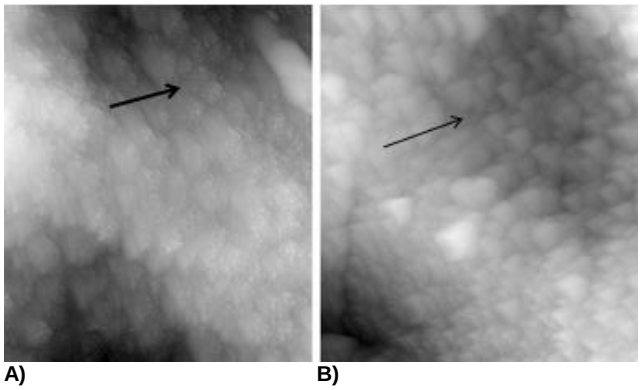
Figure 3: Surface treated with coca cola for 5 min

On smaller magnification, the untreated surface (Figure 2) is covered with an amorphous layer which may point to aprismatic enamel and parts of the pellicle. No clear borders between prisms nor existence of larger depressions can be seen. Enamel shows signs of compactness. The surface is flatter and protrusions that can be seen have a uniform structure. The granules are arranged in parallel rows, and no pores on enamel surface can be seen.

In contrast to that, slight porosity on the surface treated for 5 minutes with coca cola can be seen. Depressions and the grid-like structure of granules can be seen (Figure 3).

Figure 4: Untreated control surface Figure 5. Surface treated with coca-cola for 5 minutes

Higher magnifications confirm the compactness of untreated surface (Figure 4). The structure of densely compacted crystals on the untreated surface is more prominent, where no border can be seen between the prisms. On the treated surface depressions between the prisms are more prominent, with protruding prism heads and depressions around them.



A) **B)**

Figure 6: A) Untreated control surface; B) Surface treated with coca cola for 5 minutes

On higher magnitudes, depressions can be seen (the arrow) between the „prismatic structures“ in treated samples (Figure 6B), while on the untreated surface (Figure 6A) there is a filled-in area, which would correspond to compacted crystals. The findings point to the primary action in the area of less mineralised prism rims, where dilatation of the area can be seen.

By the analysis of all measurement points, the following mean values of average roughness, expressed in Ra, were obtained:

Table 2: Values of average roughness

	Untreated sample-N	Sample treated with coca cola
Arithmetic mean of Ra values of all measurement points Xsr	34.66	76.28

Variables	t_{emp}	t_{gr}	Statistical significance	P
N-Ra /CC5-Ra	-237.9093	3.291	VSZ	< 0.001

Based on the test of a hypothesis of existence of differences between the treated and untreated sample, with an application of a t-test, it is shown that there are statistically highly significant differences between Ra of the treated sample with a 5-minute treatment of coca cola and Ra of the same sample without the treatment, because the absolute value t is empirically higher than t – border value t_{emp} =|-237.9093|> tgr = 3.291 for p = 0.001.

Discussion

The selected studies are extremely important because non-carious damages of teeth are coming more and more in focus with the trend to place caries under control in developed countries. While in the past a great deal of such damage would even pass unnoticed, today, in developed countries, it is among the most frequent chronic diseases with the children of 5-17 years of age [2, 11, 17].

Carbonated beverages are dominant among soft drinks, with coca cola taking a leading place. An increased consumption is accounted for by the recession and a lower price of coca cola [18, 19].

The increased consumption of carbonated beverages is correlated with an increase in erosive damages of enamel [20].

Numerous studies have shown the advantage of using AFM analyses to monitor both qualitative and quantitative changes in enamel surface [21-26].

Speaking of qualitative and quantitative monitoring of enamel dissolution, the paper [27] demonstrates correspondence of AFM examination technique with other techniques (SEM, profilometry, nano carving), while the paper [28] confirms AFM as the most precise technique for such analyses.

The analysis of changes on our sample after five minutes of action of coca cola shows that there are no densely compacted crystals which are, on the contrary, clearly seen on untreated samples; this is an indication of initial demineralisation and creation of depressions, most probably on places of the lower density of crystals. Other authors have also shown similar results [29].

Many other researchers, who used different techniques, also showed negative effects of carbonated beverages on both tooth enamel and composite fillings [30-33]. In the paper [30] it was demonstrated that carbonated beverages reduce the physical properties of enamel (hardness and elasticity module) as a consequence of enamel erosion. The paper also shows that such drinks have a more prominent effect compared to, for example, orange juice. Some authors have found that unpolished enamel is less susceptible to erosions compared to the polished one and that the enamel structure itself in *vitro* conditions can significantly influence the progression of erosions. This particularly relates to the presence of aprismatic enamel, cracks and perikymata [34].

The chemical composition of acidic beverages is certainly significant in the modification of the mineral structure and thereby of the mechanical properties of enamel [34]. That acidity of carbonated cola drinks primarily originates from the phosphoric acid has been shown in the paper [32]. It has been known from earlier researches that the drinks with citric acid cause higher enamel erosion compared to the drinks that contain only phosphoric acid, such as in coca cola [34]. In addition to pH, the liquid environment around the enamel and temperature are also relevant, and they all affect the physical properties such as the elasticity module, hardness and surface roughness of human enamel.

The results obtained in *in vitro* studies, such

as ours, can only be partly transferred on what is going in clinical conditions, which largely depends on the dynamics of distortion of demineralisation-remineralisation kinetics. Remineralization periods that lead to enamel recovery, reduction of roughness and microhardness increase have not been included in our studies [21, 26, 35]. Likewise, certain studies have shown increased resistance with the application of remineralisation pastes or alternating presence of enamel in saliva and the drink [21, 27].

In conclusion, the analysis of the samples after five minutes of coca cola action shows both the qualitative and quantitative difference. On treated enamel samples, a lower density of crystals with larger areas between prisms is seen. The surface is more irregular with higher depressions. The analysis of Ra of treated and untreated surfaces confirms such findings as well as the higher roughness of enamel surface treated with coca cola. The initial irregularity (after five minutes of treatment) is the consequence of the partial destruction of the aprismatic layer and the attack with the crystals of less compacted parts, such as perikymata or lamellae. The findings correlate with the other studies [29, 36].

The results obtained indicate that the treated surfaces are statistically significantly rougher (higher Ra), which is also confirmed with the morphological signs of depressions and decrease of enamel crystals.

Under the influence of coca cola, there is statistically significant disruption of the integrity of the crystal grid which is observed after five minutes of action, which corresponds to the most common way of consumption of this carbonated drink. Enamel damages are primarily related to the decrease of the crystal thickness and creation of fissures which increase the overall roughness of the surface. Higher roughness leads to greater contact with acids thus increasing the possibility of further damage.

Our AFM researches indicate the irregular surface structure of enamel in physiological conditions, which has a certain degree of roughness, depending on the histological properties, the presence of pellicle and compactness of crystal units in prisms.

Despite its limitations, our studies have proved that the use of AFM enables successful monitoring of changes on enamel surface as well as the interpretation of the ultrastructural configuration of the crystal stage and the damage created under the influence of different external factors.

Likewise, we believe that coca cola, as the most common carbonated beverage, exerts aggressive influence on enamel surface thus endangering the mineral structure.

Further research should be directed toward the changes of ultrastructural enamel during the period two h after the action of the examined agent, which should be correlated with the physiological conditions in the oral cavity; subsequently, causal relationship between them should be established.

References

1. Al-Majed I, Maguire A, Murray JJ. Risk factors for dental erosion in 5–6 year old and 12–14 year old boys in Saudi Arabia. Community Dent Oral Epidemiol. 2002; 30: 38–46. https://doi.org/10.1034/j.1600-0528.2002.300106.x PMid:11918574

2. Attin T, Meyer K, Hellwig E. et al. Effect of mineral supplements to citric acid on enamel erosion. Arch Oral Biol. 2003; 48: 753–759. https://doi.org/10.1016/S0003-9969(03)00156-0

3. Attin T, et al. Impact of modified acidic soft drinks on enamel erosion. Oral Diseases. 2005; 11:7–12. https://doi.org/10.1111/j.1601-0825.2004.01056.x PMid:15641960

4. Bozec L, de Groot J, Odlyha M, Nicholls B, Horton MA. Mineralised tissues as nanomaterials: Analysis by atomic force microscopy. IEEE Proc. Nanobiotechnol. 2005;152:183−186.

5. Barbour ME, Finke M, Parker D.M, Hughes JA, Allen GC, Addy M. The relationship between enamel softening and erosion caused by soft drinks at a range of temperatures. J Dent. 2006; 34:207-213. https://doi.org/10.1016/j.jdent.2005.06.002 PMid:16112333

6. Barbour ME, Finke M, Parker DM, Hughes JA, Allen GC, Addy M. The relationship. between softening and erosion caused by soft drinks at a range of temperatures. J Dent. 2006;34:207-213. https://doi.org/10.1016/j.jdent.2005.06.002 PMid:16112333

7. Barbour ME, Parker DM, Allen GC, Jandt KD. Human enamel erosion in constant composition citric acid solutions as a function of degree of saturation with respect to hydroxyapatite, Journal of Oral Rehabilitation. 2005; 32:16-21. https://doi.org/10.1111/j.1365-2842.2004.01365.x PMid:15634296

8. Barbour M, Shellis RP. An investigation using atomic force microscopy nanoindentation of dental enamel demineralization as a function of undissociated acid concentration and differential buffer capacity. Phys Med Biol. 2007;52:899-910. https://doi.org/10.1088/0031-9155/52/4/003 PMid:17264360

9. Badra VV, Faraoni JJ, Ramos RP, Palma-Dibb RG. Influence of different beverages on the microhardness and surface roughness of resin composites. Oper Dent. 2005;30:213-219. PMid:15853107

10. Chandra S, et al. Textbook of Dental and Oral Histology with Embryology and MCQS. 2/E, Jaypee Brothers Medical Publishers (P) Ltd.; 2nd edition, 2010.

11. Devlin H, Bassiouny MA, Boston D. Hardness of enamel exposed to Coca-Cola and artificial saliva. J Oral Rehabil. 2006;33(1):26-30. https://doi.org/10.1111/j.1365-2842.2006.01533.x PMid:16409513

12. Fzhen-Jiang Cheng et al. The enamel softening and loss during early erosion studied by AFM, SEM and nanoindentation 2009 Biomed. Mater. 2009; 4:015020. https://doi.org/10.1088/1748-6041/4/1/015020 PMid:19193971

13. Field J, Waterhouse P, Germa M. Quantifying and qualifying surface changes on dental hard tissues in vitro. Journal of dentistry. 2010; 38:182–19. https://doi.org/10.1016/j.jdent.2010.01.002 PMid:20079800

14. Freitas RA, Jr. Nanodentistry. J Am Dent Assoc. 2000;131:1559−1565. https://doi.org/10.14219/jada.archive.2000.0084 PMid:11103574

15. Gimzewski J, Miles MJ. High-speed atomic force microscopy of dental enamel dissolution in citric acid. Arch Histol Cytol. 2009;72:328−335.

16. Holme B, Hove LH, Tveit AB. Using white light interferometry to measure etching of dental enamel. Measurement. 2005; 38:137−147. https://doi.org/10.1016/j.measurement.2005.04.003

17. Humphris ADL, Miles MJ, Hobbs JK. A mechanical microscope:

High-speed atomic force microscopy. Appl Phys Lett. 2005; 86:281−288. https://doi.org/10.1063/1.1855407

18. Kimyai S, et al. (2011), Effect of three prophylaxis methods on surface roughness of giomer. Medicina Oral, Patología Oral y Cirugía Bucal. 2011; 16(1): e110-e114. https://doi.org/10.4317/medoral.16.e110

19. Lee GJ, et al. A quantitative AFM analysis of nano-scale surface roughness in various orthodontic brackets. Micron. 2010; 41(7):775-782. https://doi.org/10.1016/j.micron.2010.05.013 PMid:20646928

20. Lussy A, Jaeggi T, Jaeggi-Scharer S. Prediction of the erosive potential of some beverages. Caries Res. 1995;29:349-354. https://doi.org/10.1159/000262091

21. Machado C, Lacefield W, Catledge A. Human Enamel Nanohardness, Elastic Modulus and Surface Integrity after Beverage Contact, Braz Dent J. 2008; 19(1): 68−72. https://doi.org/10.1590/S0103-64402008000100012 PMid:18438563

22. Maupome G, Diez de Bonilla J, Torres-Villasenor G, Andrade-Delgado LC, Casta-o VM. In vitro quantitative assessment of enamel microhardness after exposure to eroding immersion in cola drink. Caries Res. 1998;32:148-153. https://doi.org/10.1159/000016445 PMid:9580392

23. Meurman JH, Frank RM. Progression and surface ultrastructure of in vitro caused erosive lesions in human and bovineenamel. Caries Res. 1991;25:81-8. https://doi.org/10.1159/000261348

24. Mirjanic Dj, MirjanicV, Vojinovic J. AFM analysis of nanostructure enamel after the aggressive beverage. Contemporary materials. 2015;24:603-617.

25. Neville B, Damm DD, Allen CM, Bouquot J. Oral and Maxillofacial Pathology, 3rd Edition, Saunders, 2008.

26. Oliver WC, Pharr GM. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. J Mater Res. 1992; 7:1564-1583. https://doi.org/10.1557/JMR.1992.1564

27. Poggio C, et al. Impact of two toothpastes on repairing enamel erosion produced by a soft drink: an AFM in vitro study. Journal of Dentistry. 2010; 38(11): 868-874. https://doi.org/10.1016/j.jdent.2010.07.010 PMid:20673785

28. Mythri H, Chandu GN, Prashant GM, Reddy VS. Effect of four fruit juices on pH of dental plaque-A four period cross-over study. Journal of Indian Association of Public Health Dentistry. 2008;6(11):53.

29. Roveri N, Battistella E, Bianchi CL, Foltran I, Foresti E, Iafisco M, Lelli M, Naldoni A, Palazzo B, Rimondini L. Surface enamel remineralization: biomimetic apatite nanocrystals and fluoride ions different effects. Journal of Nanomaterials. 2009;2009:8. https://doi.org/10.1155/2009/746383

30. Schmid T, Burkhard J, Yeo BS, Zhang W, Zenobi R. Towards chemical analysis of nanostructures in biofilms I: Imaging of biological nanostructures. Anal. Bioanal. Chem. 2008;391:1899−1905. https://doi.org/10.1007/s00216-008-2100-2 PMid:18427786

31. Schlueter N, Hara A, Shellis RP, Ganss C. Methods for the measurement and characterization of erosion in enamel and dentine. Caries Res. 2011;45 (Suppl 1):13-23. https://doi.org/10.1159/000326819 PMid:21625129

32. Smith A. The Oxford companion to American food and drink. Oxford University Press US, 2007:451.

33. Sigusch BW, Beyer M, Heurich E, Jandt KD. Erosion des Zahnhartgewebes - Neue pathogenetische und diagnostische Aspekte durch Atom-Kraft-Mikroskopie und Nanoindentation. ZWR. 2008; 117:152-158. https://doi.org/10.1055/s-2008-1076779

34. Sigusch BW, Beyer M, Heurich E, Jandt KD. Erosion des Zahnhartgewebes - Neue pathogenetische und diagnostische Aspekte durch Atom-Kraft-Mikroskopie und Nanoindentation. ZWR. 2008; 117:152-158. https://doi.org/10.1055/s-2008-1076779

35. Šaćirović S, Imamović M, Ketin S, Milešević T, Biočanin R. Elements of Water Bacteriology with Special Reference to Public Drinking Water. Acta Medica Mediane. 2015.

36. Quartarona E, et al. Surface kinetic roughening caused by dental erosion: An atomic force microscopy study. J Appl Phys. 2008; 103: 104702. https://doi.org/10.1063/1.2927386

37. Zeng Q, Zhang YH, Xing ZH, Zhao XJ. Comparisons of the Apatite Presentation and Demineralization in Enamel and Cementum by Atomic Force Microscopy. InKey Engineering Materials. 2007; 330:733-736. https://doi.org/10.4028/0-87849-422-7.733

38. U.S. Department of Health and Human Services. Preventing Chronic Diseases: Investing Wisely in Health. National Center for Chronic Disease Prevention and Health, 2007.

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):994-999.
https://doi.org/10.3889/oamjms.2017.164
eISSN: 1857-9655
Stomatology

The Effect of Strontium Ranelate Gel on Bone Formation in Calvarial Critical Size Defects

Hani El Nahass[1], Nada Nour El Din[2], Sherine Adel Nasry[3*],

[1]Oral Medicine and Periodontology Department, Faculty of Dentistry, Cairo University, Cairo, Egypt; [2]Department of Oral Pathology, Faculty of Dentistry, Cairo University, Cairo, Egypt; [3]Orodental Division, National Research Centre, Cairo, Egypt

Citation: El Nahass H, El Din NN, Nasry SA. The Effect of Strontium Ranelate Gel on Bone Formation in Calvarial Critical Size Defects. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):994-999. https://doi.org/10.3889/oamjms.2017.164

Keywords: Strontium ranelate; Bone formation; Critical defect; Animal experiment.

***Correspondence:** Sherine Adel Nasry. Orodental Division, National Research Centre, Cairo, Egypt. E-mail: nasrysherine@yahoo.com

Received: 16-Aug-2017; **Revised:** 01-Nov-2017; **Accepted:** 22-Nov-2017; **Online first:** 12-Dec-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: The current study was designed to investigate the effectiveness of locally applied Strontium ranelate to induce bone formation.

MATERIALS AND METHODS: Forty-eight female rats were divided into six groups (eight rats in each group): The three test groups included Strontium (SR) 2.5 mg, 5 mg and 10 mg that was dissolved in methylcellulose gel. The control groups included methylcellulose, simvastatin 5 mg and a negative control where the defect was left to heal without any intervention. At 44 days the groups were sacrificed, and the bone defects were assessed histomorphometically to assess bone formation. The data was statistically analysed.

RESULTS: There was a statistically significant difference in the amount of new bone formation between all groups, where the 2.5 mg SR group showed the highest median bone percentage, is 41.95 %, followed by the 5, and 10 mg SR demonstrating a median bone are a percentage of 39.89%, and 30.19% respectively. Simvastatin showed a median bone percentage of 36.07 %, while the methylcellulose and the negative control groups demonstrated the lowest median area percentage of 23.12 and 20.70 % respectively.

CONCLUSIONS: The study showed that the local application of an SR could up-regulate the bone formation and may prove to be a cost-effective method of bone regeneration.

Introduction

Epidemiological studies have shown that 50% of the adult population between the ages of 45 and 65 years exhibited bone loss around teeth that are caused by periodontal disease. Various materials have been proposed to treat the bone defects which are a hallmark of the periodontal disease. The stimulation of local bone formation could positively affect the healing of isolated bony defects [1, 2]. Growth factors have exhibited the ability to induce bone formation [3]. On the other hand host tissues as well as a possible antibody response decreased the appeal for growth factor utilization.

The local application of systemic bone modulating drug that is commonly used to treat bone disease could offer a plausible alternative to growth factors. The advantage of local drug delivery is releasing the drug directly into the site of infection for a sufficient period without systemic exposure preventing both bacterial resistance and drug-related systemic side effects [4].

Various drugs have been studied using local delivery to improve the periodontal health and to achieve periodontal regeneration. Pharmacological agents offer great promise in this direction. Simvastatin, a widely used cholesterol-lowering drug, was shown to stimulate bone both in vitro and in vivo when applied locally, and its effects on bone metabolism favour its use in the treatment of periodontal defects. Additional advantages of simvastatin are its antioxidant and anti-inflammatory properties which could further facilitate healing of periodontal intrabony defects [5, 6].

Strontium ranelate (SR), a drug licensed for the treatment of postmenopausal osteoporosis is

composed of an organic moiety (ranelic acid) and two atoms of stable strontium. In contrast to most currently used drugs currently that inhibit bone resorption rather than stimulating bone formation, SR has a novel mode of action, both increasing bone formation and reducing bone resorption, rebalancing bone turnover in favor of bone formation and increases bone strength It has a dual mode of action, both increasing bone formation and decreasing bone resorption [7-9]. The mechanism behind its dual mode of action was explained in several studies as by enhancing osteoblastic cell replication and activity and decreasing preosteoblast differentiation and osteoclastic activity [9, 10].

In an experiment that was conducted to examine the cytotoxicity of SR on human periodontal ligament (PDL) fibroblasts, SR was found to be non-toxic at appropriate concentrations. In the experiment, PDL cells were treated with SR at 20, 10, 5 and 2.5 mg/ml. The highest SR concentrations (20 and 10 mg) had significantly lower cell viability and cell numbers than those in 5 and 2.5 mg/ml. The author recommended the need for preclinical tests to further assess its safety and effectiveness before clinical use [11].

Methylcellulose has been used as a drug carrier for releasing in several pharmaceutical preparations because it is a harmless, nontoxic material that does not sensitise the tissues [12].

The critical-size rat calvarial defect is a convenient model for evaluating bone regenerative effects of bio-materials. It is accessible, simple and is unable to regenerate spontaneously because of the distance that the progenitor cells and blood vessels must travel from the healing margin to bridge the defect is great [13, 14]. In rat calvaria, the defect is 8 mm and is well suited for placement of particulate materials [15].

The purpose of this study was to evaluate qualitatively and quantitatively the ability of strontium ranelate to induce bone regeneration in rat critical-sized bone defects

Methods

Experimental design

The animal protocol was approved by the Ethical Committee of the National Research Centre, Cairo, Egypt. Forty-eight adult Sprague-Dawley rats (275 to 300 g) were randomly divided into six groups of eight animals each: The three test groups included Strontium 2.5 (SR), 5 mg and 10 mg that was dissolved in methylcellulose gel. The control groups included methylcellulose, simvastatin 1.2 mg and a negative control where the defect was left to heal without any intervention. In all group except the negative control group the gel was applied to the defect and at 44 days the groups were sacrificed and the bone defects were assessed histomorphometically to assess bone formation: Formulation of Strontium Ranelate (SR) gel A 4.0% (w/v) methylcellulose (4,000 cps) gel, (Sigma chemicals Co., St . Louis, MO), which served as the vehicle for SR was previously prepared, by adding the required amount of polymer to hot distilled water and cooling to gel at room temperature. Then 2.5, 5 and 10 mg of SR (EVA pharmaceuticals, Cairo, Egypt)was dissolved in 1 ml of methylcellulose [16].

Surgical Protocol

Animals were sedated using an intramuscular injection of two parts ketamine (100 mg/ml) Ketamar 100 (mg/ml) Amoun and one part xylazine (20 mg/ml) Xylazine Albrecht, Germany; Ceva, Germany; Serumber, Germany at a dosage of 0.2 ml/100 g, additional sedation was given if needed. Routine infiltration anaesthesia with 2% lidocaine and 1:100,000 epinephrine (Octacaine) was used at the surgical site.

An incision was made in the sagittal plane across the cranium, and a full-thickness flap was reflected, exposing the calvarial bone. A standardised, circular, transosseous defect, 8 mm in diameter, was created on the cranium with the use of a saline-cooled trephine drill (Biomet 3i, Palm Beach Gardens, USA). An 8 mm dental trephine was used to create a standardised, circular, transosseous defect, 8 mm in diameter, of the rat calvaria. The trephined calvarial bone was then carefully removed to avoid perforation of the dura mater.

To achieve standardisation 1 ml of the formula (either methylcellulose alone, with simvastatin or with strontium) was injected within the created defect.

In the first three groups, a 2.5, 5 and 10 mg/ml dose of SR added to an inert methylcellulose gel was inserted into the defect. The two positive control groups included a group were 5 mg of simvastatin dissolved in methylcellulose and a group of one ml of methylcellulose gel that was added to critical defect size. No material was added to the last group acting as a negative control, where the defect was left to heal without any intervention. The periosteum and skin were then closed and sutured with 4-0 Egysorb, (Taisier - med, Egypt). The animals were sacrificed after 44 days using CO_2 asphyxiation.

Sample Preparation

The calvarium was removed, and the specimens were fixed in 10% formalin for 48 hours. Specimens were then transferred to the decalcifying solution (RDO Apex Engineering Products

Corporation, IL - USA). After 72 hours, specimens were processed through ascending grades of alcohol, cleared in xylene and embedded in paraffin. Formalin-fixed, decalcified, paraffin-embedded tissue blocks were obtained. Paraffin sections of 5-micron thickness were prepared from each of the paraffin blocks with a longitudinal cut to show the defect with the surrounding bone healing. For routine histopathological examination, slides were stained with Hematoxylin & Eosin (H&E) stain. Assessment of new bone formation and morphometric analysis were performed on Masson trichrome-stained sections, where the mineralised bone was stained red, osteoid dark bluish green and collagen fibres greenish blue.

Histomorphometric Analysis

A quantitative study was obtained with the aid of Leica Qwin 500 LTD (Leica Microsystem Corporation, Cambridge, England) using the software Quin 500 (England). The slides were examined using power magnification X 100 for measurement of area percent of newly formed bone detected by Masson trichrome stain. The red stained areas were outlined and then measured in micrometre square in successive fields of the section and the total new bone formation in the specimen was then calculated automatically by the morphometry software.

Statistical Analysis

Numerical data were explored for normality by checking the data distribution and using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data showed non-parametric distribution. Data were represented by median and range values. Kruskal - Wallis test was used to compare the different groups. Mann-Whitney U test with Bonferroni's adjustment was used for pairwise comparisons between the groups. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 23 for Windows.

Results

Histopathological Results

Histopathological examination showed areas filled with red blood cells, inflammatory cells and fibroblastic proliferation with neovascularisation and granulation tissue formation as well as mild osteoblastic proliferation with osteoid tissue was noticed together with few foci of disorganised woven bone formation especially at the periphery of the defect. The centre showed fewer areas of osteoid & bone formation.

Strontium Treated Group

The 2.5 and 5 mg groups showed more attempts at bone formation with less inflammatory reaction as compared to the control group. Moderate fibroblastic proliferation was seen with marked osteoblastic cells proliferation. More foci of disorganised, woven bone were observed with the more collagenous extracellular matrix. The active bone formation was suggested as newly formed osteoid was observed, in the form of spicules rimmed by numerous osteoblasts as well as osteoid tissue having viable osteocytes within widened lacunae. Changes were seen extending from the margins down to the centre of the defect (Fig. 1).

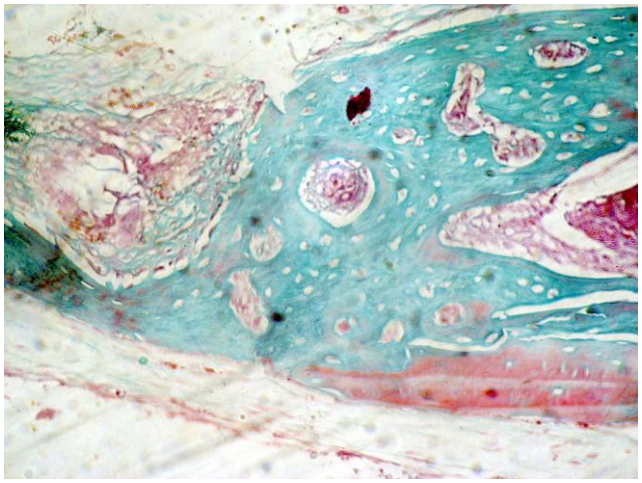


Figure 1: Photomicrograph of the methylcellulose group showing areas of osteoid formation and collagen without evidence of maturation (Masson trichrome, original magnification X 100)

The 10 mg group showed no osteoblastic proliferation at all. Large areas were filled with fibroblasts, and collagen fibres with heavy inflammatory cell infiltrate (Fig. 2).

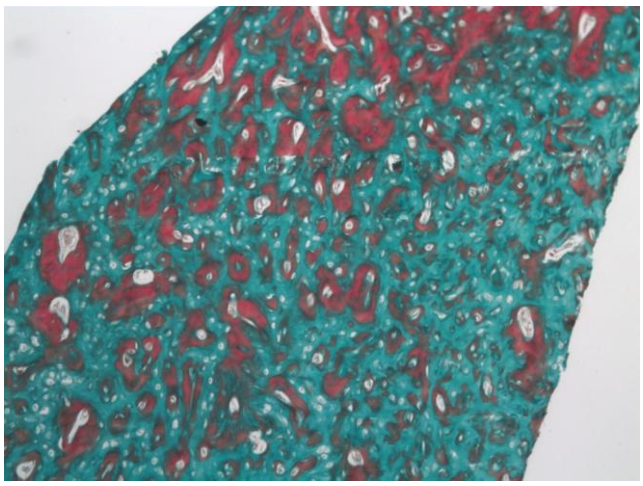


Figure 2: Photomicrograph of the 5mg strontium group showing areas of osteoid and woven bone formation with large marrow cavities (Masson trichrome, original magnification X 100)

Simvastatin Treated Group

The simvastatin group showed large, alternating areas of lamellar and woven bone formation with marked osteoblastic rimming and proliferation. No inflammatory cell infiltrate was detected. The new bone filled the margins of the defect till the centre (Fig. 3).

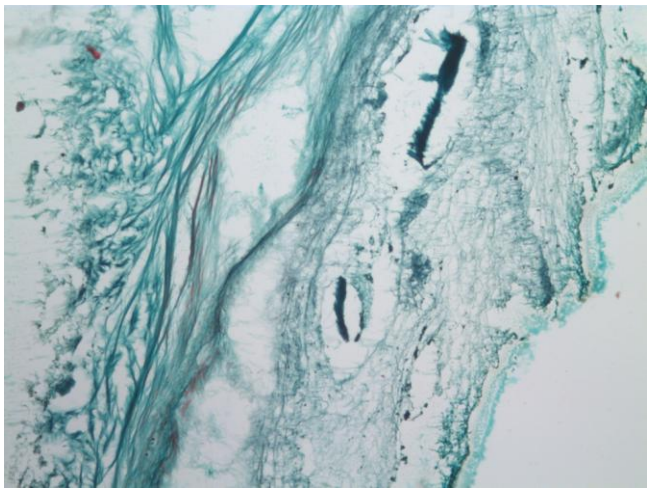


Figure 3: Photomicrograph of the 10mg strontium group showing large areas of collagen fibres with no attempts at osteoid or bone formation (Masson trichrome, original magnification X 100)

Negative control Group

In the negative control groups, the limited bone repair was observed only at the margins of the defect, as recognised by the irregularly shaped bone trabeculae of newly formed mineralised bone and newly formed osteoid tissue. No bone formation or evidence of mineralization in the centre of the defect, where only a mature and well organized fibrous tissue was noticed.

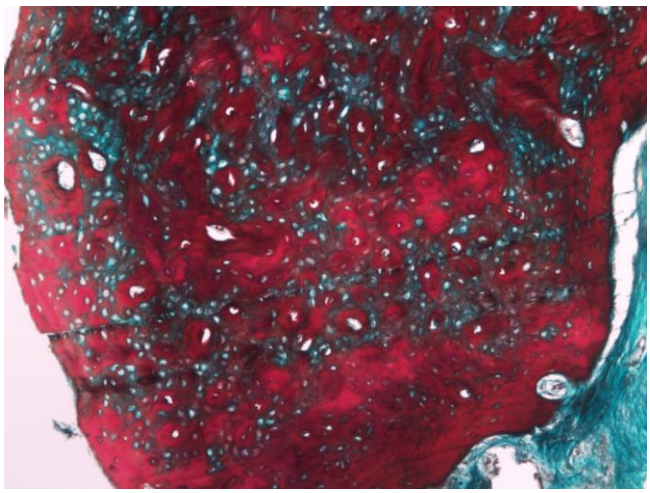


Figure 4: Photomicrograph of the simvastatin group showing large alternating areas of osteoid and woven bone formation (Masson trichrome, original magnification X 100)

Statistical Results

Descriptive statistics of area % values are presented in Table 1. There was a statistically significant difference between area % values of the different groups. Pair-wise comparisons between the groups revealed that there was no statistically significant difference between Strontium (2.5 mg), Strontium (5 mg) and Simvastatin with an as significant difference with Methylcellulose and Negative control.

Table 1: Comparison between area % of the five groups

Area %	Median	Minimum	Maximum	Rank	p-value
Strontium (2.5 mg)	41.95	22.15	44.05	a	≤0.001*
Strontium (5 mg)	39.89	19.56	57.25	a	
Strontium (10 mg)	30.19	16.09	46.96	ab	
Simvastatin	36.07	26.89	77.61	a	
Methylcellulose	23.12	11.01	30.48	b	
Negative control	20.70	10.50	23.70	b	

*: Significant at P ≤ 0.05, Different letters in the same column are statistically significantly different

For the negative control group, the maximum bone area percentage was 23.7%, while that of methylcellulose was 30.48%, both were statistically different than the strontium and simvastatin groups., where the strontium groups showed a maximum area percentage of 44.05, 57.25 and 46.96% for the 2.5, 5 and 10 mg strontium respectively while that of simvastatin showed the higher area percentage with 77.61%.

Discussion

The study aimed to evaluate the bone regenerative potential of locally applied SR in different concentrations. The rat calvarial critical-sized defect is a well-established animal model for regenerative bone studies. The bone defect of critical size is unable to regenerate. Thus it is considered to be a suitable for the evaluation of osteoinductivity of various materials. Only materials that can induce bone regeneration show signs of bone formation throughout the entire critical defect [16, 17].

SR was used as it showed promising results in osteoporosis therapy, where it is known to increase in vitro osteoblasts' differentiation from progenitors, as well as osteoblastic activity and survival. Furthermore, SR regulates osteoblast - induced osteoclastogenesis, and prevents bone resorption by decreasing osteoclastic differentiation and activity, while increasing their apoptosis [18].

The incorporation of strontium into mesoporous bioactive glass scaffolds was shown to be a viable way to stimulate the biological response of periodontal ligament cells as well as stimulate bone formation in bone defects in an osteoporotic rat model [19, 20].

Previous studies showed that 44 days represent adequate timing to analyse newly formed

bone in rat calvarial defects [2]. The bone turnover rate of rats and the ability of fast healing make the evaluation of bone formation 6 to 8 weeks after implantation of a biomaterial appropriate. Thus no major differences are expected to be observed in bone formation even if prolonged healing time is allowed [16].

All defect sites exhibited bone formation, but there was a statistically significant difference in the amount of new bone formation between all groups, where the 2.5 SR group showed the highest median bone percentage, being 41.95 %, followed by the 5, and 10 mg SR demonstrating a median bone are percentage of 39.89%, and 30.19% respectively. Simvastatin showed a median bone percentage of 36.07%, while the methylcellulose and the negative control groups demonstrated the lowest median area percentage of 23.12 and 20.70 % respectively.

The histologic evaluation of the 2.5 and 5 mg groups showed more attempts at bone formation with less inflammatory reaction as compared to the control group. This could be attributed to the anti-inflammatory effect of SR that has been proposed by due to the antagonising effect of SR to NF-κB activation. Furthermore, in unpublished data by the same group, they proposed that SR may act as TNF inhibitor which could explain its anti-inflammatory properties [21].

Bone defect healing occurs naturally after a phase of bleeding and inflammation and terminates with the formation of woven bone which is then remodelled by osteoclasts and replaced by lamellar bone by osteoblasts [3].

The osteoblasts presence was marked in the 2.5 and 5 mg SR groups compared to the control group) which may be explained due to the fact that SR has the ability to stimulate PGE2 production and osteoblastic differentiation in marrow stromal cells, which is markedly affected by inhibition of COX-2 activity or disruption of COX-2 gene expression [22]. Furthermore, [23] concluded that SR promotes the replication of osteoblasts which could explain the marked osteoblastic proliferation in the 2.5 and 5 mg SR groups.

Woven osteoid bone and mineralised bone presence were marked in the SR group than by the control group. All these observations could be clearly to the dual effect of SR that promoted the bone formation on several levels and through various pathways.

In the negative control groups, the limited bone repair was observed only at the margins of the defect, as recognised by the irregularly shaped bone trabeculae of newly formed mineralised bone and newly formed osteoid tissue. The findings of no bone formation or evidence of mineralization in the center of the defect, where only a mature and well organized fibrous tissue was noticed, is consistent with the calvarial nonunion defects study of [17] where both the central and peripheral regions of the 8 mm calvarial defects were characterized by dense fibrous tissue repair and inactive fibroblasts.

Simvastatin was used in the present study as a positive control, to compare its effect on bone formation with that of SR, where former research demonstrated that local application of simvastatin in alveolar defects could promote bone regeneration [12]. In the present study large, areas of lamellar and woven bone formation with marked osteoblastic proliferation where demonstrated histologically, where the new bone filled the margins of the defect till the centre with no detected inflammatory cell infiltrates. The local application has been demonstrated anti-inflammatory effects as well, anabolic effects on bone and a stimulatory effect on vasculogenesis which was attributed to the increase in the expression of BMP-2, and vascular endothelial growth factor [24] which may explain the results of the present study.Our findings are also in line with a former research that demonstrated the ability of local simvastatin application to enhance healing of the bone defects even in the diabetic rat models [25].

In conclusion, the current study demonstrated that the local application of a single dose of SR (2.5 and 5 mg) could up-regulate the bone formation. The local application of SR may prove to be a cost-effective and safe method to stimulate bone formation. However more in vivo studies and different concentrations are recommended to define the optimal dose to equilibrate soft tissue inflammation and bone stimulation.

Acknowledgement

The authors thank EVA Pharma Inc, Abdeen, Cairo, Egypt, for providing the Strontium Ranelate powder.

References

1. Intini G, Andreana S, Buhite RJ, Bobek L. A comparative analysis of bone formation induced by human demineralised freeze-dried bone and enamel matrix derivative in rat calvarial critical-size bone defects. J Periodontol. 2008; 79:1217–24. https://doi.org/10.1902/jop.2008.070435 PMid:18597604

2. Thylin MR, McConnell JC, Schmid MJ, Reckling RR, Ojha J, et al. Effects of simvastatin gels on murine calvarial bone. J Periodontol. 2002;73:1141-8. https://doi.org/10.1902/jop.2002.73.10.1141 PMid:12416771

3. Francis PO, McPherson JC, Cuenin MF, Hokett SD, Peacock ME, et al. Evaluation of a novel alloplast for osseous regeneration in the rat calvarial model. J Periodontol. 2003;74: 1023–31. https://doi.org/10.1902/jop.2003.74.7.1023 PMid:12931765

4. Puri K, Puri N. Local drug delivery agents as adjuncts to endodontic and periodontal therapy. J Med Life. 2013;6: 414–9. PMid:24868252 PMCid:PMC4034307

5. Montero J, Manzano G, Albaladejo A. The role of topical simvastatin on bone regeneration: A systematic review. J Clin Exp Dent. 2014;6: 286–90. https://doi.org/10.4317/jced.51415 PMid:25136432 PMCid:PMC4134860

6. Elavarasu S, Suthanthiran TK, Naveen D. Statins: A new era in local drug delivery. J Pharm Bioallied Sci. 2012; 4:S248-51. https://doi.org/10.4103/0975-7406.100225 PMid:23066263 PMCid:PMC3467872

7. Burlet N, Reginster J-Y. Strontium ranelate: the first dual acting treatment for postmenopausal osteoporosis. Clin Orthop Relat Res. 2006; 443:55–60. https://doi.org/10.1097/01.blo.0000200247.27253.e9 PMid:16462426

8. Hamdy NAT. Strontium ranelate improves bone microarchitecture in osteoporosis. Rheumatology. 2009; 48:9–13. https://doi.org/10.1093/rheumatology/kep274 PMid:19783592

9. Amini AR, Laurencin CT, Nukavarapu SP. Bone tissue engineering: recent advances and challenges. Crit Rev Biomed Eng. 2012; 40:363–408. https://doi.org/10.1615/CritRevBiomedEng.v40.i5.10 PMid:23339648 PMCid:PMC3766369

10. Zhang Y, Wei L, Wu C, Miron RJ. Periodontal regeneration using strontium-loaded mesoporous bioactive glass scaffolds in osteoporotic rats. PLoS One. 2014; 9 e104527. https://doi.org/10.1371/journal.pone.0104527 PMid:25116811 PMCid:PMC4130544

11. Er K, Polat ZA, Ozan F, Taşdemir T, Sezer U, et al. Cytotoxicity analysis of strontium ranelate on cultured human periodontal ligament fibroblasts: a preliminary report. J Formos Med Assoc. 2008; 107:609–15. https://doi.org/10.1016/S0929-6646(08)60178-3

12. Chen S, Yang JY, Zhang SY, Feng L, Ren J. Effects of simvastatin gel on bone regeneration in alveolar defects in miniature pigs. Chin Med J (Engl). 2011; 124:3953–8.

13. Gomes PS1, Fernandes MH. Rodent models in bone-related research: the relevance of calvarial defects in the assessment of bone regeneration strategies. Lab Anim. 2011; 45:14-24. https://doi.org/10.1258/la.2010.010085 PMid:21156759

14. Choi J, Jung U, Kim C, Eom T, Kang E, et al. The effects of newly formed synthetic peptide on bone regeneration in rat calvarial defects. J Periodontal Implant Sci. 2010; 40:11-8. https://doi.org/10.5051/jpis.2010.40.1.11 PMid:20498754 PMCid:PMC2872809

15. Schmitz JP, Hollinger JO. The critical size defect as an experimental model for craniomandibulofacial nonunions. Clin Orthop Relat Res. 1986; 299–308. https://doi.org/10.1097/00003086-198604000-00036

16. Intini G, Andreana S, Intini FE, Buhite RJ, Bobek LA. Calcium sulfate and platelet-rich plasma make a novel osteoinductive biomaterial for bone regeneration. J Transl Med. 2007; 5:13. https://doi.org/10.1186/1479-5876-5-13 PMid:17343737 PMCid:PMC1831762

17. Schmitz JP, Schwartz Z, Hollinger JO, Boyan BD. Characterization of rat calvarial nonunion defects. Acta Anat (Basel). 1990 ;138:185–92. https://doi.org/10.1159/000146937

18. Fonseca JE, Brandi ML. Mechanism of action of strontium ranelate: what are the facts? Clin Cases Miner Bone Metab. 2010; 7:17–8. PMid:22461285 PMCid:PMC2898000

19. Wu C, Zhou Y, Lin C, Chang J, Xiao Y. Strontium-containing mesoporous bioactive glass scaffolds with improved osteogenic/cementogenic differentiation of periodontal ligament cells for periodontal tissue engineering. Acta Biomater. 2012;8:3805–15. https://doi.org/10.1016/j.actbio.2012.06.023 PMid:22750735

20. Wei L, Ke J, Prasadam I, Miron RJ, Lin S, et al. A comparative study of Sr-incorporated mesoporous bioactive glass scaffolds for regeneration of osteopenic bone defects. Osteoporos Int. 2014; 25:2089–96. https://doi.org/10.1007/s00198-014-2735-0 PMid:24807629

21. Yamaguchi M, Weitzmann MN. The intact strontium ranelate complex stimulates osteoblastogenesis and suppresses osteoclastogenesis by antagonizing NF-κB activation. Mol Cell Biochem. 2012 ; 359:399–407. https://doi.org/10.1007/s11010-011-1034-8 PMid:21874315

22. Choudhary S, Halbout P, Alander C, Raisz L, Pilbeam C. Strontium ranelate promotes osteoblastic differentiation and mineralization of murine bone marrow stromal cells: involvement of prostaglandins. J Bone Miner Res. 2007; 22:1002–10. https://doi.org/10.1359/jbmr.070321 PMid:17371157

23. Caverzasio J. Strontium ranelate promotes osteoblastic cell replication through at least two different mechanisms. Bone. 2008; 42:1131–6. https://doi.org/10.1016/j.bone.2008.02.010 PMid:18378206

24. Maeda T, Kawane T, Horiuchi N. Statins augment vascular endothelial growth factor expression in osteoblastic cells via inhibition of protein prenylation. Endocrinology. 2003;144:681–92. https://doi.org/10.1210/en.2002-220682 PMid:12538631

25. Ezirganli Ş, Kazancioğlu HO, Mihmanli A, Aydin MŞ, Sharifov R, et al. The effect of local simvastatin application on critical size defects in the diabetic rats. Clin Oral Implants Res. 2014; 25:969–76. https://doi.org/10.1111/clr.12177 PMid:23600677

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):1000-1004.
https://doi.org/10.3889/oamjms.2017.208
eISSN: 1857-9655
Stomatology – Case Report

Prosthodontic Rehabilitation of Patient with Anterior Hyper Function Syndrome

Vesna Korunoska-Stevkovska[1], Ljuben Guguvcevski[1], Zaklina Menceva[2], Nikola Gigovski[1], Aneta Mijoska[1*], Julijana Nikolovska[1], Emilija Bajraktarova-Valjakova[1]

[1]Faculty for Dental Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]University Dental Clinic Ss Panteleimon, Skopje, Republic of Macedonia

Citation: Korunoska-Stevkovska V, Guguvcevski L, Menceva Z, Gigovski N, Mijoska A, Nikolovska J, Bajraktarova-Valjakova E. Prosthodontic Rehabilitation of Patient with Anterior Hyper Function Syndrome. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):1000-1004. https://doi.org/10.3889/oamjms.2017.208

Keywords: Anterior hyperfunction syndrome; Removable dentures.

***Correspondence:** Aneta Nikola Mijoska. Faculty of Dental Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: amijoska@yahoo.com

Received: 23-Aug-2017; **Revised:** 21-Sep-2017; **Accepted:** 27-Sep-2017; **Online first:** 05-Dec-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: The success of prosthetic rehabilitation in patients with removable dentures depends on the achievement of the aesthetics, phonetics and most of all, proper use in the mastication process. All the patients that receive removable prostheses need a feeding education program. They must cut the food into smaller pieces, extend the length of time necessary for chewing and place the food upon both the right and left sides of the mouth at once. Bilaterally chewing with dentures will contribute to increased efficiency and denture stability during mastication. Using the anterior teeth for biting, as a result of increased pressure on the anterior ridge may lead to the anterior hyperfunction syndrome.

CASE REPORT: The patient requested dental rehabilitation in our clinic for prosthetic dentistry two and a half years ago. We examined him and made therapy plan, for complete removable maxillary denture and partial mandibular denture. Besides our instructions for proper use of dentures and necessity for regular controls, his next visit was after two and a half years. He came with enlarged tuberosity and papillary hyperplasia in the pre-maxillary region. After oral surgery treatment (laser removing of hyperplastic tissue) and a healing period of four weeks, we made indirect relining on the upper denture, re-occlusion and re-articulation achieving weak contacts between the lower natural teeth and upper teeth of the complete denture. The patient was advised not to bite food with his anterior teeth, and avoid chewing very hard food which tends to imprint and displace dentures.

CONCLUSION: Anterior hyperfunction syndrome with its high incidence is a disease with the need of interdisciplinary therapy approach. Fast diagnosis, thorough clinical examination using all available diagnostic tools, and choosing the right treatment is very challenging.

Introduction

The anterior hyperfunction syndrome is associated with the combination syndrome or Kelly Sy.In 1972, Kelly presented clinical case, with the combination of edentulous maxilla opposed by natural mandibular anterior teeth, and called it the combinationsyndrome, today in his honor - Kelly`s syndrome [1]. This syndrome was presented as combination of some specificdestructive changes, in the hard and soft tissues, morphological and functional, and characterised by Kelly through fiveclinical simptoms [2] (Figure 1).

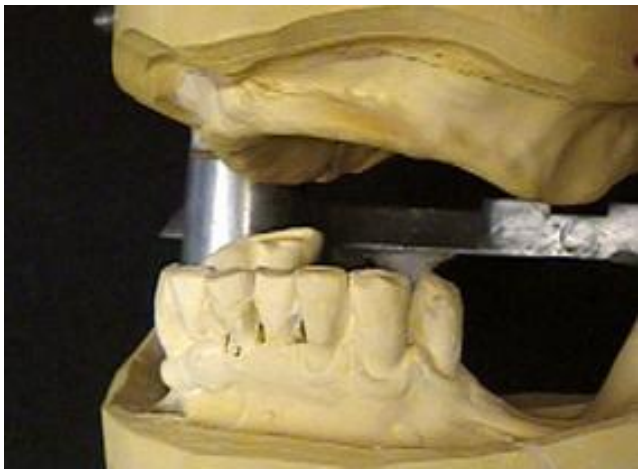


Figure 1: Kelly syndrome

The Glossary of Prosthodontic Terms defines Combination Syndrome as: The characteristic features that occur when an edentulous maxilla is opposed by natural mandibular anterior teeth, including loss of bone from the anterior portion of the maxillary ridge, overgrowth of the tuberosity, papillary hyperplasia of the hard palatal mucosa, extrusion of mandibular anterior teeth and loss of alveolar bone and ridge height beneath the mandibular removable partial denture bases, also called anterior hyperfunction syndrome [3, 4]. In 1979 Saunders, Gillis and Desjardins suggested extending the range of symptoms that characterize this syndrome by adding the following features: loss of the correct vertical dimension of occlusion, papillary hyperplasia on the hard palate, incorrect occlusal plane, patient's poor adaptation to dentures, occurrence of granuloma fissuratum and changes in the periodontium of existing natural teeth [5, 6].

The success of the prosthetic rehabilitation in patients with removable dentures depends on the achievement of the aesthetics and phonetic ability, and most of all, from the proper use of the devices in the masticatory process [7]. All the patients that receive removable prostheses need a feeding education program. They must cut the food into smaller pieces, extend the length of time necessary for chewing, and place the food upon both the right and left sides of the mouth at once [8]. The masticatory function is important for the proper preparation of food bolus in the mouth, and since dentures provide only 15-50% masticatory efficiencies, bilateral mastication is very important, for feeding and denture stability also [9]. During the adapting period with keratinisation of the residual alveolar ridge, after learning to have active control overdentures, patient's diet can change to harder food. In general, the food has to be cut in small bites and bitten with the side teeth, not the front one. The masticatory time is prolonged, and bilateral chewing is the best option. Bilateral chewing is very hard to achieve because in normal dentition there is unilateral chewing habit [10, 11]. Using the front teeth for biting hard food can cause higher pressure on the anterior residual ridge and appearance of anterior hyperfunction syndrome [12, 13].

The combination syndrome has a prevalence of 25% of individuals, wearing both complete denture opposing mandibular anterior teeth and a bilateral distal extension removable partial denture [14]. This syndrome is found in 48 8% of patients with a complete upper denture who do not wear a lower partial denture [15].

Case report

A 52-year-old male patient was received in the Clinic for Prosthodontic, two and a half year ago, for restorative treatment. On clinical examination, the patient had an edentulous maxilla and four natural mandibular anterior teeth. Initial therapy procedure included oral hygiene instructions, caries control and manufacturing two dentures, complete maxillary and partial mandibular denture.

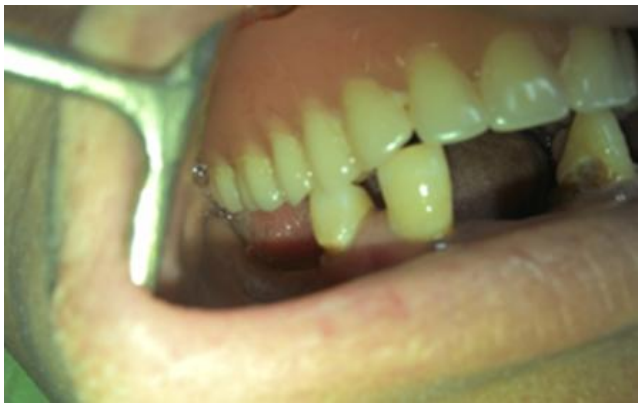


Figure 2: Super eruption of the frontal teeth and mandibular ridge resorption

Beside our advice for proper use, maintains, and regular check-ups, his first visit was after two and a half years. The patient's chief complaints were inadequate retention of maxillary complete denture and light swallowing in the upper canine region, without pain. During the intraoral examination, there was denture hyperplasia in the intercanine region of the upper jaw (Figure 2), flabby tissue.

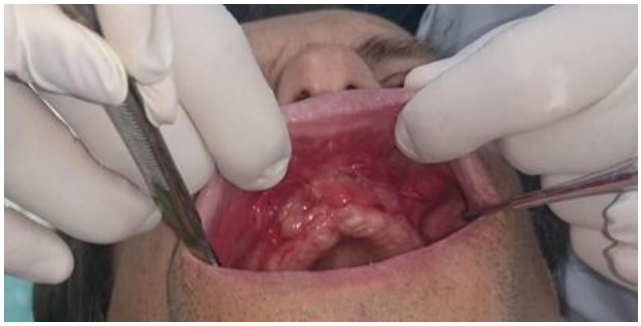


Figure 3: Denture-hyperplasia of maxilla

One of the most common tissue reactions to a chronically ill-fitting denture is this inflammatory hyperplasia. Although benign, the condition is relatively troublesome since it interferes with denture placement. Surgical excision is the treatment of choice. With conventional techniques, high levels of skill with the accurate planning of incisions and repositioning of tissues are mandatory to prevent loss of sulcus depth. A reliable alternative to conventional surgery is the use of lasers. In this case was the application of neodymium (Nd: YAG) laser by Fotona. The Nd: YAG laser has long been used for oral surgery as it was the first purpose-built laser for dentistry. The patient agreed with suggested oral surgery treatment (vestibuloplasty and excision of the flabby tissue) with soft-tissue laser by Fotona.

Figure 4: Fotona SP Dynamis

The oral surgical procedure was performed with a previous application of a 3% anaesthetic - Scandonest, in the form of local infiltration anaesthesia with the help of a carpule syringe for the maxillary nerve. The wound can be left for secondary healing without sutures or covering.

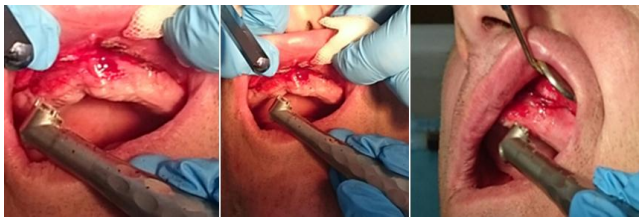


Figure 5: Use the Nd: YAG laser

Care should be taken with all soft tissue lasers to direct the laser parallel to the underlying bone or other anatomical structures like foremen or salivary glands. After the treatment, he was advised to maintain good oral hygiene and to apply Solcoseril dental adhesive three times a day, on the operation site.

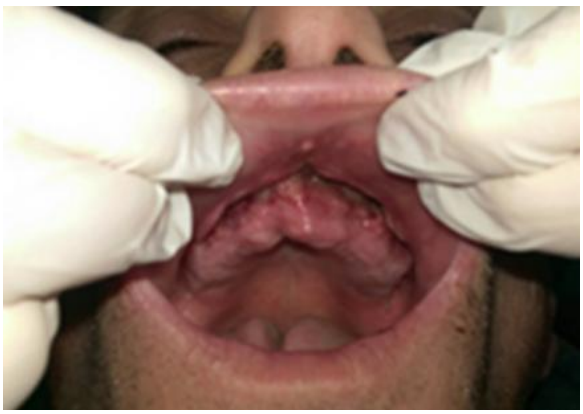


Figure 6: Condition after use of laser

After two weeks we made control chek-up, and found hyperplastic tissue still present, in some parts of the upper jaw. We decided that a revision of the surgical treatment was necessary for complete healing by (Figure 7).

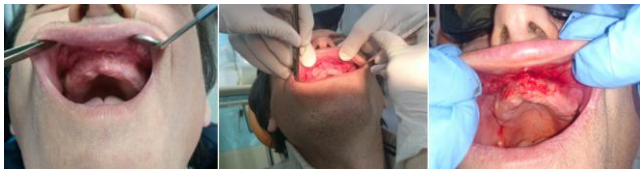


Figure 7: Revision of the surgical treatment by Nd: YAG

It took over four weeks for tissue consolidation and healing of the treatment wounds, and after that our prosthodontic treatment was indirect relining of the upper complete maxillary denture (Figure 8) to achieve retention and stability.

Figure 8: Postoperative situation without flabby tissue

Next part of the therapeutic procedure was achieving and maintaining good occlusal bilateral balance with proper re-occlusion and re-articulating of the dentures (Figure 9).

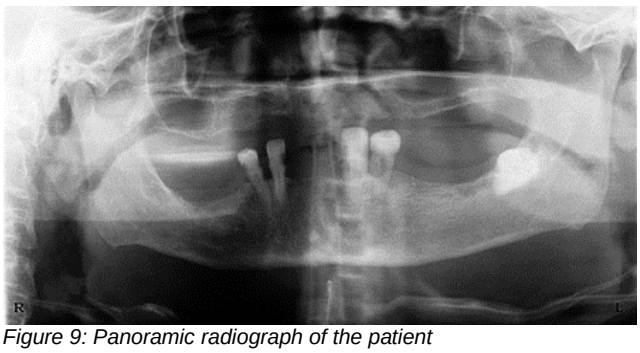


Figure 9: Panoramic radiograph of the patient

Panoramic radiograph showed a typical case of combination syndrome with resorption of the anterior maxillary bone, periodontal changes with super-eruption of opposed mandibular anterior teeth and severe resorption of the residual mandibular alveolar ridge (Figure 10).

Figure 10: New situation with relined existing dentures

The patient was given instructions about food diet habits; he was warned not to bite hard food with front teeth because very hard food can imprint his dentures in the soft tissue and interfere with their stability. Regular chek-ups were appointed in two weeks, once a month, and every three months during the first year. The dentures were with satisfactory retention and stabilisation, and soft tissue without hypertrophic changes.

Discussion

The constant occlusal pressure of natural teeth on the opposing alveolar ridge can cause a bone atrophy of the edentulous region. A reverse effect of hypertrophy of the alveolar bone with an extrusion of teeth opposed by an edentulous jaw segment is also evident and usually develops synchronously. There are several classifications of the most dominant changes within this syndrome in three classes and subclasses [16].

Occlusal forces can cause remodelling of the jaw bonelike atrophy or hypertrophy and intensity of the changes vary from mild, moderate, to severe. Different factors, including presence or absence of teeth, history of tooth loss, the periodontal condition of present teeth, previous prosthetic treatments, bone density some parafunctional and dietary habits, influence the changes [17].

When treating the patients with anterior hyperfunction syndrome, we must make attempts to minimise the destructive changes by distributing occlusal stress over the hard and soft tissues and developing balanced and stable occlusion [18]. The maximal supporting surface under the dentures base, mandibular posterior support and balanced bilateral occlusion are proposed for longevity without typical clinical problems.

Treatments are different, and they have to be directed towards removing of the hypertrophic tissue with conventional or laser surgery (vestibuloplasts and excision of flabby tissue followed by metallic denture base prosthesis).

Planned extractions followed by immediate dentures is treatment when arch requires alveolectomy along with the extraction of the anterior teeth for patients with the severe prognathic maxilla, periodontally compromised proclined anterior teeth present in the maxillary arch and missing mandibular posterior teeth [19]. In patients, without surgical treatment, we have to use some modified impression techniques over the flabby area, during prosthodontic treatment. Best materials for this are zinc oxide eugenol impression paste, green stick and elastomeric [20].

Implant supported overdentures, and the fixed metal-ceramic prosthesis is also a satisfactory, good treatment option, but they are far more expensive [21]. Another new therapy treatment is to substitute resorption of the bone with bone augmentation, grafting procedure (autogenous iliac crest, autogenous rib grafts, calvarial bone grafting, tibiae grafting), or use of the Osseo-inductive effect of bone morphogenic protein within endosseous dental implants, etc. [22-24].

When we have to choose the type of prosthodontic treatment the most important factor is the distance from the residual ridge to the occlusal plane. This distance is increased by the vertical loss of bone and of soft tissue that occurs in this patient. When this distance is higher than 15 mm, the most indicated prosthesis is a removable type (overdenture), because resorpted tissue is compensated with the acrylic material [25]. The use of fixed restorations of metal porcelain type is compromised because it can result in the production of elongated teeth, which are not very aesthetic and also lead to increased leverage forces [26].

In conclusion, anterior hyperfunction syndrome with its high incidence is a disease with the need of interdisciplinary therapy approach. Fast diagnosis, thorough clinical examination using all available diagnostic tools, and choosing the right treatment is very challenging. Oral surgery as part of the therapeutic protocol, proceed with proper prosthodontic treatment brought our long-term patient solution for all of his problems. Our procedures were also successfully directed towards the preservation of the health of the natural dentition and their masticatory function.

References

1. Murariu CM, Preoteasa E. The anterior hyperfunction syndrome – FEM Simulation. Romanian Journal of Oral Rehabilitation. 2009; 1(4):54-58.

2. Kelly E. Changes caused by a mandibular removable partial denture opposing a complete maxillary denture. J Prosthet Dentistry. 1972;27(2): 140-50. https://doi.org/10.1016/0022-3913(72)90190-4

3. SW Feng, PB Liao, MS Chen. Prosthodontic Treatment of a Patient with Combination Syndrome: A Clinical Case Report. Journal of Prosthodontics and Implantology 2012; 1 (1): 22-25.

4. The glossary of prosthodontic terms. J Prosthet Dent. 2005; 94: 10-92. https://doi.org/10.1016/j.prosdent.2005.03.013

5. Saunders TR, Gillis RE Jr, Desjardins RP. The maxillary complete denture opposing the mandibular bilateral distal-extension partial denture: treatment considerations. J Prosthet Dent. 1979; 41: 124-8. https://doi.org/10.1016/0022-3913(79)90292-0

6. Thiel C, Evans D, Burnett R. Combination syndrome associated with a mandibular implant.The Journal of Prosthetic Dentistry. 1996; 75: 107-113. https://doi.org/10.1016/S0022-3913(96)90084-0

7. Uçtali S, Hasanreisolu U, Ieri H. Cephalometric evaluation of maxillary complete, mandibular fixed-removable partial prosthesis:

a 5-year longitudinal study. J Oral Rehabil. 1997; 24:164-9. https://doi.org/10.1046/j.1365-2842.1997.00444.x

8. Arcelino FN, Wilson MJ, Adriana da Fonte Porto C.Masticatory Efficiency in Denture Wearers with Bilateral Balanced Occlusion and Canine Guidance. Braz Dent J. 2010; 21(2):165-169. https://doi.org/10.1590/S0103-64402010000200013

9. Rajendran S, Baburajan. Combination Syndrome. Int J Prosth Restor Dent. 2012; 2(4): 156-160. https://doi.org/10.5005/jp-journals-10019-1066

10. Schmitt SM. Combination syndrome: a treatment approach. J Prosthet Dent. 1985;54(5):66471. https://doi.org/10.1016/0022-3913(85)90246-X

11. Kelly E. Changes caused by a mandibular removable partial denture opposing a maxillary complete denture. J Prosthet Dent. 1972; 27:140-50. https://doi.org/10.1016/0022-3913(72)90190-4

12. Palmqvist S, Carlsson GE, Owall B. The combination syndrome: A literature review. J Prosthet Dent. 2003; 90:270-5. https://doi.org/10.1016/S0022-3913(03)00471-2

13. Carlsson GE. Responses of jawbone to pressure. Gerontology. 2004; 21: 65-70. https://doi.org/10.1111/j.1741-2358.2004.00005.x

14. Shen K, Gongloff RK. Prevalence of the 'combination syndrome' among denture patients. J Prosthet Dent. 1989; 62:642-4. https://doi.org/10.1016/0022-3913(89)90582-9

15. Kumar L, Shefali S."Kelly's Syndrome"- prevention, using Implant Supported Hybrid Denture: Clinical Considerations and Case Report with 5 year follow up. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2017; 16(6): 21-26. https://doi.org/10.9790/0853-1606042126

16. Pal KS, Sarapur S, Gaikwad A, Ali Z. Combination Syndrome: A Review of Classification and Treatment Modalities. J Res Adv Dent 2015; 4:1:11-17.

17. Tolstunov L. Combination syndrome: classification and case report. Journal of Oral Implantology. 2007; 33(3):139-51. https://doi.org/10.1563/1548-1336(2007)33[139:CSCACR]2.0.CO;2

18. McFadden DD. Pre-prosthetic surgery options for fixed dental implant reconstruction of the atrophic maxilla. Ann R AustralasColl Dent Surg. 2000; 15:61–64. PMid:11709979

19. Toolson LB, Smith DE. A two year longitudinal study of overdenture patients, Part 1: Incidence and control of caries on overdenture abutments. J Prosthet Dent. 1978; 40:486-9. https://doi.org/10.1016/0022-3913(78)90078-1

20. Keltjens H, Kayser A, Hertel R, Battistuzzi P. Distal extension removable partial dentures supported by implants and residual teeth: considerations and case reports. The International Journal of Oral & Maxillofacial Implants. 1993; 8(2):208-13. PMid:8359879

21. Thiel C, Evans D, Burnett R. Combination syndrome associated with a mandibular implant.J Prosthet Dent. 1996 ;75(2):107-13. https://doi.org/10.1016/S0022-3913(96)90084-0

22. Schoeman R, Subramanian L. The use of orthognathic surgery to facilitate implant placement: a case report. Int J Oral Maxillofac Implants. 1996; 11:682–684. PMid:8908869

23. Boyne P, Jones SD. Demonstration of the osseoinductive effect of bone morphogenic protein within endosseous dental implants. Implant Dent. 2004; 13:180–184. https://doi.org/10.1097/01.ID.0000127520.06443.42 PMid:15179095

24. Lynch C, Allen P. Management of the flabby ridge: using contemporary materials to solve an old problem. British Dental Journal. 2006; 200(5):258-61. https://doi.org/10.1038/sj.bdj.4813306 PMid:16528326

25. Jivraj S, Chee W, Corrado P. Treatment planning of the edentulous maxilla. British Dental Journal 2006; 201(5):261-80. https://doi.org/10.1038/sj.bdj.4813952 PMid:16960607

26. Tallgren A. The continuing reduction of the residual alveolar ridges in complete denture wearers: a mixed longitudinal study covering 25 years. J Prosthet Dent. 1972; 27:120-32. https://doi.org/10.1016/0022-3913(72)90188-6

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):1005-1010.
https://doi.org/10.3889/oamjms.2017.183
eISSN: 1857-9655
Public Health

Demographic and Clinical Features of Thyroid Carcinomas in Republic of Macedonia (1999-2010)

Tanja Makazlieva[1*], Olivija Vaskova[1], Venjamin Majstorov[1], Sinisha Stojanoski[1], Nevena Manevska[1], Rubens Jovanovic[2]

[1]Institute of Pathophysiology and Nuclear Medicine, Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]Institute of Pathology, Medical Faculty, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

Citation: Makazlieva T, Vaskova O, Majstorov V, Stojanoski S, Manevska N, Jovanovic R. Demographic and Clinical Features of Thyroid Carcinomas in Republic of Macedonia (1999-2010). Open Access Maced J Med Sci. 2017 Dec 15; 5(7):1005-1010. https://doi.org/10.3889/oamjms.2017.183

Keywords: thyroid carcinoma; histopathological types; Papillary thyroid carcinoma; Follicular thyroid carcinoma.

***Correspondence:** Tanja Makazlieva. Institute of Pathophysiology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: tmakazlieva@gmail.com

Received: 11-Aug-2017; **Revised:** 20-Sep-2017; **Accepted:** 27-Sep-2017; **Online first:** 17-Oct-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Thyroid carcinomas (TC) are the most common endocrine malignancies. In some parts of the world, the incidence of TCs has increased over the past few decades, especially in females according to some studies.

AIM: We have set as the objective for our study to analyse the demographic, ultrasound features, thyroid hormonal status and frequency of thyroid carcinomas in the Republic of Macedonia according to histopathological type.

MATERIALS AND METHODS: Retrospective analysis of medical data from all diagnosed and treated patients with TC at the Institute of Pathophysiology and Nuclear Medicine for the period 1999-2010 was performed. Demographic characteristics: age at diagnosis, gender, histopathological type of TC and from clinical features: US findings and thyroid hormonal state at initial examination and their distribution in eight state regions were evaluated.

RESULTS: Total number of 204 patients with TC in the Republic of Macedonia was registered. Papillary thyroid carcinoma (PTC) was the most frequent with 131 pts (64.21%), follicular (FTC) with 13 pts (6.37%) was second thyroid malignoma, followed by medullary (MTC) with 12 pts (5.88%), anaplastic (ATC) 11 pts (5.39%) and the rarest types were Hurtle cell carcinoma and intrathyroid sarcoma with only 1 diagnosed case. Age varied widely from 7 yrs to 88 yrs age (average 47.9 ± 16.6 yrs). PTC was more prevalent in younger age groups, while ATC was diagnosed in elderly patients. In all of the eight-country regions, the prevalence rate was higher for females than males (3:1) or $15.21/10^5$ female to $5.03/10^5$ male prevalence rate. According to US appearance mostly TC was unilateral in 42.65% and multifocal in 7.84% with dimensions from 15 to 50 mm.

CONCLUSION: There is an increase in incidence and prevalence rate of TCs in our country, mostly PTC, while reduction exists in the number of diagnosed cases of ATC and FTC, comparing with previous studies before iodine prophylaxis program. Different from described in the literature is female: male (4:1) ratio for ATC. According to US features, we can conclude that introduction of more detailed reporting system may improve diagnostic accuracy.

Introduction

Thyroid carcinomas (TC) are the most common endocrine malignancies. In some parts of the world, the incidence of TC has increased over the past few decades, especially in female patients according to some studies [1-3]. Thyroid malignancies are a heterogeneous group of tumors, with papillary (PTC) and follicular thyroid carcinomas (FTC) known as follicular cell-derived or differentiated thyroid carcinomas, medullary thyroid carcinoma (MTC) originating from neuroendocrine calcitonin-producing C – cells and less common anaplastic thyroid carcinoma (ATC) with poor prognostic outcome and very rare intrathyroid lymphomas originating from intrathyroid lymphoid tissue and sarcomas arising from intrathyroid connecting tissue [4-6].

Advances in histopathology techniques, immunohistochemical staining and molecular pathology reveal that there are further subtypes in differentiated thyroid carcinomas, PTC and FTC, with different genetic alterations, different histopathological features and different prognosis and outcome. Described subtypes of PTC are: typical variant, follicular variant of PTC, tall cell, oncocytic, columnar cell, diffuse sclerosing, solid, clear cell, cribriform morular, macrofollicular, PTC with fasciitis-like stroma,

PTC with prominent hobnail features, Warthin – like PTC, mixed papillary and medullary thyroid carcinoma and PTC with dedifferentiation to ATC [6]. FTC is divided into subtypes according to the patterns of growth related to capsular and vascular invasions into minimally invasive follicular carcinoma (MIFC) and widely invasive follicular thyroid carcinoma (WIFC), and according to the World Health Organisation (WHO) Hürthle cell, adenocarcinoma is also included as a variant of FTC [7-9]. Poorly differentiated thyroid carcinoma (PDTC) as a distinct group between differentiated thyroid carcinoma and ATC was originally described in1983 and included in the WHO classification of thyroid tumours in 2004.

A consensus conference held in Turin in 2006 confirmed geographical differences among claimed forms of PDTC according to suggested diagnostic algorithm based on the presence of a solid/trabecular/insular pattern and high-grade features. Evaluation recognised some overlap with solid and tall cell variants of PTC and FTC with predominant solid and trabecular growth pattern [10]. Insular thyroid carcinoma (ITC) was firstly described in 1984 by Carcangiu et al., as a distinct clinicopathological entity. ITC is characterised by insulae consisted of small, uniform cells sometimes associated with small thyroglobulin-containing follicules and according to its aggressive biology and the worst prognosis is included into the PDTC group of thyroid tumours [11,12]. MTC include sporadic (not inherited), MEN 2A and MEN 2B (multiple endocrine neoplasias, genetic syndromes that involve other parts of the endocrine system), and familial (inherited, but not linked to other MEN-related endocrine tumours). Metastatic thyroid tumours (ms) are also included in thyroid malignancies, mostly resulting from contiguous infiltration from carcinoma of the larynx, oesophagus or by hematogenous dissemination from breast, lung, kidney carcinoma, metastatic deposits from melanoma, etc. [13].

Due to the deficit of state cancer register and absence of published papers about TC in the population of Republic of Macedonia we have set as the objective for our study to analyse the demographic, ultrasound features and the frequency of thyroid carcinomas in the Republic of Macedonia according to histopathological type for the period 1999-2010.

Materials and Methods

Retrospective analysis of data of all diagnosed and treated patients with TC at the Institute of Pathophysiology and Nuclear Medicine for the period 1999 – 2010 was performed. For the analysed period our Institute was the healthcare institution that treats most of the patients with thyroid carcinomas. From the total number of diagnosed patients with TC, their demographic characteristics were evaluated: age at diagnosis, gender, histopathological type of TC, while from the clinical features: US findings and thyroid hormonal state at initial examination.

Using the basic demographic indicators from Statistical State Office and data from census in 1994 and 2002 we assessed the yearly incidence rate per 100.000 female and male, differences in incidence rate and prevalence rate of different histopathological types between gender and their distribution in age groups, dividing the patients into four age groups (0-19, 20-40, 41-60 and >61 years). Regional distribution of histopathological types of TC in R Macedonia was analysed also. Incidence and prevalence rate of distinct types of TC were evaluated for eight geographical regions of the whole country according to data from the Republic State Statistical Office from 2009 [14].

Initial US features were analysed, and from the initial US representation were recorded dimensions of the thyroid nodules, echogenicity, the presence of calcifications, enlarged neck lymph nodes and localisation of a tumour. According to the dimensions, thyroid nodules were divided into four groups (occult tumours in the absence of the clearly defined lesion in the thyroid gland, small tumours <15 mm, 15 – 50 mm, gross tumours > 50 mm). Multifocal or multicentric TCs were also described as the distinct group according to findings from the histopathological report. Another described US feature was the echogenicity and according to this characteristic we have described anechoic, hyperechoic, hypoechoic, isoechoic and heterogeneous nodules, also the presence/absence of calcifications, localisation (isthmic, right, left lobe and bilateral) and detection of enlarged neck lymph nodes at the initial representation of the patients.

Results

A total number of 204 patients with TC in R Macedonia was registered. The Annual incidence rate showed the slight increase of thyroid malignomas comparing with previous studies for the period 1966-1980, with the highest incidence rate in 2000 when $1.18/10^5$ was recorded. The evaluated prevalence rate for our population increased from $6.5/10^5$ in 1966-1980 to $10.15/10^5$ in 1999-2010 [15]. Average disease-specific mortality for the period 1999-2010 was $0.044/10^5$ [15]. Distribution according to the histopathological type of TC showed that papillary thyroid carcinoma (PTC), for the analysed period including all histopathological variants of PTC was the most frequent with 131 pts (64.21%). From known

histopathological variants of PTC in our population most common was typical PTC with 97 pts (47.55%) then second according to frequency was follicular variant of PTC 29 pts (14.21%), 4 cases (1.96%) PTC with osteoclast-like multinucleated giant cells PTC and PTC with nodular fasciitis-like stroma 1 case (0.49%). Follicular thyroid carcinoma (FTC) with 13 pts (6.37%) was the second thyroid malignoma according to frequency, followed by MTC with 12 pts (5.88%), anaplastic (ATC) 11 pts (5.39%) and rarest types were Hurtle cell variant of follicular carcinoma and intrathyroid sarcoma with only 1 diagnosed patient respectively for the evaluated period (Figure 1).

Ca papillare	47.55%
Ca papill-foll var.	14.21%
Ca papill osteocl.	1.96%
Ca papill fascii.	0.49%
Ca folliculare	6.37%
Ca medullare	5.88%
Ca anaplasticum	5.39%
Hurtle cell Ca	0.49%
Lymphoma	1.47%
Sarcoma	0.49%
Secondary (ms)	1.96%
Without data	13.72%

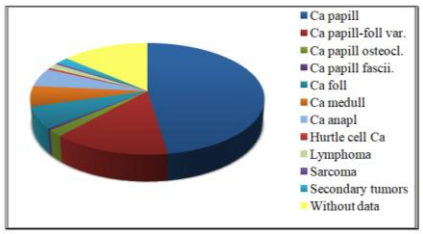


Figure 1: Annual frequency of TC by histopathological type

From the demographic features, we have analysed age of the patients at the time of diagnosis. Age of the patients varies widely from 7 yrs to 88 yrs age (average 47.9 ± 16.6 yrs) (Figure 2a). Annual average age of the patients in the moment of diagnosis was calculated and according to this data youngest patients were diagnosed in 2002 with an average age of 36 years (± 16.18) and oldest in 2001 with an average age of 57.9 years (± 10.87) (Figure 2b).

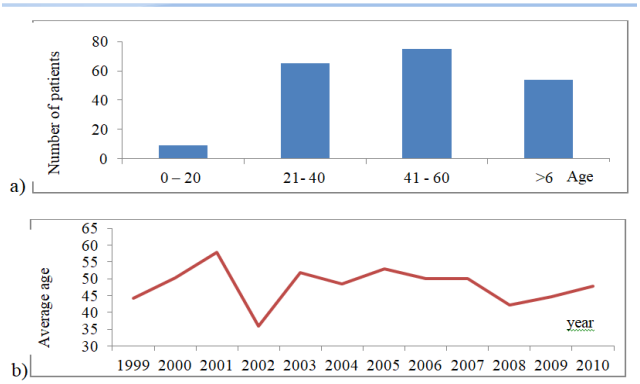


Figure 2: Distribution of TC by age groups. a) Frequency of all TC by age groups; b) Average age of the patients at the diagnosis yearly

Analysis of the distribution of histopathological types by age indicates that PTC was more prevalent in younger age groups, while ATC was diagnosed in age groups above 40 years and the highest frequency above 61 years in elderly patients. The average age for PTC was 43 (± 14.7) yrs, 51.8 yrs (± 16.6) for FTC, 47.5 (± 18.9) yrs for MTC and 67 (± 8.3) yrs for ATC (Figure 3).

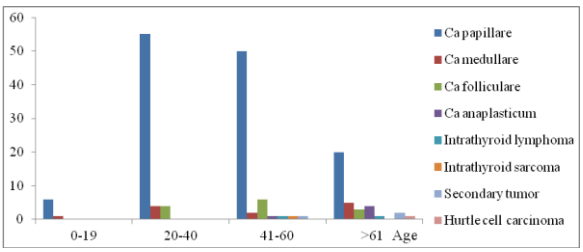


Figure 3: Distribution of different histopathological types by age groups

Age-standardized distribution of TC in both genders indicates that in females TCs were most frequently in age groups from 20-40 years and with almost similar frequency in the age group 41-60, while in male most TCs were distributed in the age group 41-60 and older age above 61 years (Figure 4).

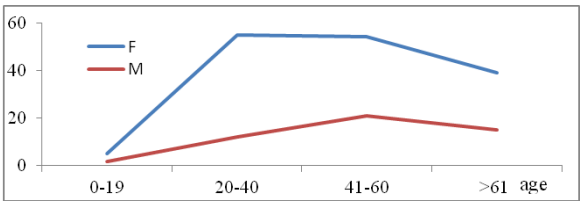


Figure 4: Distribution of TC by gender according to age groups

The incidence rate of TC related to gender was evaluated and around three times higher incidence rate was recorded in female population than a male with the exception in 1999 when slightly higher incidence rate in the male population of $0.69/10^5$ compared to a female with an incidence rate of $0.59/10^5$ (Figure 5). Analysis revealed three times higher prevalence rate ($15.21/10^5$) of TC in female compared to ($5.03/10^5$) male gender.

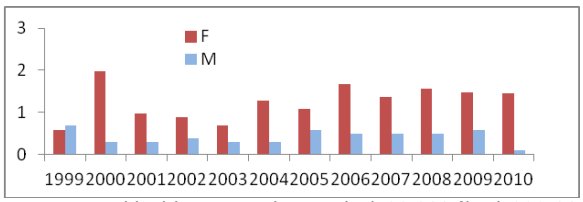


Figure 5: Annual incidence rate by gender/100.000 f/m (1999-2010)

Distribution of histopathological types by gender revealed a similar pattern in female and male population, with PTC type as most frequent in both genders and MTC second most prevalent TC in female and FTC in the male population. Anaplastic thyroid carcinoma was more frequently diagnosed in female than male in our population (Figure 6).

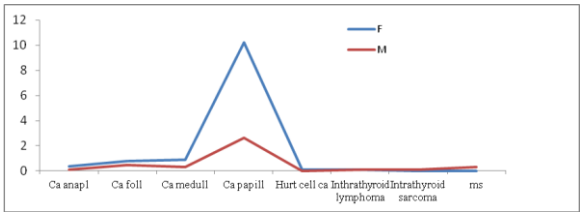


Figure 6: Distribution of different histopathological types by gender

The prevalence rate of TC by gender per 100.000 female and male individuals in eight regions of our country were analysed. In all regions, prevalence rate was higher for female population than male, especially in Vardar region where only female TC pts were diagnosed for the analysed period. The highest prevalence rate for male population was detected in East region, while for a female in Skopje region (Figure 7).

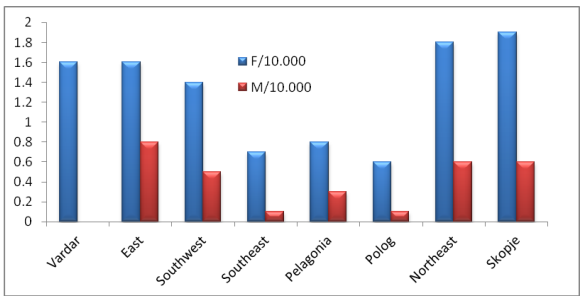


Figure 7: Prevalence rate by gender in eight regions of our country

According to ultrasound (US) appearance of TC at initial presentation, 12 cases were diagnosed as occult thyroid carcinoma because of absence of clearly defined lesion in thyroid gland, 149 pts were with detected thyroid nodules on the US, from which 93 pts were with one nodule, struma nodosa athyreotic (SNE) and 56 pts with multinodular goiter, struma multinodosa athyreotic (SMNE) and 43 pts lacking first US examination descriptions in medical files, mostly due to the fact that their first examination at our Institute was preformed after the surgical treatment or TC was incidentally diagnosed after thyroidectomy of multinodular goiter. Another parameter that derived from US examination and additional pathology report features, was the largest diameter of the thyroid nodule at first examination and thereafter at intervention and appropriately according to dimensions we have divided pts in to four groups, respectively as small thyroid tumors less than 15 mm, 15 – 50 mm, large tumors > 50 mm and multifocal carcinoma. Mostly TCs were unilateral and with dimensions from 15 to 50 mm with 42.65% and the rarest were multifocal with 7.84%. According to US appearance, most nodules were described as hypoechoic (29.9%), and with similar representation heterogeneous (29.4%), hyperechoic solid nodules (5%), isoechoic (3.4 %) and rarest anechoic cystic lesions (1.5%), in 30.9% description of echogenicity of the nodule was missing. In 19% of TC presence of calcifications were described. Nodules were usually localised in thyroid lobes more frequently in the right lobe (34.8%), than left lobe (26.5%), rarely with bilateral involvement (8.3%) and lesions with thyroid isthmus involvement with only 4.4%. In 30% of patients on the initial US were detected enlarged neck lymph nodes. According to thyroid hormonal status at initial examination most of the patients were euthyroid 153/204 (75%), subclinical hypothyroidism had 11/204 (5.39%), subclinical hyperthyroidism had 8/204 (3.92%), hyperthyroid state was detected in 4/204 (1.96%) and hypothyroid only in 1/204 (0.49%) (Table 1).

Table 1: The US features at initial examination of diagnosed thyroid carcinomas 1999-2010

Nodules	**Pts**	**(%)**	**Echogenicity**	**Pts**	**(%)**
Occult	12	5.88%	Anechoic	3	1.471%
SNE	93	45.59%	Hyperechoic	10	4.902%
SMNE	56	27.45%	Hypoechoic	61	29.902%
Without data	43	21.08%	Isoechoic	7	3.431%
			Heterogeneous	60	29.412%
			Without data	63	30.882%
Dimensions	**Pts**	**(%)**	**Localisation**	**pts**	**(%)**
<15 mm	20	9.804%	Isthmic	9	4.412%
15 - 50 mm	87	42.647%	Right lobe	71	34.804%
> 50 mm	23	11.275%	Left lobe	54	26.471%
Multifocal	17	8.333%	Bilateral	17	8.333%
Without data	57	27.941%	Without data	53	25.980%
Neck lymph nodes	**Pts**	**(%)**	**Calcifications**	**pts**	**(%)**
Present	55	26.961%	Present	39	19.118%
Without	125	61.275%	Without	165	80.882%
Without data	24	11.765%			

Discussion

Thyroid carcinoma comprises a spectrum of different tumours with the wide range of biological behaviour and prognosis and from highest priority is the early differential diagnosis, which leads to appropriate treatment of the patient [16]. The importance of epidemiological data is in the possibility of giving the true distribution of the disease in the population and using different scientific methods, which indicate of possible etiological influences in the pathogenesis. Many studies have analysed epidemiological trends in TC. Gathered data showed that as in other countries, in our country there was a trend of increase in the incidence and prevalence rate, compared with the published data for the period 1966-1980, from $6.5/10^5$ to $10.15/10^5$, or around 1.6 times increase in prevalence rate between two analysed decades [15, 17]. This prevalence rate is lower than reported in many countries and may be due to environmental, genetic factors or due to underdiagnosis of TC [3- 5].

From all TCs in R Macedonia, PTC predominates with 64.21% representation, females affected more frequently than males in ratio 3.3:1. Cancer gender disparity in incidence, disease aggressiveness and prognosis has been observed for a variety of cancers, and according to literature, thyroid cancer has 2.9-times higher rate in women (data from USA study), but in the literature this gender disparity in TC is also specific to the histologic subtype, data indicates that more aggressive types of TC, ATC and MTC have similar rates of incidence in men and women. Meanwhile, differentiated thyroid cancer of follicular cell origin, such as FTC and PTC, are more common in women [18, 19]. We found same female/male ratio for PTC and MTC 3:1 and even

higher ratio for ATC of 4:1 and almost similar prevalence for FTC by gender. According to V. LiVolsi and other data from the literature, almost 80 – 85% of all TC are PTC, with a female: male ratio varying 2:1 to 4:1 [3, 18, 20]. A similar distribution of histopathological types of TC was described in the Ellis Fischel Cancer Center Registry Data for the period 1998-2012, with registered 57% of PTC in all TC. Furthermore, it is concluded that this frequency of PTC is recorded both in iodine deficiency and iodine sufficiency regions and it is recognised as the most indolent type of TC. Evaluations of epidemiological trends showed that papillary microcarcinomas are more frequently diagnosed. One probability is that global increase in the incidence of TC is due to improved diagnosis of papillary microcarcinomas [21].

The important fact is that papillary carcinomas are also subdivided into different subtypes, with different biological behaviour. In the current study from 204/97 pts or 47.55% were typical PTC, then the second was a follicular variant with 14.21%, 1.96% tall cell and only 0.495 or 1 patient was PTC with nodular fasciitis-like stroma type [20].

Second most common TC is FTC with 6.37% representation of all TC in our population for 1999-2010 periods. In literature, the incidence rate varies greatly from 10 to 32 %, the known environmental factor is dietary iodine intake, and it is recognised that greater frequency of FTC exists in iodine deficiency regions [22]. B. Karanfilski et al. (1990), published data about the distribution of TC for period 1966 – 1988 in our country and according to those data FTC was represented with 17.5% in all TC. We found 2.8 times reduction in the representation of FTC in all TC [17]. This observation is recorded in other studies in countries after the introduction of iodine deficiency correction programs. The territory of R Macedonia was iodine deficient region till 1956 when iodine prophylaxis program was started with the introduction of salt iodination with 10 mg KJ/1kg salt till 1999 when the second program was initiated with 20 - 30 mg KJO_3/1 kg salt. In 2003 expert team by WHO, UNICEF, ICCID and National Committee conducted an evaluation and concluded in their final report that iodine deficiency in R Macedonia was corrected. Change in iodine intake in the analysed period is a possible mechanism for the lowered frequency of FTC in our population comparing to the study for previous decades [23, 24]. We assume that correction of iodine deficiency in R Macedonia is also the reason for the detected reduction in the frequency of ATC from 17.9% (second commonest) in 1966-1988 to 5.39% in 1999-2010. On the other hand increase in the number of MTC, cases were detected. This increase in MTC, as third most common TC with 5.88%, compared to 2.2% in 1966-1988 published data, may be due to advanced diagnostic facilities.

Age distribution of histopathological types showed younger age at the moment of diagnosis of PTC, compared to ATC in our study, described in other studies as well. Thyroid cancer is the only malignancy with age as a prognostic indicator included in the staging systems. The theory of the pathogenesis of ATC describes the possibility of development of this poorly differentiated TC from undifferentiated follicular cells of PTC with the process of genetic alterations leading to loss of TSH receptor expression and loss of iodine avidity of a tumour [25].

According to US features and histopathological data, TC was usually unilateral and with dimensions from 15 to 50 mm with 42.65% representation and most rarely was multifocal with 7.84%. Mostly nodules were described as hypoechoic (29.9%) and inhomogeneous (29.4%). 30% of patients were detected with enlarged neck lymph nodes on the initial US. Many studies indicate that detection of nonpalpable thyroid nodules has increased with wider application of US and other imaging modalities [26]. The study revealed mostly thyroid tumours with dimensions from 15 to 50 mm (42.65%), while only 9,8% were smaller than 15 mm. According to literature one of a possible reasons for the increase in the incidence of TC is improved diagnosis of microcarcinomas, less than 1 cm.

From all patients only in 19.11% were described calcifications in the thyroid nodules at initial US examination. According to Hoang et al., one of the most specific US features for malignancy is the presence of calcifications with a described specificity of 85.8%–95% and positive predictive value of 41.8%–94.2% [27, 28]. Microcalcifications represents psammoma bodies and are usually described in PTC, while coarse calcifications more likely to be found in MTC. This discordance between our data and literature may be due to the absence in the detailed description of US examinations according to Thyroid imaging reporting and data system (TIRADS) criteria [29].

In conclusion, there is an increase in the incidence and prevalence rate of TCs in R Macedonia for the period 1999-2010 compared with data from the previous study (1966-1980). According to histopathological type, the most common was PTC, second FTC, and third MTC, followed by ATC and most rare were Hurtle cell adenocarcinoma and intrathyroid sarcoma with only 1 case. Comparing with the data from the previous study for period 1966-1988 in our country, there is a reduction of diagnosed ATC and FTC. We conclude that this change in the distribution of histopathological types may be due to the introduction of iodine prophylaxis and elimination of the problem of iodine deficiency in our country. All histopathological types were more common in females than males, (f/m ratio 3:1). Age-standardized distribution of TC in both genders indicates that in females TCs were more frequently diagnosed in younger age than in males. PTC was usually diagnosed in younger age (average 43 yrs), while ATC was mostly diagnosed in elderly people (average

age at diagnosis 67 yrs). Analyzed US features indicates that introduction of more detailed reporting system may improve the diagnostic accuracy. Further prospective studies with the introduction of TIRADS system on our populations are needed for evaluation of diagnostic accuracy of this method and selecting suspicious thyroid nodules for more detailed examinations.

References

1. Burns WR, Zeiger MA. Differentiated thyroid cancer. Semin Oncol. 2010;37(6):557-66. https://doi.org/10.1053/j.seminoncol.2010.10.008 PMid:21167375

2. Slijepcevic N, Zivaljevic V, Marinkovic J, Sipetic S, Diklic A, Paunovic I. Retrospective evaluation of the incidental finding of 403 papillary thyroid microcarcinomas in 2466 patients undergoing thyroid surgery for presumed benign thyroid disease. BMC Cancer. 2015;15:330. https://doi.org/10.1186/s12885-015-1352-4 PMid:25925164 PMCid:PMC4423135

3. Vecchia C La, Malvezzi M, Bosetti C, Garavello W, Bertuccio P, Levi F et al. Thyroid cancer mortality and incidence: A global overview. Int J Cancer. 2015:136, 2187–2195. https://doi.org/10.1002/ijc.29251 PMid:25284703

4. Curado MP, Edwards B, Shin HR, Storm H, Ferlay J, Heanue M, et al. Cancer incidence in five continents, Vol. IX. Lyon: IARC Scientific Publications No 160, 2007.

5. British Thyroid Association, Guidelines for the management of thyroid Cancer,Third edition, 15th February, 2014.

6. Lloyd RV, Buehler D, Khanafshar E. Papillary thyroid carcinoma variants. Head and Neck Pathol. 2011; 5:51–56. https://doi.org/10.1007/s12105-010-0236-9 PMid:21221869 PMCid:PMC3037461

7. Collini P, Sampietro G, Rosai J, Pilotti S. Minimally invasive (encapsulated) follicular carcinoma of the thyroid gland is the low-risk counterpart of widely invasive follicular carcinoma but not of insular carcinoma. Virchows Arch. 2003; 442:71. PMid:12536317

8. D'Avanzo A, Treseler P, Ituarte PH, et al. Follicular thyroid carcinoma: histology and prognosis. Cancer. 2004; 100:1123. https://doi.org/10.1002/cncr.20081 PMid:15022277

9. Volante M, Papotti M. Poorly differentiated thyroid carcinoma: 5 years after the 2004 WHO classification of endocrine tumours. Endocr Pathol. 2010;21(1):1-6. https://doi.org/10.1007/s12022-009-9100-4 PMid:19960273

10. Pellegriti G, Giuffrida D, Scollo C, Vigneri R, Regalbuto C, Squatrito S et al. Long-Term outcome of patients with insular carcinoma of the thyroid the insular histotype is an independent predictor of poor prognosis. Cancer. 2002; (95): 2076-2085. https://doi.org/10.1002/cncr.10947 PMid:12412160

11. Kini H, Nirupama M, Rau AR, Gupta S, Augustine A. Poorly differentiated (insular) thyroid carcinoma arising in a long-standing colloid goitre: A cytological dilemma. Journal of Cytology/Indian Academy of Cytologists. 2012;29(1):97-99. https://doi.org/10.4103/0970-9371.93237 PMid:22438634 PMCid:PMC3307470

12. Volante M, Collini P, Nikiforov YE, Sakamoto A, Kakudo K, Katoh R et al. Poorly differentiated thyroid carcinoma: the Turin proposal for the use of uniform diagnostic criteria and an algorithmic diagnostic approach. Am J Surg Pathol. 2007;31(8):1256-64. https://doi.org/10.1097/PAS.0b013e3180309e6a PMid:17667551

13. Schumberger M, Pacini F, Tutle RM. Thyroid tumors, Forth edition, Paris: Institute Medico-Educatif, 2015.

14. State statistical office of Republic of Macedonia, Regions of the Republic Macedonia, 2009. ISSN 1857-6141, 2010.

15. Makazlieva T, Vaskova O, Tripunoski T, Majstorov V. Incidence and prevalence of thyroid carcinoma in Republic of Macedonia (1999-2010). Arch Pub Health. 2017; 9(1): 66 - 73.

16. Lukasa J, Drabekb J, Lukasc D, Dusekd L, Gateke J. The epidemiology of thyroid cancer in the Czech Republic in comparison with other countries, Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2013; 157(3):266-275

17. Karanfilski B, Serafimov N, Dolgova-Korubin V, Tadzer I, Shestakov Gj, Simova N et al. Klinichki I laboratoriski karakteristiki na karcinomot na tiroidnata zhlezda vo SR Makedonija, Prilozi. 1982; 3(2):13-27.

18. Rahbari R, Zhang L, Kebebew E. Thyroid cancer gender disparity. Future Oncol. 2010; 6(11): 1771–1779. https://doi.org/10.2217/fon.10.127 PMid:21142662 PMCid:PMC3077966

19. Chen GG, Vlantis AC, Zeng Q, Van Hasselt CA. Regulation of cell growth by estrogen signaling and potential targets in thyroid cancer. Curr Cancer Drug Targets. 2008; 8:367-77. https://doi.org/10.2174/156800908785133150 PMid:18690843

20. Li Volsi VA. Papillary thyroid carcinoma: an update. Modern Pathology. 2011; 24:S1–S9. https://doi.org/10.1038/modpathol.2010.129 PMid:21455196

21. Ito Y, Miyauchi A, Inoue H, Fukushima M, Kihara M, Higashiyama T et al. An observational trial for papillary thyroid microcarcinoma in Japanese patients. World J Surg. 2010; 34:28-35. https://doi.org/10.1007/s00268-009-0303-0 PMid:20020290

22. Crea CD, Raffaelli M, Sessa L, Ronti S, Fadda G, Bellantone C et al. Actual incidence and clinical behaviour of follicular thyroid carcinoma: An institutional experience. The Scientific World Journal. 2014; 2014:Article ID 952095.

23. Davies, H Gilbert Welch. Increasing Incidence of Thyroid Cancer in the United States, 1973- 2002 .JAMA. 2006;295(18):2164-2167. https://doi.org/10.1001/jama.295.18.2164 PMid:16684987

24. Haymart M. Understanding the relationship between age and thyroid cancer. The Oncologist. 2009;14:216–221. https://doi.org/10.1634/theoncologist.2008-0194 PMid:19270027

25. Vaskova O, Kuzmanovska S, Josifovska T, Bogdanovska A, Majstorov V, Zdravkovska M, Stojanoski S, Tripunoski T, Zdravevska M.Thyroid malignomas before and after correction of mild iodine deficit. Radioactive Isotopes in clinical Medicine and Research 27th International Symposium, Bad Gastein, Austria. Nuklearmedizin 6, 2005. PMCid:PMC1112585

26. Butros R, Boyvat F, Ozyer U, Bilezikci B, Arat Z, Aytekin C et al. Management of infracentrimetric thyroid nodules with respect to ultrasonographyc features. Eur Radiol. 2007;17: 1358 -1364. https://doi.org/10.1007/s00330-006-0413-0 PMid:17021705

27. Hoang JK, Lee WK, Lee M, Johnson D, Farrell S. US features of thyroid malignancy: Pearls and pitfalls. Radio Graphics. 2007; 27(3):847-860. https://doi.org/10.1148/rg.273065038

28. Kwak JY, Han KH, Jung MS,Yoon H, Moon HJ, Son EJ, Park SH et al. Thyroid imaging reporting and data system for US features of nodules: A step in establishing better stratification of cancer risk. Radiology. 2011; 260(3): 892-899. https://doi.org/10.1148/radiol.11110206 PMid:21771959

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):1011-1015.
https://doi.org/10.3889/oamjms.2017.191
eISSN: 1857-9655
Public Health

The Value of Mobile Ultrasound Services in Rural Communities in South-South Nigeria

Anthonia Ikpeme[1*], Nchiewe Ani[1], Boniface Ago[2], Emmanuel Effa[3], Omofolasade Kosoko-Lasaki[4], Andrew Ekpenyong[5]

[1]Department of Radiology, University of Calabar Teaching Hospital, Calabar, Nigeria; [2]Department of 0bstetrics and Gynaecology, University of Calabar Teaching Hospital, Calabar, Nigeria; [3]Department of Internal Medicine, University of Calabar Teaching Hospital, Calabar, Nigera; [4]Creighton University School of Medicine, Omaha, NE 681 78, USA; [5]Department of Physics, Creighton University, Omaha, NE 681 78, USA

Citation: Ikpeme A, Ani N, Ago B, Effa E, Kosoko-Lasaki O, Ekpenyong A. The Value of Mobile Ultrasound Services in Rural Communities in South-South Nigeria. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):1011-1015. https://doi.org/10.3889/oamjms.2017.191

Keywords: Mobile ultrasound; Abdomino-pelvic scans; Uterine fibroids; Prostatic enlargement.

***Correspondence:** Anthonia Asanye Ikpeme. Radiology Department, University of Calabar, Calabar, Nigeria. E-mail: ikpemeanthonia@yahoo.com

Received: 12-Aug-2017; **Revised:** 25-Sep-2017; **Accepted:** 29-Sep-2017; **Online first:** 26-Oct-2017

Funding: This work was partially supported by Friends of Joseph Ukpo Hospitals and Research Institutes, a nonprofit corporation and a public charity with Tax Exempt Status under section 501(c) [3] of the Internal Revenue Code of the United States of America, whose mission is to bring the benefits of modern medical science to individuals in South-South Nigeria.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: This paper examines the activities of mobile services units including ultrasound services in rural and urban communities in the Calabar region of South-South, Nigeria.

MATERIALS AND METHODS: Consenting individuals were invited and attended five medical outreach activities in rural and urban areas of the Calabar region between January and June 2016. Abdomino-pelvic scans were done. Subsequently the results were analyzed.

RESULTS: Five hundred and seventy-four (574) individuals had Abdomino-pelvic scans done, using a curvilinear probe to assess the abdomino-pelvic organs. The female to male ratio was 1.46:1. The age ranged from 1-78 years with a mean of 40.63 (standard deviation of 17.5). The commonest sonographic finding was uterine fibroids, 21 (8.1%). Fifty-four percent of the scans were normal. The commonest sonographic finding in men was prostatic enlargement.

CONCLUSION: Medical outreach activities provided by mobile units provide much needed ultrasound services in poor resource settings in Nigeria. Significant clinical pathologies were identified at fairly high rates.

Introduction

The benefits of diagnostic ultrasound are no longer debatable [1]. Since the practice of diagnostic ultrasound became established and popular in the 1950s [2] the field of medicine and medical care worldwide has been greatly revolutionized. Compared with the earlier imaging modalities, diagnostic ultrasound is safe, uses sound waves with no ionizing radiation, is cheap, simple to operate and widely available [2, 3]. It can be used both for diagnosis and therapeutic purposes [3].

In developing countries such as Nigeria, the rural communities have limited access to diagnostic centres [1]. Besides the unavailability of ultrasound and other diagnostic facilities, misconceptions, fears and poverty have further limited access to medical care [4]. Over the past decade however, the use of clinician performed, hand-carried bed side ultrasound has gained increasing popularity as a useful imaging modality worldwide, helping to boost the diagnostic capacity of rural district hospitals in resource–limited settings [5]. Many new lap-top-based machines (mobile ultrasound machines) are now available [5]. Improvements in battery life for hand-carried machines and the lack of film, chemical developers and dedicated technicians allow for use of ultrasound in health care missions to remote areas of the developing world [5-8].

The use of mobile health care services and mobile ultrasound services is not new in the world and even within the country [7-10]. West D et al. [11] reported that the use of mobile devices and mobile ultrasound services help with maternal care, chronic disease management and disease epidemics. They improve the efficiency and effectiveness of the medical system through patient tracking and reporting

and they extend critically needed health services to underserved areas [10-12]. The report also showed that the mobile ultrasound patrol program in Morocco uses portable ultrasound machines and 3D smart phones to improve diagnostic times for expectant mothers [11]

Nigeria is the most populous country in Africa and many areas have little or no access to adequate healthcare, well below that of other African countries. The country represents a potentially important example of mobile health solution with more than 174 million residents [10-12].

The aim of this study is to examine the medical outreach activities of portable mobile ultrasound services units, donated by Joseph Ukpo Hospital and Research Institute (JUHRI) in rural and urban communities in the Calabar region of South-South Nigeria.

Materials and Methods

This is a descriptive study of clinical outreach activities that took place in January, March and June 2016 in five Cross River State rural and urban communities. Two portable ultrasound systems (Mindray DP-30, each with 12.1 inch LED monitor, high-resolution 1024 x 768 control panel, dual transducer ports, enabling THI (Tissue Harmonic Imaging) and transducer dependent TSI (Tissue Specific Imaging).

The ultrasound systems included two convex probes (Mindray 35C50EA, 2.0/3.5/6.0 MHz convex array transducer). The two mobile ultrasound systems were donated by Joseph Ukpo Hospital and Research Institute (JUHRI) with the aim of setting up mobile clinics and hospitals with emphasis on the rural/urban settings who do not have access to medical care. Other items donated to set up the mobile clinics included two Ambulance vehicles (Ford E 350 Super duty Type II Ambulance), a medical library, first aid equipment, drugs, clinical thermometers, blood pressure equipment, glucometers, urinalysis kits and haemoglobinometers [13]. This year (2016) mobile clinics have been set up in three rural communities including Akpabuyo, Akamkpa and Mfamosing. Urban centres were Saint Patrick's Catholic Church, Ikot Ansa, Calabar and a correctional facility in the city of Calabar.

The St. Patrick's Church, Ikot Ansa is located along the Old Odukpani Road, one of the major Roads in Calabar, it has a population of 1410 families. Akamkpa Local Government Area has a population of 151,125 people. Akamkpa has a land mass of 4,300 km2, bounded by Odukpani and Akpabuyo Local Government Areas to the west and south. There are 260 villages including Mfamosing and is serviced by one Primary Health Center at Oban, one General Hospital at the headquarters and several private health clinics. Akpabuyo, also a local Government Area of Cross River State, Nigeria, has an area of 1,241 km^2 and a population of 271,395 [14, 15]. These go to show how massively populated these areas are and how underserved they are in terms of healthcare. The correctional facility had 800 in-mates as at the time of the medical outreach. All communities involved in the outreaches were duly informed by their parish priests two to three weeks before each exercise. The correctional facility outreach exercise was requested for by their Chaplain in-charge, the date and time was agreed upon. Consent was obtained from the correctional facility authorities, the Cross River State ethical committee and the individual in-mates. Formal letters were written to the correctional facility authorities and to the village or town heads of these communities for this purpose and this included seeking consent from them for the ultrasound examination.

A verbal consent was also obtained from the individuals just before commencement of the medical outreach by explaining to them what was to be done and by whom as well as the overall benefit of this exercises. All the outreaches were carried out within the parish premises or within the correctional facility. Four other parishes were invited to each community outreach and subjects were encouraged to come along with their sick family members.

The medical personnel consisted of a consultant radiologist who specializes in ultrasonography, senior residents in radiology and internal medicine as well as hematology. They assisted the consultant in doing the scans, consulting with the subjects and carried out the basic laboratory investigations respectively. Others included senior nurses and laboratory scientists whose roles were to ensure that proper records were kept, vital signs and laboratory investigations accurately documented.

With the aid of a detailed questionnaire, subject data such as age, sex, body mass index clinical history, examination and diagnosis were recorded on paper initially before transferring them to a computer. Some of the outreaches were purely for screening and the others based on clinical presentation of the participants. Ultrasound scans were carried out on all participants using the portable ultrasound systems for the screening activities. For the selective treatment activities, scans were carried out on individuals with clinical indications such as abdominal pain, infertility, pregnancy and undiagnosed chronic illness.

Data were entered into Excel format and analyzed using SPSS v. 20 (SPSS Inc. Chicago. IL, USA). Frequency tables, histograms and bar charts were used to analyze the data.

Results

Five Hundred and Seventy Four (574) patients were seen in Five (5) different Medical outreaches and five (5) different communities in Cross River, South South Nigeria (Table 1).

Table 1: Participating communities and female/male ratio

Communities	Frequency (%)	Female (%)	Male (%)
Akpabuyo Local Government Area	95 (16.5)	75 (79)	20 (21)
St. Patrick's Parish Ikot Ansa	91 (15.8)	66 (72.5)	25 (27.5)
Correctional facility	113 (19.7)	30 (26.5)	84 (73.5)
Akamkpa	163 (28.5)	101 (62)	62 (38)
Mfamosing	112 (19.5)	69 (61.6)	43 (38.4)
Total	574 (100)	341 (59.4)	233 (40.6)
	Female/male ratio 1.46:1		

The female to male ratio was 1.46:1, with females 341 and males 233 (Table 1 and Figure 1). The ages ranged from 1-78 years with a mean of 40.63 ± 17.15 (standard deviation).

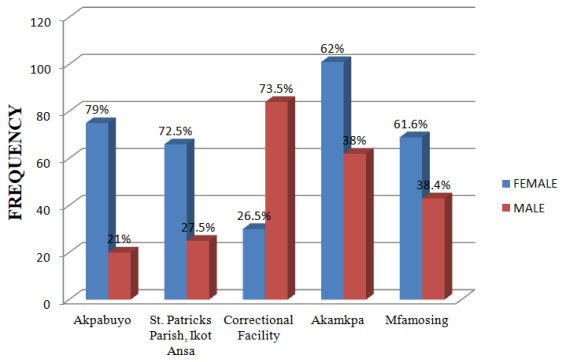


Figure 1: Participating Communities and Female/Male Ratio

The five communities and their frequencies included; Akpabuyo 95 (17.6%), St Patrick's, Ikot Ansa 91 (16.8%), Correctional facility 113 (19.7%), Akamkpa 163 (28.4%), Mfamosing 112 (19.5%). (Table 1), (Figure 1).

Table 2: Common sonographic findings

Sonographic findings	Frequency	Percentage (%)
Normal study	140	54
Uterine fibroids	21	8.1
Fatty liver	16	6.2
Pelvic inflammatory disease	-14	6.2
Prostatic enlargement	8	3.1
Splenic enlargement	5	1.9
Normal pregnancy	4	1.5
Increased bladder wall thickness (cystitis)	4	1.5
Nephrocalcinosis	4	1.5
Epigastric hernia	3	1.2
Liver calcification	3	1.2
Renal parenchymal disease	3	1.2
Renal cysts	3	1.2
Ovarian cysts	3	1.2
Small uterus for age	2	0.7
Pyelonephritis	2	0.7
Inguinal hernia	2	0.7
Umbilical hernia	2	0.7
Abdominal malignancy	2	0.7
Utero-vaginal prolapse	2	0.7
Polycystic ovary disease	1	0.4
Pelvi-ureteric junction obstruction	1	0.4
Lipoma (upper limb)	1	0.4
Intestinal obstruction	1	0.4
Renal calculus	1	0.4
Early onset menopause	1	0.4
Ectopic pregnancy	1	0.4
Kidney enlargement	1	0.4
Post surgical, Abdominal incisional hernia	1	0.4

The commonest sonographic finding over all by far was uterine fibroids 21 (8.1%) (Table 2), whereas more than 50% of scans done were normal (54%).

Table 3: Gender with common sonographic findings

Sonographic findings	Female (%)	Male (%)
Normal	65 (46.4)	75 (54.6)
Uterine fibroids	20 (100)	-
Fatty liver	14 (87.5)	2 (12.5)
Pelvic Inflammatory Disease	14 (100)	-
Prostate enlargement	-	8 (100)
Renal pathology	7 (100)	-
Normal cyesis	4 (100)	-
Splenic enlargement	3 (60)	2 (40)
Ovarian cysts	3 (100)	-
Small uterus for age	3 (100)	-
Liver calcification	-	3 (100)
Abdominal hernias	2 (50)	2 (50)
Utero-vaginal prolapse	2 (100)	-
Polycystic ovaries	2 (100)	-
Renal cysts	1 (33)	2 (66)
Post surgical incisional hernias	1 (50)	1 (50)
Abdominal malignancy	1 (50)	1 (50)
Ectopic pregnancy	1 (100)	-

The commonest sonographic finding in the females was uterine fibroids while in the males it was prostatic enlargement (Table 3).

A typical sonographic scan being carried out is shown in Figure 2.

Figure 2: Pelvic-Ultrasound scanning during the medical outreach

Discussion

In much of Sub-Saharan Africa, diagnostic imaging in patient care is limited to urban settings and lack of adequate healthcare facilities, personnel and diagnostic tools remain a major barrier to healthcare delivery. The use of Ultrasound and x-rays are ideal diagnostic tools because they can meet 70-80% of all clinical diagnostic needs [15] Their absence increases the risk of misdiagnoses, treatment delays, and negative healthcare outcomes [15]. Few prior studies of ultrasound services in remote settings exist. With non-governmental organizations efforts to strengthen and scale-up existing public sector health care models in rural international settings, attention has focused on appropriate placement of cost effective, durable technology that will assist local care providers in the clinical care of their patients [16].

This motive is seen in this initiative by the

Joseph Ukpo Hospital and Research Institute (JUHRI), through their provision of ambulances, drugs and mobile ultrasound machines in order to reach rural settings with little or no access to medicare. JUHRI is a faith-based charity in Nigeria funded by Friends of Joseph Ukpo Hospital and Research Institute, a nonprofit corporation and a public charity with Tax Exempt Status under section 501(c) [3] of the Internal Revenue Code of the United States of America, whose mission is to bring the benefits of modern medical science to individuals in South South Nigeria and beyond.

The number of scans done was determined by whether or not health screening was carried out. Scans were therefore limited to those who required them in the first instance. On the other hand screening exercises were carried out in two of these communities and hence all participants were scanned. Types of scans were limited to the abdomen and pelvis because of unavailability of other transducers. The commonest finding was uterine fibroids in women and prostate enlargement in men. Majority of scans done turned out to be normal during the screening exercises.

Uterine fibroids were the commonest pelvic tumor in women [17, 18]. Several studies have documented increased incidence of uterine leiomyoma in black women and women of African descent [18, 19]. This probably explains why it is the commonest finding in the women in the index study. Our study reveals that the age bracket commonly affected was 40-59 years. This is quite unlike other studies conducted within and outside Nigeria where the younger age groups were most affected [17, 20, 21].

Prostatic enlargement is a common disease of aging men worldwide [22]. Men in the seventh decade are most commonly affected [22-24]. The index study showed that majority of men affected were in the 40th and 50th decades, in younger age groups when compared with the above mentioned studies done within the country [22-24].

Comparison of past medical history and ultrasound findings could not be accurately done. Past medical history such as tuberculosis, sickle cell disease, human deficiency virus, bronchial asthma and glaucoma were probed for. We could not however rely on the clinical history given because a good number of them had not sought any form of medical care before and if they did, it was from a local primary health care center or general hospitals which are not well equipped to evaluate and handle such severe and chronic illnesses. The general healthcare status in these areas is quite poor and basically similar. The overall healthcare facilities include a primary healthcare center, a non-functional general hospital, several small private health clinics and patent drug stores. The only tertiary healthcare institution, a teaching hospital, is in the city of Calabar which is about 35 to 44 km (about three to five hours drive by road considering the bad roads) from these aforementioned towns or villages. Basically any proper and affordable healthcare can only be obtained from the teaching hospital in Calabar. However, the far distance, the fact that access to treatment is not free and the long period of waiting to be attended to, make these new mobile healthcare services preferable since it is free of charge, they do not have to travel far for it and the waiting time is drastically reduced.

Some literature have attempted to measure the impact of ultrasound services in these low resource settings and innovations in teleradiology and portable ultrasound have been shown to offer opportunity for improved ultrasound access in these areas [25, 26].

Sachita P. Shah et al., [16] suggests that ultrasound is a useful modality that particularly benefits women's health and obstetrical care in the developing world. They also believe that ultrasound services significantly impact patient management plans especially with regards to potential surgical interventions.

In conclusion, these outreaches have indicated the need for ultrasound services in these communities as significant pathologies were identified at fairly high rates. It is recommended that other ultrasound transducers be acquired to further increase the scope of diagnoses made in these outreaches and further the advantage therefrom.

Acknowledgements

The author wish to thank Professors Emmanuel Ekanem and Ikpeme Ikpeme of the University of Calabar Teaching Hospital, Nigeria (UCTH) for reading and correcting the manuscript as well as Marcus Iyama (UCTH) and Theresa Ekpenyong (General Hospital, Calabar, Nigeria) who assisted during the outreaches.The authors also thank Akan Otu (UCTH) who helped with the initial planning of the mobile clinics.

References

1. Ostensen H. Developing Countries. Ultrasound Med Biol. 2000; (26 Suppl 1): 159-61. https://doi.org/10.1016/S0301-5629(00)00194-0

2. Goldberg BB, Gramiak R, Freimans AK. Early History of Diagnostic Ultrasound. The Role of American Radiologists. AJR. 1993; 160: 189-197. https://doi.org/10.2214/ajr.160.1.8416623 PMid:8416623

3. Carovac A, Smajlovic F, Junuzovic D. Application of Ultrasound in Medicine. Acta Inform Med. 2001; 19(3): 168-171. https://doi.org/10.5455/aim.2011.19.168-171 PMid:23408755

PMCid:PMC3564184

4. Iliyasu G, Ogoina D, Otu AA, Dayyab FM, Habib AG. Multi-Site Knowledge Attitude and Practice Survey of Ebola Virus Disease in Nigeria. PLos One. 2015;10(8). https://doi.org/10.1371/journal.pone.0135955 PMid:26317535 PMCid:PMC4552659

5. Sippel S, Muruganandan K, Shah S. Review article: Use of Ultrasound in the Developing World. Int J Emerg Med. 2011; 4:7. https://doi.org/10.1186/1865-1380-4-72 PMid:22152055 PMCid:PMC3285529

6. Ault MJ, Rosen BT. Portable Ultrasound; the next generation arrives. Crit Ultrasound J. 2010;2(1): 39-42. https://doi.org/10.1007/s13089-010-0031-6 PMid:21151494 PMCid:PMC2994643

7. Liang D, Schnittger I. Accuracy of hand-carried Ultrasound. Echocardiography. 2003; 20(5): 487-490. https://doi.org/10.1046/j.1540-8175.2003.03072.x PMid:12848872

8. Gorcsan J. Hand-carried Ultrasound for Consultative Cardiology. Echocardiography. 2003; 20(5): 463469. https://doi.org/10.1046/j.1540-8175.2003.03082.x

9. Director General, World Health Organization: World Health Report: Fighting Disease, Fostering Development. WHO, Geneva vi, 1996.

10. World Health Organization. Baseline Country Survey on Medical Devices 2010. Geneva: WHO press, 2011.

11. West DM. Using Mobile Technology to Improve Maternal Health and Fight Ebola: A case study of mobile innovation in Nigeria, 2015.

12. Filkins M, Halliday S, Daniels B, Bista R, Thapa S, Schwarz R, Schwars D, Guuchan BK, Maru D. Implementing Diagnostic Imaging Services in a Rural Setting of Extreme Poverty: five years of x-ray and ultrasound service delivery in Achham Nepal J Glob Radiol. 2015; 1(1): Article 2.

13. Wright EE, Schellekens O. Achieving Universal Health coverage in Nigeria one State at a time. Brookings Global Economy and Development, June, 2013.

14. Ekpenyong A, Effa E, Ikpeme A, Inyama M, Ago B, Ekpenyong T, Out A, Ani N, Egbe W, Tom EA, Prathivadhi S, Nichols M, Kosoko-Lasaki S. Mobile Clinics Deployed in Rural Nigeria for free Patient Care and Clinical Research. Global Health Conference Midwest Research Symposium. Omaha, NE. 2016;pg. 18.

15. "Institutions". National Board for Technical Education. Retrieved 2010-03-20.

16. Nwabueze BO. A constitutional History of Nigeria. C Hurst and Co LTD, UK, 1982.

17. Baird DD, Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. Am J Obstet Gynecol. 2003;188(1):100. https://doi.org/10.1067/mob.2003.99 PMid:12548202

18. Shah SP, Epino H, Bukhman G, Umulisa I, Dushimiyimana JM, Reichman A, Noble VE. Impact of the introduction of ultrasound services in a limited resource setting: rural Rwanda 2008. BMC international health and human rights. 2009;9(1):4. https://doi.org/10.1186/1472-698X-9-4 PMid:19327157 PMCid:PMC2667437

19. Eze JC, Ugwu AC, Ohagwu CC, Imoh OC. The value of ultrasonography in the diagnosis leiomyoma in the South East Nigeria. Journal of Asian Scientific Research. 2013; 3(2): 151-156.

20. Szlark JE, Choi TH, Vithya VV. Imaging of common and uncommon large pelvic masses. Radiographics. 2003; 23:403-424. https://doi.org/10.1148/rg.232025089 PMid:12640156

21. Taran FA, Brown HL, Stewart EA. Racial diversity in uterine leiomyoma clinical studies. Fertile Steril. 2010; 94:1500-1503. https://doi.org/10.1016/j.fertnstert.2009.08.037 PMid:19969296

22. Zimmermann A, Bernuit D, Gerlinger C, Schaefers M, Geppert K. Prevalence, symptoms and management of uterine fibroids: An International Internet-based survey of 21,746 women. BMC Women's Health. 2012; 12:6. https://doi.org/10.1186/1472-6874-12-6 PMid:22448610 PMCid:PMC3342149

23. Shah SP, Epino H, Bukhman G, Umulisa I, Dushimiyimana JM, Reichman A, Noble VE. Impact of the introduction of ultrasound services in a limited resource setting: rural Rwanda 2008. BMC international health and human rights. 2009;9(1):4. https://doi.org/10.1186/1472-698X-9-4 PMid:19327157 PMCid:PMC2667437

24. Ma'aji SM, Adamu B. Transabdominal Ultrasonogrpahic assessment of prostate size and volume in Nigerians with Clinical diagnosis of benign prostatic hyperplasia. Niger J Iliin Pract. 2013; 16:404. https://doi.org/10.4103/1119-3077.113476 PMid:23771473

25. Ajayi I, Aremu A, Olajide A, Bello T, Olajide F, Adetiloye V. Correlation of transrectal and transabodominal ultrasound measurement of transition zone volume with post-operative enucleated adenoma volume in benign prostatic hypertrophy. The Pan African Medical Journal. 2013;16. https://doi.org/10.11604/pamj.2013.16.149.2532

26. Tahir A. Ahidjo A. Variation of prostatic volume with advancing adult Nigerians. Arch Nig Med. 2004; 1:12-14.

27. Stanton K, Mwanri L. Global Maternal and Child Health Outcomes: the role of obstetric ultrasound in low resource settings. World Journal of Preventive Medicine. 2013;1(3):22-9.

28. Ali QN, Roth J, Abdel-Rahum IM, Franke D, Kardoff R, Schwasforth H, Doehring E. Cost saving with ultrasonography in a developing country district hospital. East Afr Med. J. 1999; 76(5):272-4. PMid:10750508

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):1016-1020.
https://doi.org/10.3889/oamjms.2017.103
eISSN: 1857-9655
Public Health

Risk Factors for Early Preterm Birth at King Salman Armed Force Hospital in 2010

Badriah Ali Alansi[1*], Hytham Bahaeldin Mukhtar[1], Maher Ahmad Alazizi[2], Amjad Ahmad Zuiran[3], Areej Mohammed AL-Atawi[3], Badriah Abdulrahman Al-Sabah[3], Shrooq Salem Al-Yami[3]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tabuk, Tabuk, Kingdom of Saudi Arabia; [2]Department of Obstetrics and Gynecology, King Salman Armed Force Hospital (KSAFH), Tabuk, Kingdom of Saudi Arabia; [3]Students in Faculty of Medicine, University of Tabuk, Tabuk, Kingdom of Saudi Arabia

Citation: Alansi BA, Mukhtar HB, Alazizi MA, Zuiran AA, AL-Atawi AM, Al-Sabah BA, Salem Al-Yami SS. Risk Factors for Early Preterm Birth at King Salman Armed Force Hospital in 2010. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):1016-1020. https://doi.org/10.3889/oamjms.2017.103

Keywords: early preterm birth; risk factors for preterm birth; preterm birth; neonatal morbidity.

***Correspondence:** Badriah Ali Alansi. Assistant Professor of Obstetrics and Gynecology, Faculty of Medicine, University of Tabuk, Tabuk, KSA. PO Box 71471, Tabuk, KSA. Phone: +966559297667. E-mail: drbadriah@hotmail.com

Received: 16-May-2017; **Revised:** 21-Aug-2017; **Accepted:** 22-Aug-2017; **Online first:** 18-Nov-2017

Funding: This research receives financial support from the Deanship of Scientific Research, the University of Tabuk under grant number (S-1436-0108).

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: To investigate risk factors for early preterm birth.

METHODS AND MATERIAL: A retrospective comparative study was conducted at Tabuk, Kingdom of Saudi Arabia during the period from January to December 2010. Five hundred and ninety-five patient's files and delivery registry logbooks were reviewed, the following information was collected; demographic data, current and past obstetric histories. Then the early and late preterm births were compared for various risk factors. The Statistical Package for Social Sciences (SPSS version 22) was used. The Chi-square and t-test were used to test the statistical significance and a P-value<0.05 considered significant.

RESULTS: Prevalence of early preterm birth was found to be 2.5% in our study group. Women at risk for early preterm birth were: primigravidas (33.7% vs. 26.2% for control), P-value 0.039, OR 1.429 and 95% CI 0.982 - 2.079); multiple gestations (87.7% vs. 95.1% for control, P-value 0.002, OR 0.368 and 95% CI 0.196 - 0.688); and patients with a prior history of placental abruption (3.7% vs. 1.0% for control, P-value 0.027, OR3.928 and 95% CI 1.1360 - 13.586).

CONCLUSIONS: Current study indicated that early preterm births differed from preterm as a whole; primigravida, multiple gestations and a history of placental abruption are independent risk factors for them.

Introduction

Preterm birth defines as the delivery of an infant between 24 and 37 weeks gestation [1, 2]. It remains a big challenge in the perinatal health care and is associated with considerable mortality and short and long-term morbidities for the surviving infants. With the improvement in neonatal intensive care, late preterm infants (born after the 34 weeks) were at far lower risk for these complications when compared to early preterm infants (born between 24 and 34 weeks) [3-5].

Neonates born before 34 weeks experienced considerable morbidity and often necessitate admission to neonatal intensive care units [6]. These morbidities include cerebral palsy, mental retardation and risk for neonatal respiratory distress syndrome [7, 8]. Identification of risk factors before conception or early in pregnancy would lead to interventions that could help to prevent those complications. Many obstetricians decided to postpone delivery after the thirty-fourth week to ensure fetal lung maturity [12].

In Saudi Arabia, the literature is scarce concerning risk factors for early preterm birth. So this study was undertaken, in this survey, we sought to determine the risks associated with early preterm birth which can be prevented if they are fully assessed and appropriately managed.

Subjects and Methods

This retrospective comparative study conducted at King Salman Armed Force Hospital, Kingdom of Saudi Arabia (KSA). The patient's files and delivery registry logbooks in the year 2010 were approached. The ethical committees of both the University of Tabuk and King Salman Armed Force Hospital approved the research.

For this research, the following definitions were adopted: early preterm birth is the delivery at 24 to 33 6/7 weeks; late preterm birth is delivery at 34 to 36 6/7 weeks [4, 5].

During the study period, the number stands at 595 preterm deliveries (between 24 and 36 6/7 weeks of gestation), while 641 of neonates were recorded. The ethics of data collection were strictly followed, and the collected information was used only for research. Babies with chromosomal anomalies, congenital anomalies, and outborn infants were excluded. Comparisons were then undertaken between early and late preterm deliveries. Data have been collected from patient's files and delivery registry log book, which include the following information; maternal age, parity, gestational age, booking status, mode of deliveries, maternal disease, birth weight, multiple gestations and their sex.

The data also include previous pregnancy complications like; abortion, stillbirth, preterm premature rupture of membrane (PPROM), premature rupture of membrane (PROM), placenta abruption, placenta previa and caesarean section. The signs and symptoms of previous pregnancy from the patient record of the entire study group were carefully revised by the authors to reach the above diagnoses. "For example, placenta abruption was distinguished by Vaginal bleeding, abdominal pain, uterine tenderness, rapid contractions, Fetal heart rate abnormalities and confirmation after delivery by the presence of a retroplacental haematoma".

Statistical analysis was carried out by using the Statistical Package for Social Sciences (SPSS version 22). The Chi-square and t-test were used to test the statistical significance; the data were presented as number (percentages), mean (± SD) and a P-value < 0.05 considered statistically significant.

Results

The total numbers of deliveries were 7444. Among them, 595 patients delivered preterm, out of them 187 (31.4%) patients had early preterm delivery (Figure 1).

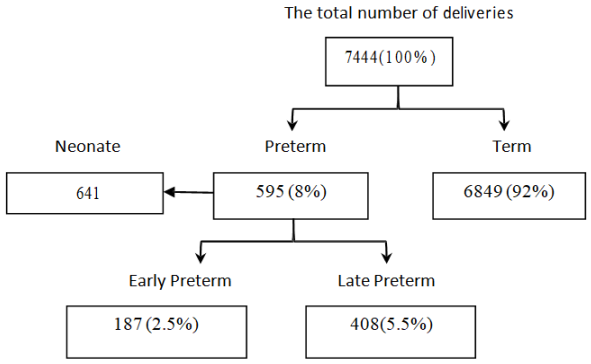


Figure 1: Methodology flowchart

Regarding the general characteristics of the study participants, mean (± SD) age of women in early (case) and late (control) preterm was 27.91 (± 6.110) and 28.15 (± 6.121), respectively. Other characters were shown in Table 1.

Table 1: A comparison between early and late-term deliveries

	Early preterm Mean (± SD)	Late preterm (Control) Mean (± SD)	P-Value*
Maternal age/year (n = 595)	27.91 (± 6.110)	28.15 (± 6.121)	0.660
Gestation age/weeks (n = 595)	30.11 (± 2.696)	35.44 (± 0.739)	0.000
Birth weight/g (n = 641)	1443.42 (± 638.863)	2539.92 (± 547.925)	0.000

*t-test.

Table 2 illustrated that parity (P-value 0.039, OR 1.429 and 95% CI 0.982-2.079) was the only obstetrics risk factor for early preterm birth during pregnancy but booking status, mode of delivery and maternal disease were not.

Table 2: Risk factors for early preterm birth in the current pregnancy (n = 595)

Obstetric factors		Early Preterm group N (%)	Control group (Late preterm) N (%)	P-value*	OR†	95%CI‡	
						lower	upper
Maternal age	< 19 y	7 (3.7%)	13 (3.2%)				
	19-39	147 (93%)	372 (91.2%)	0.423			
	>39	6 (3.2%)	23 (5.6%)				
Booking status	Booked	150 (80.2%)	333 (81.6%)	0.382	0.913	0.589	1.415
	Unbooked	37 (19.8%)	75 (18.4%)				
Parity	PG	63 (33.7%)	107 (26.2%)	0.039	1.429	0.982	2.079
	Multi	124 (66.3%)	301 (73.8%)				
Mode of delivery	Vaginal	113 (41.2%)	270 (66.2%)	0.195	0.780	0.546	1.116
	CS	74 (39.6%)	138 (33.8%)				
Maternal disease	DM	3 (1.6%)	16 (3.9%)				
	HTN	11 (5.9%)	11 (2.7%)				
	Cardiac	1 (0.5%)	2 (0.5%)				
	Anemia	0	1 (0.2%)	0.24			
	HTN and DM	0	3 (0.7%)				
	Cardiac and DM	0	1 (0.2%)				

*Chi-square; †OR = odds ratio; ‡CI =confidence interval.

Comparing late preterm birth, history of placenta abruption (P-value 0.027, OR 3.928 and 95% CI 1.136-13.586) was significantly high in cases of early preterm birth. Histories of abortion, PPROM, PROM, placenta previa, caesarean section, and stillbirth were the other associated factors in late preterm births, but they were not found to be significant when compared with early preterm birth (as shown in Table 3).

Table 3: Risk factors for early preterm birth in Previous obstetrics history (n = 595)

Previous maternal history		Early Preterm group N (%)	Control group (Late preterm) N (%)	P-value*	OR	95%CI	
						Lower	Upper
History of Abortion	Yes	67 (35.8%)	130 (31.9%)	0.194	0.838	0.582	1.206
	No	120 (64.2%)	278 (68.1%)				
History of PPROM†	Yes	13 (7.0%)	16 (3.9%)	0.085	1.83	0.862	3.888
	No	174 (93.0%)	392 (96.1%)				
History of PROM‡	Yes	3 (1.6%)	12 (2.9%)	0.254	0.538	0.150	1.930
	No	184 (98.4%)	396 (97.1%)				
History of Placenta Abruption	Yes	7 (3.7%)	4 (1.0%)	0.027	3.928	1.136	13.586
	No	180 (96.3%)	404 (99.0%)				
History of Placenta Previa	Yes	4 (2.1%)	7 (1.7%)	0.473	1.252	0.362	4.331
	No	183 (97.9%)	401 (98.3%)				
History of caesarean section	Yes	25 (13.4%)	54 (13.2%)	0.529	0.988	0.594	1.645
	No	162 (86.6%)	354 (86.8%)				
History of still birth	Yes	23 (12.3%)	37 (9.1%)	0.143	0.711	0.409	1.235
	No	164 (87.7%)	371 (90.9%)				
History of preterm birth	Yes	5 (2.7%)	11 (2.7%)	0.613	0.992	0.340	2.895
	No	182 (97.3%)	397 (97.3%)				

*Chi-square; †PPROM: preterm premature rupture of membrane; ‡PROM: premature rupture of membrane.

Table 4 depicted that the multiple pregnancies is a risk factor for early preterm birth which is statistically significant (P-value 0.002, OR 0.368 and 95%CI 0.196-0.688), where the sex of the baby was not (P-value 0.622 OR 0.92 and 95%CI 0.662-1.28).

Table 4: Risk factors for early preterm birth in newborn

Neonate characters		Early Preterm group N (%)	Control group (Late preterm) N (%)	P-value*	OR	95%CI	
						Lower	Upper
Multiple gestation (n = 595)	Yes	164 (87.7%)	388 (95.1%)	0.002	0.368	0.196	0.688
	No	23 (12.3%)	20 (4.9%)				
Sex of the baby (n = 641)	Male	121 (56.3%)	231 (54.2%)	0.622	0.92	0.662	1.28
	Female	94 (43.7%)	195 (45.8%)				

*Chi-square.

Discussion

In this study, we assessed the risk factors for early preterm birth as this group might be differing from overall preterm birth. Very few efforts have been tried to estimate the causative factors of early preterm births. If addressed, this can help to decrease the occurrence of preterm birth.

Factors such as maternal age, booking status, parity, multiple gestation, mode of delivery, sex of the babies and maternal disease in the current pregnancy and other factors in the previous obstetrics history like abortion, PPROM, PROM, placental abruption, placenta previa, caesarean section and stillbirth are known risk factors for preterm birth [12-14]. In our study, the factors mentioned above were studied with its relation to early preterm birth among deliveries at King Salman Armed Force Hospital (KSAFH) during the year 2010.

The prevalence of early preterm birth in our study group was found to be 2.5% which is more than the prevalence reported by Lo CC et al. (1.4%) because they excluded iatrogenic preterm deliveries which were included in our study [15]. The prevalence of total preterm birth in our study found to be (8%). Compared to other cities of Kingdom of Saudi Arabia, Similar findings were reported in Jazan (8.24%) [16], while others in Riyadh and Abha cities reported lower rates (5%) [17, 18]. Compared to another part of the world, it is found to be more or less equal to that in Asian (9.8%) and European countries (6.7%), but less than in African (12.6%) and Vietnam (11.8%) [19].

In our study, experience previous history of placenta abruption was identified as the most significant risk factor for preterm birth with odds ratio of 3.928 and 95% CI 1.136-13.586 found in 3.7% cases of early preterm birth Comparing to 1% in late preterm birth (control), which indicated by P-value 0.027 as a highly significant. The mechanism for this has not been well understood, however, the associated placental insufficiency and abruption and it recurrent nature may explain this relation. Our study also revealed significant associations between early preterm birth and primigravidas (33.7% vs 26.2% for control, P-value 0.039, OR 1.429 and 95% CI 0.982 - 2.079).

Multiple gestations are found to be high in both groups 87.7% for the early preterm vs 95.1% for the control group, although, it is found to be statistically significant (P-value 0.002) when the Odd ratio (0.368) done they found to be not significant. Therefore, it is an important risk factor in both early and late preterm birth.

Most prior studies found that risk factors for early preterm birth include a prior preterm delivery, history of fetal demise, history of placental abruption, history of abortions and maternal age (< 20 years and > 34 years). Although, these were contradictory to our results, except the prior history of placental abruption was in line with the findings of our study [20-23].

In support of our findings, a study from Mandruzzato et al. and Dey et al. reported that parity was a risk factor for the early preterm birth. They did not find any significant association with late preterm birth [24, 25].

Our study showed that there was no association between early preterm birth and other risk factors in the current pregnancy as maternal age (P -value 0.423), booking status (P -value 0.382, OR 0.913 and 95% CI 0.589 - 1.415), mode of delivery (P - value 0.195, OR 0.78 and 95% CI 0.546 - 1.116), maternal disease (P -value 0.24, OR 1.065 and 95% CI 0.82 - 1.385), number of fetus (P -value 0.002, OR 0.368 and 95% CI 0.196 - 0.688) and sex of the baby (P -value 0.622, OR 0.92 and 95% CI 0.662-1.28).

Dey et al. indicated that some maternal

medical conditions, including hypertensive disorders of pregnancy and diabetes, are associated with an increased risk for preterm birth at the same time increase the incidence of late preterm birth [25]. The s e results support our findings that no significant association between maternal medical diseases and early preterm birth.

The present study showed that mothers with previous maternal histories of abortion (P -value 0.194, OR 0.838 and 95% CI 0.582 - 1.206), PPROM (P -value 0.085, OR 1.83 and 95% CI 0.862 - 3.888), PROM (P -value 0.254, OR 0.538 and 95% CI 0.15 - 1.93), placenta previa (P -value 0.473, OR 1.252 and 95% CI 0.362 - 4.331), cesarean section (P -value 0.529, OR 0.988 and 95% CI 0.594 - 1.645), still birth (P -value 0.143, OR 0.711 and 95% CI 0.409 - 1.235) and preterm birth (P -value 0.613, OR 0.992 and 95% CI 0.340 - 2.895) found to be not statistically significant with early preterm birth when compared to late preterm birth.

The strengths of this study are a comparative design and first to describe early preterm birth in Tabuk, KSA. The limitation is that it is retrospective and done in one centre cannot be considered representative of all early preterm births.

In conclusion, parity, multiple gestations and a history of placental abruption were identified as important risk factors for early preterm birth. Identifying pregnant women with these risks may decrease the rate of preterm birth and its consequences

Acknowledgements

The authors would like to acknowledge the financial support of this work from Deanship of Scientific Research, the University of Tabuk under grant number (S-1436-0108). Also, our special thanks for the technical help of Dr Hyder Osman Mirghani.

References

1. Khalid O, Hassan A, Abdullah M, Saad A, Ali A Mohammed A, Faisal A. Ministry of health, King Saudi Arabia, manual booklet in Obstetrics & Gynaecology page 30 -34. Available from: (http://www.moh.gov.saDocuments/MOH %20Pocket %20Manual %20in %20 Obstetrics% 20and %20Gynaecology.pdf).

2. Zeitlin J, Szamotulska K, Drewniak N, Mohangoo AD, Chalmers J, Sakkeus L, et al. Preterm birth time trends in Europe: a study of 19 countries. BJOG. 2013; 120(11):1356 -65. https://doi.org/10.1111/1471-0528.12281 PMid:23700966 PMCid:PMC4285908

3. Hack M, Taylor H, Klein N, Eiben R, Schatschneider C, Mercuri -Minich N. School -age outcomes in children with birth weights under 750 g. N Engl J Med. 1994; 331:753 –9. https://doi.org/10.1056/NEJM199409223311201 PMid:7520533

4. Moster D, Lie RT, Markestad T. Long -Term Medical and Social Consequences of Preterm Birth. N Engl J Med. 2008; 359(3): 262 -273. https://doi.org/10.1056/NEJMoa0706475 PMid:18635431

5. Rees S, Inder T. Fetal and neonatal origins of altered brain development, Early Hum Dev. 2005;81(9):753 -61. https://doi.org/10.1016/j.earlhumdev.2005.07.004 PMid:16107304

6. Marret S, Ancel PY, Marpeau L, Marchand L, Pierrat V, Larroque B, et al. Neonatal and 5 -year outcomes after birth at 30 -34 weeks of gestation. Obstet Gynecol. 2007;110(1):72 -80. https://doi.org/10.1097/01.AOG.0000267498.95402.bd PMid:17601899

7. Raju TN, Higgins RD, Stark AR, Leveno KJ. Optimizing care and outcome for late -preterm (near - term) infants: a summary of the workshop sponsored by the National Institute of Child Health and Human Development. Pediatrics. 2006; 118(3):1207 –14. https://doi.org/10.1542/peds.2006-0018 PMid:16951017

8. Lewis DF, Futayyeh S, Towers CV, Asrat T, Edwards MS, Brooks GG. Preterm delivery from 34 to 37 weeks of gestation: is respiratory distress syndrome a problem? Am J Obstet Gynecol. 1996;174(2):525 -8. https://doi.org/10.1016/S0002-9378(96)70421-0

9. McGowan J, Alderdice F, Doran J, Holmes V, Jenkins J, Craig S, Johnston L. Impact of neonatal intensive care on late preterm infants: developmental outcomes at 3 years. Pediatrics. 2012;130(5):e1105 -12. https://doi.org/10.1542/peds.2012-0745 PMid:23027176

10. Bhutta AT, Cleves MA, Casey PH, Cradock MM, Anand KJ. Cognitive and behavioral outcomes of school -aged children who were born preterm: a meta -analysis. JAMA. 2002;288(6):728 -37. https://doi.org/10.1001/jama.288.6.728 PMid:12169077

11. Sibai B. Magnesium sulfate for neuroprotection in patients at risk for early preterm delivery: not yet. Am J Obstet Gynecol. 2011;205(4):296 -7. https://doi.org/10.1016/j.ajog.2011.02.084 PMid:22083053

12. Ekwo E, Gosselink C, Moawad A. Unfavorable outcome in penultimate pregnancy and premature rupture of membranes in successive pregnancy. Obstet Gynecol. 1992; 80(2):166 -72. PMid:1635725

13. Butali A, Ezeaka C, Ekhaguere O, Weathers N, Ladd J, Fajolu I, et al. Characteristics and risk factors of preterm births in. Pan Afr Med J. 2016; 1; 24:1.

14. Robert L, Jennifer F, Culhane J, Roberto R. Epidemiology and causes of preterm birth. Lancet. 2008;5;371(9606):75 - 84.

15. Lo CC, Hsu JJ, Hsieh CC, Hsieh TT, Hung TH. Risk factors for spontaneous preterm delivery before 34 weeks of gestation among Taiwanese women. Taiwan J Obstet Gynecol. 2007;46(4):389 -94. https://doi.org/10.1016/S1028-4559(08)60008-X

16. Kamel RM. A clinical epidemiology study of spontaneous preterm birth in Jazan, Saudi Arabia. J Reprod Med. 2010; 55(9 -10):395 -403.

17. Youssef A, Al -Eissa S. Ba'Aqeel. Author links open the author workspace.b. Risk factors for spontaneous preterm birth in a Saudi population. Eur J Obstet Gynecol Reprod Biol. 1994; 57(1):19 -15. https://doi.org/10.1016/0028-2243(94)90104-X

18. Ali K, Ali M, Khalid M. High altitude and spontaneous preterm birth. Int J Gynaecol Obstet. 1996;54(1):11 -5. https://doi.org/10.1016/0020-7292(96)02673-2

19. Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH, et al. The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull World Health Organ. 2010;88:31 –38. https://doi.org/10.2471/BLT.08.062554 PMid:20428351 PMCid:PMC2802437

20. Chin LO C, Hsu JJ, Hsieh CC, Hsieh TT, Hung T. Risk factors for spontaneous preterm delivery before 34 weeks of gestation among Taiwanese women. Taiwan J Obstet Gynecol. 2007; 46:389 –393. https://doi.org/10.1016/S1028-4559(08)60008-X

21. Martius JA, Steck T, Oehler MK, Wulf KH. Risk factors

associated with preterm (< 37 + 0 weeks) and early preterm birth (< 32 + 0 weeks): univariate and multivariate analysis of 106 345 singleton births from the 1994 statewide perinatal survey of Bavaria. Eur J Obstet Gynecol Reprod Biol 1998; 80:183 –9. https://doi.org/10.1016/S0301-2115(98)00130-4

22. Beta J, Akolekar R, Ventura W, Syngelaki A, Nicolaides KH. Prediction of spontaneous preterm delivery from maternal factors, obstetric history and placental perfusion and function at 11 –13 weeks. Prenat Diagn. 2011; 31: 75 –83. https://doi.org/10.1002/pd.2662 PMid:21210482

23. Chen CP, Wang KG, Yang YC, See LC. Risk factors for preterm birth in an upper middle class Chinese population. Eur J Obstet Gynecol Reprod Biol. 1996; 70:53 –9. https://doi.org/10.1016/S0301-2115(96)02555-9

24. Butali A, Ezeaka C, Ekhaguere O, Weathers N, Ladd J, Fajolu I, et al. Risk Factors for Late Preterm Births: A Case - Control Study. Gynecology & Obstetrics. 2013; 3:182.

25. Dey M, Agarwal R, Maji D, Kohli UA. Maternal and fetal factors observed with late preterm births. Int J Res Med Sci. 2015; 3(8):1969 -73. https://doi.org/10.18203/2320-6012.ijrms20150310

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):1021-1027.
https://doi.org/10.3889/oamjms.2017.200
eISSN: 1857-9655
Public Health

Management, Diagnostic and Prognostic Significance of Acetylcholinesterase as a Biomarker of the Toxic Effects of Pesticides in People Occupationally Exposed

Mitar Lutovac[1], Olga V. Popova[2], Zoran Jovanovic[3], Hatidza Berisa[4], Radoman Kristina[5], Sonja Ketin[6*], Marko Bojic[7]

[1]University Nikola Tesla, Belgrade, Serbia; [2]Southern Federal University, Russia; [3]High School Dositej Obradovic, Belgrade, Serbia; [4]Military Academy, Belgrade, Serbia; [5]High Medical School, Podgorica, Montenegro; [6]University of Novi Sad, Square Dositeja Obradovica 6, 21000, Novi Sad, Serbia; [7]Privredna Akademija Novi Sad, Novi Sad, Serbia

Citation: Lutovac M, Popova OV, Jovanovic Z, Berisa H, Kristina R, Ketin S, Bojic M. Management, Diagnostic and Prognostic Significance of Acetylcholinesterase as a Biomarker of the Toxic Effects of Pesticides in People Occupationally Exposed. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):1021-1027. https://doi.org/10.3889/oamjms.2017.200

Keywords: Toxic effects; Pesticides; Organophosphorus compounds; Occupational exposure.

***Correspondence:** Sonja Ketin. University of Novi Sad, Square Dositeja Obradovica 6, 21000, Novi Sad, Serbia. E-mail: ketin.sonja@gmail.com

Received: 22-Aug-2017; **Revised:** 20-Oct-2017; **Accepted:** 28-Oct-2017; **Online first:** 01-Dec-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: The paper presents research on the most common causes of exposure that leads to disorders of cholinesterase activity, as well as an overview of the results of cholinesterase activity with the poisoned people.

MATERIAL AND METHODS: In a group of 35 acute poisoned patients by organophosphate compounds has led to inhibition of AchE. A total number of examined workers are 175 in the chemical industry and agricultural production in the area of Rasina District-Serbia.

RESULTS: The results showed that among workers who are constantly exposed to pesticides, acetylcholinesterase is within the reference value. Having examined the medical records of these workers, it is noted that, at 72%, there is a slight fall of AchE activity, each year. The workers who had been exposed to pesticides at the time of testing had acetylcholinesterase regarding reference value, but 52% of them had a few years ago significantly reduced the value of the activity of acetylcholinesterase, which was treated and then transferred to other jobs. The 48% of these workers had acetylcholinesterase regarding benchmarks or were transferred to other jobs, for a variety of other health problems.

CONCLUSION: Using each pesticide should only deal with people who are well versed in the way of its use, as well as the way of protecting them from poisoning.

Introduction

Organophosphorus compounds (OPC) inhibit both serum and erythrocyte cholinesterase. According to chemical composition, these are esters of phosphoric acid. Organophosphorus compounds smoothly penetrate the uninjured skin and all mucous membranes. Poisoning can occur at inhalation, contact through is the dermal penetration route and inhalation, and the lower character has a penetration through the digestive tract. Organophosphorus insecticides are causes about 80-90% of all cases of acute pesticide poisoning of the people. Annually, in the world is registered about 100,000 victims of acute organophosphate poisoning. About 20% were accidental poisoning, and suicidal is approximately 70%, with 30% of the cases are with fatal outcome [1-4]. Exposure to organophosphate can cause four clinical syndromes that have different pathogenesis, prognosis and requiring different treatment. They occur after a long latency different intervals after exposure to the organism of a man to organophosphorus compounds. These are: acute cholinergic crisis, intermediate syndrome, delayed peripheral neuropathy and other specific lesions of organs. Symptomatology of acute cholinergic crisis occurs immediately after exposure or to a maximum of 12 hours from the moment of exposure. How is in poisoning by organophosphates the physiological function of the enzyme acetylcholinesterase inhibited, the image of poisoning has the characteristics of intoxication by endogenous acetylcholine. Depending on the degree of inhibition of acetylcholinesterase and increase acetylcholine content, organophosphates lead to stimulation or paralysis transmission of nerve impulses in the central and neuro-effector synapses of the cholinergic nervous. In the clinical picture of poisoning are being distinguished: muscarinic effects (bradycardia, bronchoconstriction, bronchorrhea,

hypotension, increased gastrointestinal motility, abdominal cramps, miosis, hypersalivation), nicotinic effects (hypertension, tachycardia, fasciculation, skeletal muscle necrosis) and the central toxic phenomena (tremor, incoordination movements, convulsions, central respiratory depression, coma and death) [5, 9-11]. The therapy of organophosphate poisoning includes aspiration of the upper respiratory tracts, cardiopulmonary reanimation, gastric lavage, provision of specific drugs and antidotes. In the context of specific therapies are delivered as atropine, as an antagonist of a muscarinic receptor and oximes as inhibited cholinesterase reactivators. Conventional therapy involves the use of benzodiazepines, which are effective anticonvulsants [1, 5-7].

Organophosphate compounds easily penetrate into the body through the skin and of all lining. These compounds then react with an acetylcholinesterase to form a complex of enzyme phosphate. The phosphorylated enzyme is very stable due to the phosphorus atom, which is not electrophilic enough to react in the presence of water, so the spontaneous reactivation does not take place or is very slow, that is, inhibition of acetylcholinesterase is practically irreversible. Therefore, there is a buildup of acetylcholine at the synapses of nerve endings and to the clinical picture of toxic effects of endogenous acetylcholine.

In this study, on workers of occupationally exposed pesticides, will be monitored biochemical and hematological parameters in evaluating the impact of pesticides on the health of exposed workers: identify the toxic effects of pesticides using appropriate standardized biochemical and hematological parameters; the activities of some specific-organ enzymes; and determine the cholinesterase activity as a biomarker of toxicity of organophosphorus compounds; and assess the importance of changes in cholinesterase activity under the influence of pesticides from the group of organophosphorus compounds, as an indicator of the degree of toxicity in exposed workers.

All the above parameters can be determined in some biochemical laboratories and for their determination is using the standard equipment. This means that they can be monitored with any systematic review which is regularly subjected to workers in occupational exposure. It is planned, therefore, that from the obtained results, by the made findings, the device protocols for monitoring the early toxic effects of pesticides when still there was no visible damage to health. Thus prepared protocols can be used in medicine work within the healthcare of the workers, with the aim of helping the employees to be timely moved from those jobs to which they are endangered, so as not to cause lasting impairment of their health and working ability.

Material and Methods

A total number of examined workers are 175 in the chemical industry and agricultural production in the area of Rasina District. Of these, the two control groups were 47 workers who were not exposed to pesticides. Other groups consisted the workers who are (or previously were) exposed to pesticides. And that: 78 workers in the chemical industry who are working in the production process of pesticides in the plant of the "Pesticides" and 50 workers working in agriculture. Workers of the "Pesticides" plant were divided into three groups according to the length and duration of occupational exposure:

Industrial workers who are constantly exposed to pesticides (N = 42);

Industrial workers who are occasionally exposed to pesticides (N = 17);

Industrial workers who have previously been exposed to pesticides, and then were transferred to other jobs (N = 19);

A control group of industrial workers (CGI, N24) consisting of 24 industrial workers who are not exposed to pesticides;

Agricultural workers who are in the process of plant protection continually exposed to pesticides (N = 50). 35 of them were acutely poisoned OPC, 40 days after the systematic review, so we get the Va subgroup; and A control group of workers in agriculture (CGa, N = 23) comprised of 23 agricultural workers who are not exposed to pesticides.

Basic data which detail the examined groups of workers are presented in Table **1**. All data were obtained during regular periodic examinations of workers, which mean that they can be monitored in a systematic review of each whom undergoes occupationally exposed workers.

Table 1: Average values of the age, of the total working experience (TWE) and the exposed working experience (EWE) of the observed groups

Group	Average age	TWE	EWE
I (N = 42)	37,4 ± 6,28	15,7 ± 4,1	9,4 ± 4,5
II (N = 17)	38,3 ± 7,7	14,2 ± 5,2	7,9 ± 5,8
III (N = 19)	48,6 ± 6,4	20,3 ± 5,2	15,3 ± 5,6
IV (N = 24)	34,9 ± 8,3	12,3 ± 5,7	9,9 ± 3,4
V (N =50)	35,7 ± 5,9	11,9 ± 2,7	8,2 ± 4,4
VI (N = 23)	36,8± 3,1	14,7 ± 8,4	11,9 ± 7,7

Termination of exposure to pesticides for the III-rd group is 5.45 years, with a range of minimum and maximum values in 1-13 year.

The study made use of standard methods:

- Hemoglobin concentration, the number of erythrocytes, the number of leukocytes, leukocyte formula, thrombosis and reticulocytes were determined by counting

blood elements of the company Coulter counter. (Commercial instruction of the device).

- Chemical examination of urine was made for urine test strips of the Lachema company.
- Urine sediment was carried out microscopically.
- Blood glucose concentration was determined by GOD/PAP method.
- The concentration of cholesterol in the blood was determined using cholesterol oxidase with phenol and 4-aminoantipyrine as chromogens.
- The concentration of triglycerides in the blood was determined by glycerol oxidase/PAP without correction in blood and urine was determined by Jaffe's method with alkaline picrate.
- Urea in the blood was determined using urease and hypochlorite.
- Uric acid in blood was determined with an alkali phosphatase
- The concentration of bilirubin in the blood was determined by Jendrassik-Groff's method
- The activity of transaminases in the blood was determined by a discontinuous colourimetric method with 2,3-dinitrophenylhydrazine.
- Alkaline phosphatase activity in the blood was determined with p-nitrophenyl phosphate as a substrate.
- Glutamyltransferase activity in blood was determined by reaction with L γ glutamyl-3-carboxy-p-nitroanilide as a substrate.
- Cholinesterase activity in blood was determined by a kinetic method with Propionaldehyde as substrate and 5,5'ditio-bis-2-nitrobenzol acid (DNTB) which gives a stained reaction with tioholinom.

For the determination of the above parameters were used appliances: Counter of blood elements "Coulter Counter"; Centrifuge "Technics"***;*** Biochemical analyser "Selectra"; Microscope "Carl Zeiss" and Spectrophotometer Gilford 300 T and LKB.

Statistical methods

From the obtained results was calculated the mean value, standard deviation, coefficient of variation and medians. The significance of differences between mean values was tested by Student's t-test and analysis of variance for a probability level of 0.05.

Results

In modern agriculture, as in public health, for the destruction of pests of the plant and animal origin are used various means, all more or less toxic, known by the common name of pesticides. The chemical composition of pesticides is chlorinated hydrocarbons; organophosphorus products; dinitrophenols; nicotine; arsenical preparations; fumigation; organic mercury compounds; coumarin preparations; zinc phosphide; talium sulphate; sodium fluoroacetate; some solvents and others. Many of these are commonly known in the trade under the trade name.

Pesticides are very effective in the fight against plant and animal pests, but those, especially poisonous, are not without danger to the people who apply them. To poisoning usually comes from improper handling of pesticides, and due to an error or accident, but are known cases of deliberate poisoning. Pesticides can penetrate into the body through the digestive tract through the respiratory organs, or through the skin. For many pesticides, poisoning is possible through any of these routes, but one of them is still the most common. Signs of poisoning may, depending on the type of pesticide to be very different. Most of them, more or less, effect to the nervous system and cause a headache, dizziness, paralysis, anxiety and other nervous disorders. Many, however, cause stomach pains, vomiting and diarrhoea (organophosphorus preparations, dinitrophenols, nicotine, arsenic preparations, etc.). Some of them cause choking or increased sweating, salivation, tears, etc. Finally, a large number of pesticides and solvents irritate the skin and mucous membranes with which it comes into contact, causing a variety of changes on them.

Recovering of patients poisoned with organophosphorus compounds (OPC) is a long and slow process. Degradation of acetylcholine occurs only after the synthesis of new quantities of acetylcholinesterase (AchE), which is a small daily percentage. During the treatment, acetylcholinesterase was determined every 72 hours while monitoring the return of the acetylcholinesterase in the range of the reference value. When the enzyme activity is within the reference values and did not show the presence of the OPC is discontinued administration of an antidote. This fact points to the great importance of laboratory testing enzyme activity both in the aspect of treatment and in economic terms. In acute poisoning, the poisoned ones will be temporarily unable to work, until the rehabilitation of clinical manifestations. After complete healing, workers need to be transferred to other jobs until cholinesterase activity reaches a level that it had before exposure (approximately 2-3 months depending on the degree of inhibition). After that, workers will be able to return to their previous jobs with the rehabilitation of working conditions and

adequate implementation of protection measures. In cases of poisoning with permanent consequences or recurrent poisoning, the changes are indicated in the workplace [6].

Diagnosis of poisoning organofluorine compounds can be confirmed by determination of acetylcholinesterase activity in the serum. According to data of the Poison Control Center of Serbia in 2011 examined 58 patients due to acute exposure and pesticide poisoning, and the clinic has treated 35 patients. The most common toxic agents were organofluorine insecticides (71%). There were treated approximately an equal number of men and women. Of hospitalised patients without clinical symptoms of poisoning (PSS0) were 14 people (40%). Poisoning light degree (PSS1) is manifested in 6 patients (17%) of moderate severity (PSS2) in 3 patients (9%), and because of severe poisoning (PSS3) were treated nine patients (25%). The lethal outcome of acute poisoning organophosphate insecticides was noted in 2 patients [13].

In 2012, due to acute exposure and pesticide poisoning in OPR CKT, were examined 97 patients (2.3%) of the total number of examined patients, which was significantly higher than in the previous year. At the hospital treatment was received 54 patients, accounting for 6.5% of all hospitalised patients. About gender, there were more men (65%) than women (35%). The most common toxic agents, this year it was organophosphate insecticides (54%). Of hospitalised patients without clinical symptoms of poisoning (PSS0) were 18 people (33%). Poisoning light degree (PSS1) was demonstrated in 15 patients (28%) of moderate severity (PSS2) in 6 patients (11%), and because of severe poisoning (PSS3) were treated ten patients (19%). The lethal outcome occurred in 3 patients who were poisoned by organophosphate insecticides [14].

Compared to 2012 in 2013 was recorded a decline in the number of acute pesticide poisoning (81 patients). At the hospital treatment was received, 38 patients. Were treated 60.5% men and 39.5% women. Of hospitalised patients poisoning light degree (PSS1) is manifested in 22 patients (7.9%), due to a severe poisoning (PSS3) were treated eight patients (21.1%). The lethal outcome occurred in 5 patients [15].

In acute poisoning is usually a correlation between the degree of inhibition of AchE activity and severity. In the inhibition of AChE activity by 50% relative to the initial, one can expect light poisoning, from 60% to 70% of medium-heavy, 80% and 90% in the heavy and AChE activities designed from 1 to 4% lethal. The appearance of symptoms of poisoning depends more on the speed of cholinesterase inhibition than the absolute level of found activity [24, 25, 26].

Due to the significant presence of pesticides in the Rasina district has been investigated the clinical status of 175 workers in the chemical industry and agricultural production in the area of Rasina District. Workers were divided into six groups. Of these, the two control groups were 47 workers who were not exposed to pesticides. Other groups consisted of workers who are (or were) exposed to pesticides, such as 78 workers in the chemical industry who are working in the production process of pesticides in the plant of "Pesticides" and 50 workers working in agriculture. Workers in the "Pesticides" plant were divided into three groups according to the length and duration of occupational exposure, namely: the workers who are constantly exposed to pesticides (N=42); workers who are occasionally exposed to pesticides (N=17); and workers who have previously been exposed to pesticides, and then were transferred to other jobs (N=19).

At the time of the test, the results showed that among workers who are constantly exposed to pesticides, acetylcholinesterase is within the reference value. Having examined the medical records of these workers, it is noted that, at 72%, there is a slight fall of AchE activity, each year.

In industrial workers who are occasionally exposed to pesticides acetylcholinesterase in serum is in the framework of reference values, and there is a declining trend over the past years.

The workers who had been exposed to pesticides at the time of testing had acetylcholinesterase in terms of reference value, but 52% of them had a few years ago significantly reduced the value of activity of acetylcholinesterase, which were treated and then transferred to other jobs. The 48% of these workers had acetylcholinesterase in terms of benchmarks, or were transferred to other jobs, for a variety of other health problems.

For workers who are working in agriculture being exposed to pesticides, the results showed that the activity of acetylcholinesterase is also in terms of the reference value. A review of their medical records showed that the workers during their years of work in 87% of cases have acetylcholinesterase activity in the framework of the reference value, which over the years has not significantly decreased, and 13% had over the years, the value of slight decreased activity of acetylcholinesterase.

Acute toxicity, however, caused a significant decrease in the activity of acetylcholinesterase, which is shown in Table 2. The cause of toxicity has been malathion, a preparation which is dissolved erroneously by supervisor and given to the use of workers. In 89% of workers value of the enzyme activities of AchE were lower after the use of such prepared preparation of those values that they had during the systematic review. The percentage of inhibition ranged from 1% to 59%, indicating varying degrees of poisoning. The highest degree of poisoning had older workers. Only four of them from

this shift, they had reduced activity of acetylcholinesterase.

Table 2: Percentage of reduction in serum cholinesterase activity of 35 workers of your group

Percentage of AchE activity reduction in a patient group	AchE activity in a group before acute poisoning	AchE activity in a group after acute poisoning
1. - 49%	2938	1442
2. - 38%	3058	1170
3. - 58%	2458	1436
4. - 59%	3128	1842
5. - 41%	2432	1408
6. - 16%	2099	1770
7. - 19%	2338	1884
8. - 20%	2372	1908
9. - 34%	2648	2002
10. - 2%	2110	2072
11. –32%	2587	2008
12. –36%	3130	2014
13. –33%	3040	2328
14. - 18%	2858	2342
15. – 7%	2229	2070
16. - 22%	2788	2172
17. – 14%	3087	2666
18. ----	2093	2094
19. -15%	2598	2070
20. -15%	2449	2092
21. - 3%	3003	2928
22. ----	3015	3004
23. - 5%	1898	1805
24. ----	2008	2000
25.. ----	1992	1980
26. - 3%	2222	2148
27. -1%	2412	2384
28. - 3%	2143	2079
29. -17%	2998	2489
30. -4%	2089	2002
31. -29%	2924	2088
32. -5%	2118	2014
33. -23%	3008	2311
34. -11%	2525	2000
35. -18%	2768	2260

The reference values for serum cholinesterase are from 2618 to 6971 U / L.

In order to look at the general state of health of workers exposed to pesticides, we are, in addition to determining the activity of acetylcholinesterase, as a biomarker of toxicity of organophosphorus compounds (OPC), set the number of haematological and biochemical parameters as indicators: hematotoxicity; nephrotoxicity and hepatotoxicity. The results showed that the number of red blood cells are higher in industrial workers exposed to the constant action of pesticides, than in all other groups of industrial workers and that there is no statistically significant difference in the number of red blood cells between the II and III groups of industrial workers. There was a statistically significant difference between the number of red blood cells for agricultural workers who are exposed to pesticides and the control group of workers in agriculture. Increased number of erythrocyte in groups of workers compared to the control group is probably a result of higher feeding these workers. Hemoglobin concentration was not significantly different in either group of tested workers, except in the Vath subgroup in which the acute organophosphate poisoning of workers caused a statistically significant decrease in haemoglobin concentration. White blood cell count was not significantly different in either group of examined workers. In the leukocyte, the formula was observed a slight decrease in segmented granulocytes at V-th group compared to the control group of workers in agriculture. In the same group of workers are slightly increased lymphocytes.

The results lead us to conclude that exposure to pesticides has not caused permanent haematotoxicity in workers exposed to pesticides. However, acute pesticide poisonings caused a statistically significant decrease in haemoglobin concentration.

Hepatotoxicity of pesticides in our patients we evaluated by determining the activity of some enzymes (transaminases, alkaline phosphatase, gamma-glutamyl transferase) and bilirubin. The results showed that transaminase does not exceed the limit reference value in either group of respondents. Among the groups of industrial workers exposed to pesticides no significant differences in the activity of transaminases. Alkaline phosphatase does not exceed the limit of the reference value in any group of patients, and no statistically significant difference in the alkaline phosphatase activity between the control group and any group of examined workers. Gama deficient does not exceed the limit reference value in either group of respondents. No statistically significant differences in the activity of GGT between the control group and any group of examined workers. The concentration of bilirubin is in all groups within the reference value. No statistically significant differences in the concentration of bilirubin between two groups and the control group.

Discussion

The obtained results show that the exposure of workers to pesticides did not cause hepatotoxicity.

Nephrotoxicity in workers exposed to pesticides, we evaluated by determining creatinine, urea and uric acid in their serum, as well as physical-chemical analysis and the examination of their urine sediment. The obtained results showed that creatinine in the blood does not exceed the limit of the reference value in any study group. There was no statistically significant difference in creatinine concentration among the test groups. It regulates the blood does not exceed the reference value in any study group. Among the groups of workers exposed to pesticides, there is no significant difference in the concentration of urea. Uric acid in any group does not exceed the limits of reference values.

Among the groups of workers exposed to pesticides, there were no statistically significant differences in the concentration of uric acid. Chemical and microscopic examination of urine in all groups is clear. 60% of workers first and the fourth group had calcium oxalate crystals in the urine sediment. The presence of crystals of calcium oxalate is most likely a result of the so-called "hard water" you drink these workers.

Pesticide poisoning

Treatment in case of poisoning varies and also is applied to each concrete case. However, whenever it comes to some of the pesticide poisoning we should quickly call a physician or transfer the patients to the hospital, but before the doctor arrives should be taken, mainly, the following general measures:

1. It should come as soon as possible to establish which pesticides caused the poisoning. If the poisoning occurred at work, in most cases, it is not difficult, but if it comes to deliberate poisoning, then it is much more difficult. In any case, to take all necessary measures will be important to preserve the sputum of patients, the vessel in which the pesticide is found, as well as the clothes of workers, if needed chemical analysis of pesticides.

2. If poisoning is caused by the digestive tract through the mouth, should be as soon as the appropriate way to induce vomiting, and if possible and wash the stomach. Then, giving some laxative means to discharge hoses. If the poisoning is caused by some chlorinated hydrocarbons (DDT, hexachlorocyclohexane - NSN, dieldrin, toxaphene, etc.), for the cleaning should be given neither castor nor any other oil because the oil absorbs these toxins. If poisoning is caused by the skin or via the conjunctiva of the eye, then with the basic wash of contaminated skin and eyes should be removed remains of poison. For such washing are the best water and soap*.*

3. If it is poisoning some of asphyxiating, for example, hydrocyanic acid or the like, then the patient should immediately administer artificial respiration. To the patient, in this case, is much more useful artificial respiration on the spot, without losing time, then the transfer to the hospital. Of course, doctors should be in this case, immediately call or by telephone notify the measures taken and ask for advice. However, with artificial respiration in patients to continue until the patient is fully restored, or until the doctor arrives.

4. In case of poisoning at work, should urgently be taken the necessary measures to prevent the occurrence of poisoning to others, new cases;

5. Any case of pesticide poisoning at work, and otherwise, the application must be submitted to the appropriate authorities of sanitary inspection and inspection at work.

When poisoning with pesticides, as well as in other poisonings, are given if any, the so-called antidotes. Then are given drugs to maintain heart and medicines for calming of disturbances etc. In the case of choke is given oxygen, and when it is necessary and the blood transfusion.

In conclusion, the fact is that today we can hardly survive without pesticides. However, there is an alternative, which consists of biological possibilities to eliminate pests and diseases, from an attempt which is collectively called as organic agriculture. It is certain that we must try to replace as many pesticides and produce healthy food. Pesticide should be taken as a necessary evil at this point helps humanity to ensure a sufficient food source, but the real challenge is precise to seek ways of production which will not poison the land, water and air by these compounds. The main problem associated with the use of pesticides and their usage is a knowledge of a very narrow circle of negative effects because users are content with the current result (the destruction of pests) and often do not think about all the interactions and interdependencies that such action has on the long run List of unwanted operation of DDT or other pesticides to human health, or the environment would be lengthy. And while the overall use of pesticides can be individually badly affect on each of us remains to try to reduce the use of those pesticides in 12% of households. Maybe your imagination will be stimulated by the following examples.

Against weeds are recommended mechanical (e.g. surface treatment of land grabbing and pulling of weeds) and thermal methods (targeted incineration), as well as the alternate sowing of various crops on the same parcel.

Fighting insects includes a selection of healthy, robust varieties of crops that are adapted to climatic conditions, alternating sowing of different crops on the same parcel of land, the natural balance of the soil microorganisms by avoiding synthetic chemical and soil improvers, as well as surface treatment of the soil. In the case of occurrence of stronger insects are recommended mineral substances such as algae powder, and insecticides based on plant extracts.

Instead of fungicide, antifungal is recommended to use copper salts or preparations of sulphur.

About pesticide poisoning should, above all, take appropriate protective measures. It is necessary, first, that pesticides are stored properly and by the regulations on poisons stored properly. Storage should be organized in such a way that by pesticides, especially more toxic, can be reached only on permitted, regulated manner. Also, the warehouse operator and the vendor must be well acquainted with the nature of the toxic properties of each pesticide sold.

Depending on the way in which the highest possible poisoning, it is recommended for the application of any pesticide or group of pesticides with adequate protection (protective clothing, mask, gloves, boots, etc.).

Then, it is necessary that each pesticide is applied to the mandatory taking of prescribed hygienic and technical protection measures. Instructions on taking these measures should be attached to each

package of each pesticide. If such instructions are missing when packaging, should be asked from the store when purchasing pesticides. - Instructions must be strictly observed.

Finally, using each pesticide should only deal with people who are well versed in the way of its use, as well as the way of protecting them from poisoning.

References

1. Risher JF, De Rosa CT, Jones DE, Murray HE. Summary report for the expert panel review of the toxicological profile for mercury, Toxicol Ind Health. 1999; 15: 483 – 516. https://doi.org/10.1177/074823379901500504 PMid:10487360

2. Lauwerys RR, Hoet P. Industrial chemical Exposure: Guidelines for Biological Monitoring Boca Raton: Lewis Publisher, 1993.

3. Mertz W, Khan K, Long SG, et al. Symposium: Between a Rock and a Hard Place: Dietary and Toxicological Standards for Essential Minerals, New Orleans, 1997.

4. WHO Trace elements in human nutrition and health, Geneva, Switzerland, 1996.

5. Mertz W. J Nurt. 1998; 128: 712 – 7.

6. Vidakovic A. Professional toxicology. Belgrade: Association of Toxicologists of Yugoslavia, 2000.

7. Hulka BS. Overview of biological markers, in Biological Markers in Epidemiology, Hulka BS, Griffith JD, Wilcosky TC, Eds., Oxford University Press, New York, NY, USA, 1990: 3-15.

8. Naylor S. Biomarkers: current perspectives and future prospects. Expert Review of Molecular Diagnostics. 2003; 3(5):525–529. https://doi.org/10.1586/14737159.3.5.525 PMid:14510173

9. Manno M, Viau C, Cocker J, et al., Biomonitoring for occupational health risk assessment (BOHRA), Toxicology Letters. 2010; 192(1):3–16. https://doi.org/10.1016/j.toxlet.2009.05.001 PMid:19446015

10. Knudsen LE, Hansen ÅM. Biomarkers of intermediate endpoints in environmental and occupational health, International Journal of Hygiene and Environmental Health. 2007; 210(3-4):461–470.

11. Majkic-Singh NT, Ignjatovic S, Kovacevic R, Ilic M, Lalic N. Prognostic significance of biomarkers. Yugoslav Medical Biochemistry. 2002; 21(1):1-8.

12. Gudenus, R. and Granzer, U.: Biomarker und Surrogatendpunkte. In: transkript. 7, 2010, S. 31-32.

13. Majkic-Singh N. Osteoporosis-The significance of clinical-biochemical parameters for detection and monitoring. Inpharm. 1997; 10: 11.

14. Chen JT, Hosoda K, Hasumi K, Ogata E, Shiraki M. Serum N-terminal osteocalcin is a good indicator for estimating responders to hormone replacement therapy in postmenopausal women. J Bone Min Res 1996; 11: 1784'92.

15. Ilic M, Majkic-Singh N. The importance of clinical, biochemical parameters for monitoring osteoporosis. Yugoslav Med Biohem. 1996; 15: 216.

16. Christenson RH, Azzary HME. Biochemical markers of the acute coronary syndromes. Clin Chem. 1998; 44: 1855-86. PMid:9702995

17. Majkic-Songh N. Biochemical parameters for the diagnosis of myocardial infarction. Infarm. 1998; 4:18-19.

18. Majkic-Singh N. New biochemical markers of myocardial damage. Jugoslov Med Biohem. 1999; 18(2-3): 63-70.

19. Ignjatovic S, Mirkovic D, Majkic-Singh N, Ignjatovic D. Phospholipase A, lipase, amylase and pancreatic amylase activity in taurocholate induced experimental acute necrotizing pancreatitis. Jugoslov Med Biohem. 1998; 17(2): 69-74.

20. Ignjatovic S, Majkic-Singh N, Mitrovic M, Gvozdenovic M. Biochemical evaluation of patients with acute pancreatitis. Clin Chem Lab Med. 2000; 38:1141-44. https://doi.org/10.1515/CCLM.2000.173 PMid:11156345

21. Majkic-Singh N. Metabolic changes and laboratory diagnosis of diabetes. Jugoslov Med Biohem. 1998; 17(1): 1-9.

22. Vessella LR, Lange P. Issues in the assessement of PSA imunoassays. The Urologic Clinics of North America. 1993; 20. 607-17. PMid:7505968

23. Maksimovic R. Clinical Biochemistry, Pharmacy and medical faculties Travnik, 2016.

24. Varagic V, Milosevic M, Pharmacology, 23.edition, Elit Medica, Beograd, 2009.

25. Barton L, Parker K, Blumenthal D, Buxton I, Goodman and Gilman's Manual of Pharmacology and Therapeutics, The McGraw Hill Companies, New York, 2008.

26. Katzung B, Masters S, Trevor A, Basic and clinical pharmacology, 12th Edition, The McGraw Hill Companies, New York, 2012.

27. Hartman DE. Missed diagnoses and misdiagnoses of environmental toxicant exposure. The psychiatry of toxic exposure and multiple chemical sensitivity. Psychiatr Clin North Am. 1998;21(3):659-70. https://doi.org/10.1016/S0193-953X(05)70030-7

28. Mikov M. Industrial Toxicology. Belgrade: Scientific Book, 1980.

29. Stankovic D. Occupational medicine. Beograd -Zagreb: Medical Book, 1986.

30. Stankovic M, Milic S. Analysis of biological material in industrial toxicology, Nis: Prosveta, 1970.

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):1028-1035.
https://doi.org/10.3889/oamjms.2017.171
eISSN: 1857-9655
Public Health

Experiences of Barriers and Motivators to Weight-Loss among Saudi People with Overweight or Obesity in Qassim Region - A Qualitative Study

Abdulrahman A. Al-Mohaimeed*, Abeer Abuzeid Atta Elmannan

Department of Family & Community Medicine, College of Medicine, Qassim University, Buraidah, Saudi Arabia

Citation: Al-Mohaimeed AA, Elmannan AAA. Experiences of Barriers and Motivators to Weight-Loss among Saudi People with Overweight or Obesity in Qassim Region - A Qualitative Study. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):1028-1035. https://doi.org/10.3889/oamjms.2017.171

Keywords: Obesity; Weight management; Barriers; Motivators; Qualitative study; Saudi Arabia.

***Correspondence:** Abdulrahman A Al-Mohaimeed. Department of Family & Community Medicine, College of Medicine, Qassim University, Buraidah, Saudi Arabia. E-mail: armoh@qumed.edu.sa

Received: 24-Jul-2017; **Revised:** 06-Aug-2017; **Accepted:** 13-Aug-2017; **Online first:** 04-Dec-2017

Funding: This research was supported by Deanship of Scientific Research, on the material support for this research under the number (1736 med 2016 1 12 S) during the academic year 1438 AH/2016 AD.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Obesity has become a global health threat. Saudi Arabia ranks among the countries with high obesity and overweight rates. This study aims to explore experiences of Saudi people with overweight or obesity with a particular focus on the perceived barriers and motivators to weight loss.

MATERIAL AND METHODS: We used a qualitative approach to recruiting a purposive sample using maximum variation sampling technique. Those who had previously attempted weight loss at least once were included in the study. In-depth interviews were conducted, transcribed and/ or audiotaped. Interviews continued until saturation was reached. The qualitative content analysis was performed.

RESULTS: A total of 19 males and 18 females participated in this study with a mean Body Mass Index (BMI) of 32.6 kg/m^2. Their main triggers to weight loss were concerns about overall health and the desire to improve their looks. Declining motivation, lack of family support and unhealthy eating during social gatherings were perceived as the main barriers. Motivating factors included concerns about health, family support, and availability of exercise facilities.

CONCLUSION: Factors responsible for a successful weight- loss is context-specific. This study has shown several barriers as well as motivators, which play an important role in weight reduction and maintenance.

Introduction

Over the past three decades, the world has witnessed an alarming escalation in the rates of obesity and overweight [1]. Estimates show that the prevalence of obesity has doubled since 1980. In 2014, more than 1.9 billion adults aged 18 years and older were overweight, among whom 600 million were obese. The increasing prevalence of obesity is mainly related to sedentary lifestyle, dietary habits, and the effects of environmental changes [2, 3]. These alarming facts have made obesity as one of the central health care issues to the extent that the World Health Organization (WHO) has declared it as an epidemic [4]. The related consequences manifesting as high vulnerability to morbidity and mortality complicate the situation [5]. The huge spending associated with obesity-related medical problems compromises health care allocations in national and regional budgets [6]. Prior evidence, however, shows that it is controllable. A large descriptive study about obesity and overweight indicates that weight loss can successfully be achieved and maintained through healthy diet & physical activity [7].

The Gulf countries (Saudi Arabia, Kuwait, United Arab Emirate, Bahrain, Qatar and Oman) have the highest rate of obesity worldwide [8]. The latest national health survey of 2013 conducted in collaboration with the Institute for Health Metrics and Evaluation estimates the prevalence of obesity in Saudi Arabia at 28.7% [9].

Obesity is a complex state that results from the interaction of multiple factors including genetic, biological, social, behavioural, cultural, and environmental. Behaviors such as high intake of calories and lower levels of physical activity contribute to its development. The accelerating rates of obesity have been partly linked with unhealthy behaviours such as excessive snacking and increased portion sizes [10]. A recent study has shown that a combination of cultural practices and economic prosperity has contributed to the development of an obesogenic environment in Saudi Arabia [11]. Another study has shown that traditional clothing, foods, hospitality norms and limited outdoor activities were considered as barriers to weight loss among Saudi females [12].

Qassim region is situated in the centre of Saudi Arabia. A previous study on the regional variation in the prevalence of obesity among Saudi children and adolescents found out that the central region has a higher prevalence (21%) compared to the southwest and the Northern regions [13].

Obesity exposes individuals to a higher risk of Non-Communicable Diseases (NCDs). However, this risk is considered to be modifiable & preventable [3]. Consequently, and given the current global situation, the prevention of obesity has become one of the world`s top five health priorities set by the Lancet NCD Action Group and the NCD Alliance [14].

Evidence indicates that there is a need to understand the local socio-cultural and environmental factors to develop successful weight loss programs [15]. The available data about weight loss interventions or the factors determining their success are insufficient, generally for Saudi Arabia and Qassim in particular. In this study, we aimed to explore the participants' experience of barriers and motivators for weight loss in the Qassim region to gain deeper insight into distinct factors central to the success of weight loss interventions, thus, contributing to mitigate the existing information gap. The exploratory nature of this study necessitated the use of a qualitative approach. Despite the local pool of evidence available on obesity in Saudi Arabia, a qualitative approach has rarely been used. Weight loss interventions typically target modifications in the individual behaviour related to diet and physical activity. Many behaviour change theories have been described in the literature to provide a conceptual framework for their determinants and causal factors. Our research study was particularly informed by the Social Cognitive Theory (SCT), which explains human behaviour as reciprocal interactions between personal, social and environmental factors [16]. We hope that the results of this study will help inform decisions making processes about weight loss interventions and provide a basis for further research into this field.

Material and Methods

Study Design

This is a qualitative research study having an exploratory purpose primarily. This approach was used to gain an understanding of underlying reasons, opinions, barriers, and motivations of individuals with overweight or obesity regarding their weight control attempts.

Participants, Sampling and Recruitment Process

Purposive sampling was used to recruit participants. The aim was to recruit individuals who were overweight or obese, and who had attempted to lose weight at least once. Buraidah, the capital city of the Qassim region, making about half of the region's population, was the focus of the study. The required study sample was drawn using the maximum variation sampling technique to ensure heterogeneity. A sampling grid of the potential major factors (age, gender, education & co-morbidity) was created. The target was to recruit at least one person from each group. This technique was used to reduce bias and ensure that the study sample included groups of different combinations of variables. Participants were approached directly and asked about their interest in participation in the study. Weight eligibility was determined by three different methods; self-reporting in the majority of cases, extracting information from patient records, or using a scale and a BMI calculator, particularly for the overweight participants. Furthermore, multiple recruitment strategies were used to reach as broad a range of individuals as possible. Participants were selected from a variety of sources including outpatient clinics, parks and fitness clubs. Interviews were arranged during daytime at a mutually convenient time and place for both the interviewer & participants. However, interviews were conducted in the evenings particularly for participants recruited from fitness clubs and walking tracks in public parks. These participants were likely to be physically active. However, this recruitment was purposively done. Physically active people would probably disclose valuable information particularly about motivators for leading and maintaining physically active lifestyles. Those who had a history of bariatric surgery or were unable or unwilling to describe their experiences were excluded from the study. Considering the cultural values in Saudi Arabia and gender preferences, care was taken to ensure that interviews were conducted by a person of the same gender. Interviewing continued till data saturation was reached, which occurred after 37 participants were interviewed (18 women, 19 men). Saturation was determined by the point at which no new information was generated from interviews.

In-Depth Interviews

One-to-one semi-structured interviews were conducted between January and July 2016. These were audio-taped with the consent of interviewees. Preliminary data were obtained from participants concerning their gender, age, level of education, and co-morbidity status (chronic diseases like diabetes, hypertension, ischemic heart disease and hyperlipidemia).

Researchers obtained the ethical approval from the Regional Research Ethics Committee of the Qassim region. Before starting the interviews, participants were apprised about study goals and inclusion criteria. The interviewers introduced themselves and studied goals, received written informed consent, and selected an appropriate place for the interview. The participants were assured of the confidentiality and were given the option to refuse to answer any question or quit at any time during the interview. All interviews were recorded and/or written verbatim. In the end, participants' permission was obtained for calling him/her for further information or clarification. Data were collected by using an interview guide. Questions were open-ended and intended to elicit information about the participants' personal experience of barriers and motivators of weight loss. The development of the interview questions was based on the main research question about the perceived barriers and motivators, and they were informed by prior research in the region [17]. The interview guide consisted of two sections. The first section was introductory and included background questions. The second section encompassed questions about initiating a weight loss program, barriers, motivators and a final advice to other people based on the participants' personal experience. Probing was used for further clarification.

Data analysis

The study used an inductive approach to generate meaning from the collected data to identify patterns. Data were analysed using the qualitative content analysis. Initially, all interviews were listened to and/or read to get a general sense of the collected material that was followed by descriptive coding. During this stage, raw textual data from the interviews were summarised to generate as many codes as necessary to cover all content. Finally, codes were grouped into categories, and three major themes emerged from the data: personal, social, and environmental barriers and motivators. All data were analyzed by hand. No further validation of the analysis was performed.

Results

Participants were in the age range of 21–57 years. All had a BMI over 25 K/m^2; the mean BMI was 32.6 K/m^2. Participants' demographic characteristics are summarized in Table1.

All participants had attempted to lose weight several times in their lives. Although weight loss was their ultimate goal, they reported two main triggers that motivated them to start their weight loss program. These included concerns about overall health and wellbeing (n = 27.77%) and the desire to have an ideal body image (n = 16.45.7%). Equally, the female participants also reported the same triggers though for the male participants the primary trigger was a concern for their health and wellbeing. (n = 17.85 % of males).

Table 1: Participants Characteristic (N = 37)

Participants Characteristic	N = 37
Mean Age (Min- Max)	37.59 years (21-57)
Mean BMI (Min -Max)	32.6 (25.20- 46.80)
Obesity Status	
Pre-obese	13 (35.1%)
Obese class I	13 (35.15%)
Obese class II	8 (21.6%)
Obese class III	3 (8.1%)
Gender	
Male	19 (51.4%)
Female	18 (48.6 %)
Education	
Basic	18 (48.6%)
Higher	19 (51.4%)
Comorbidity	
Yes	23 (62.2%)
No	14 (37.8%)

Despite the initial motivation to reduce weight, the majority of participants stated that their efforts were inconsistent and often interrupted for extended periods of time. Declining motivation, lack of family support, lack of time, and failure to achieve the desired goal were perceived as common reasons for discontinuing weight loss efforts. These factors will be further explained under barriers to weight loss.

Barriers and motivators in this study were classified under the three major headings: personal, social, and environmental.

Personal barriers

Interviews revealed multiple factors related to individual behaviour. Increased appetite was perceived as a major barrier to the success of weight loss. Some participants reported extreme difficulty in controlling their appetite.

‘‘Whenever I see food I feel like [crave for] eating’’ (Male, High Education, BMI: 31.0).

Healthy eating, however, was not a priority for overeaters. Limited choices of healthy foods available at grocery stores near to their home, and lack of time (and motivation) needed to prepare healthy meals were the main reasons. “First of all, you cannot easily find stores offering healthy foods. Although there are

some stores that sell low-calorie food, they are not close to where I live. Additionally, preparing healthy food at home requires a lot of time" (Female, High Education, BMI: 27).

As a commitment to healthy eating patterns was weak, skipping meals and eating out became the alternatives. Participants perceived the pervasive fast food marketing in the Kingdom coupled with low nutritional awareness among the public as the real challenge in controlling weight.

"Fast food and soft drinks are commonly consumed by people who could be the leading cause of obesity'' (Female, High Education, BMI: 28.7).

Lack or reduced motivation to diet was also reported. Incorrect assessment of weight status prevented some participants from initiating efforts to reduce weight. Among the surveyed, some reported perception of their weight as normal, and hence didn`t attempt to lose weight.

"I feel that my weight is within the normal range" (Male, High Education, BMI: 32.5).

Moreover, some participants commented that dieting had a negative effect on their mood and reported experiencing unpleasant feelings once they started doing it:

"I feel depressed when I start dieting" (Male, Basic Education, Hypertension, BMI: 46.8).

On probing further, one participant added that feeling depressed was not associated only with the initiation of the diet program. However, it lasted longer while dieting and eventually led to the disruption of the weight loss program:

''When I feel depressed I discontinue my weight loss program'' (Female, Basic Education, BMI: 36.7)

On the other hand, some participants linked disturbances in their weight reduction program with stress and commented that they find it difficult to adhere to their program when they are under stress.

"When I'm under the pressure of study, I stop dieting" (Female, High Education, BMI: 27)

The impact of adverse personal habits like smoking was also highlighted. Some participants indicated that their schedules were too busy to follow a special program.

"Being a smoker, I felt some difficulty in exercising" (Male, Basic Education, Hypertension, BMI: 32.1)

A gender-related barrier was also reported in this study. Some women indicated that childbearing and childrearing hindered their efforts to lose weight.

"Before pregnancy and childbirth I used to exercise regularly, however, after I became a mom I found it very difficult to continue my program. I just can`t find the time as before" (Female, Basic Education, BMI: 39.5).

Social barriers

Many participants reported social gatherings as an important barrier to adhering to the weight loss program. They found it difficult to resist sweets, dates and other types of desserts offered in these gatherings. The occurrence of these gatherings and social events more frequently than they used to be in the past, further exaggerated their negative impact.

"In our social gatherings, a variety of foods and desserts are served" (Female, basic education, BMI: 33.3).

"Eating with groups (family or friends) may encourage unhealthy eating" (Female, Basic Education, Hypertensive, BMI: 25.2).

Furthermore, some participants pointed out that lack of family support demotivated hem.

"My family would not encourage me to lose weight'' (Female, High Education, BMI: 33.4).

Physical environmental barriers

Regular physical activity is essential for keeping a healthy weight. Participants were asked about their physical activities, and what were the perceived barriers to a physically active lifestyle. Walking was seemingly the most popular type of physical activity reported by participants. Although neighbourhood sidewalks and parks are widely available, personal safety remained an issue for some participants. The activity of females participants was particularly influenced by some factors. Some mentioned that access to workout facilities is limited. Others added that joining gyms and fitness clubs is fairly costly. ''few and expensive available facilities for exercising'' (Female, Basic Education, BMI: 36.7).

"Ladies need more accessible and safe walking tracks" (Female, High Education, BMI: 28.7).

The weather was also perceived as a barrier to being active. The weather in Qassim is hot and dry during summer, rainy during winter and in either situation, it makes outdoor activities troublesome.

''It is difficult to walk in hot weather'' (Female, Basic Education, Hypertensive, BMI: 38.8)

"Weather changes such as when it is hot or dusty often interrupts my weight loss program" "(Male, Basic Education, Hypertension, Dyslipidemia BMI: 26.9).

Motivators

Discussions with participants revealed a group of factors that were perceived as enablers for

weight reduction.

Personal motivators

Some participants initiated a weight loss program owing to their ill health. Doctor`s advice to patients with chronic disease such as hypertension and joint problems often prompts them to lose weight and make other healthful lifestyle changes.

“Following the birth of my third child, my weight increased significantly, and I began to suffer from joint pains. Due to the pain, I could not stand during prayers. My doctor told me that I must reduce my weight. So, I started a diet plan” (Female, Basic Education, Joint Pain, BMI 35.9).

“Having chronic medical problems like hypertension and knee pain motivated me to start my weight loss program" (Male, Basic Education, Hypertension, BMI: 46.8)

“My motive to start dieting was joint problems" (Male, basic education, Hypertension, BMI: 32.1).

“I felt that I am disabled by obesity, I cannot clean my body properly" (Male, Basic Education, Hypertension, BMI: 46.8).

“As it is said: why we should live the last 20 years of our lives suffering from diseases" and the other saying “why should we dig our graves with our teeth” (Male, High Education, BMI: 34.5).

Concerns about body shape and general appearance were a strong motivator. Participants reported that their efforts to reduce weight were triggered by their dissatisfaction with body size and their desire to pursue the ideal weight.

Clothing was also an issue. Smaller size clothing is more easily available in markets compared to plus-size clothing.

“Having thin body will give me more options in clothing" (Male, Basic Education, BMI: 26.6).

Interestingly, one female participant pointed out that clothing can be used as a good strategy to reduce weight.

“When shopping for clothes, I buy smaller size outfits and tell myself it is a motivation to lose weight and fit into them" (Female, High Education, BMI: 27).

Being aware of the nutritional value of various types of food and their calorie content was perceived as helpful motivators.

“Knowledge of different foods and their calories could be the most important motivator" (Male, High Education, BMI: 32.5).

However, for some participants, this awareness did not always result in making healthy decisions.

“I sometimes inquire about the calorie content of the food I eat, but, I don`t always use that information when making choices about what to eat” (Female, Basic Education, Joint Pain, BMI 35.9).

Losing weight for some participants was triggered by their desire to act as a good role model, self-satisfaction about producing significant weight reduction, or keenness to lead an active lifestyle.

“I want to be a role model for my family" (Female, High Education, BMI: 28.7).

“My activities are restricted by my heavyweight" (Male, Basic Education, BMI: 42.5).

“Achieving my goal in weight reduction motivated me to continue" (Male, Basic Education, Hypertension, BMI: 46.8).

Social motivators

Social support is the key to maintaining weight reduction efforts. Participants commented that the encouragement they received from family and friends was very helpful. Also, positive experiences with weight loss inspired some participants to begin a regular weight loss program.

Social support: ‘‘I feel that people encourage each other, especially for walking’’ (Female, High education, Diabetic and Hypertensive, BMI: 38.8).

‘‘Experiences of friends in weight reduction were encouraging for me" (Male, High education, BMI: 32.5).

Physical environmental motivators

The wide availability of sidewalks and neighbourhood parks made group exercise easy and convenient. “Availability of walking tracks has encouraged me to walk" (Male, High Education, BMI: 25.8)

At the end of the interview, participants were asked about what advice they would offer others for weight reduction, based on their own experience. Their responses reflected a comprehensive and correct understanding of the basics of an effective weight loss program.

Majority of participants recommended performing regular exercise, ‘‘we need to exercise regularly". Also considered important were to avoid bakery food and desserts, fast foods, soft drinks, high-fat diet and overeating, ‘‘dietary habits should be modified to eat more vegetables and fruits, and avoid high fat/calorie diet".

They also advised being educated about the benefits of having ideal body weight and diet contents and calories. Some participants advised not to aim for short programs of weight reduction. While others

stated that, it is important to have a target weight in mind. Follow up with a physician was also stated by some as a facilitating action.

Discussion

In the present study, some barriers and motivators related to weight reduction and its maintenance were identified. Motivators were mainly the concerns of individuals about the impact of weight on their health and wellbeing and the desire to have a good body image. On the other hand, the participants identified several personal, social and physical barriers that hindered their efforts to lose weight.

Weight loss programs primarily include lifestyle modifications in the form of dietary changes and physical activity. Adherence to weight loss programs has been recognised as an essential component for successful weight loss [18]. A previous study conducted in Saudi Arabia showed that adherence to dietary changes was higher compared to exercise adherence [19]. Variation in adherence could be explained regarding setting high weight loss goals, lack of social support, full-time employment, or stress. Similar findings were reported in other studies [20]. Poor adherence has also been associated with increased rates of weight regain [21]. Analysis of possible barriers to initiating and or maintaining weight loss is hence essential for providing an in-depth understanding of factors necessary for long-term adherence.

Unhealthy eating behaviour is a well-known barrier to weight loss. In our study, participants listed such behaviour as an important barrier: Having uncontrollable appetite, excessive consumption of fast food and overeating when feeling stressed were reported as examples of factors leading to unhealthy eating. The Saudi society has witnessed observable dietary changes over the years. Local studies reported overconsumption of calorie-dense foods compared to vegetables and fruits [22, 23]. Another study revealed high rates of unhealthy eating practices among female nursing students in Saudi Arabia such as watching television while eating, not sharing meals with family, and excessive consumption of soft drinks and junk food [24]. Furthermore, the wide availability of the fast food restaurants which often offer high caloric meals at an affordable cost has adversely resulted in promoting unhealthy eating practices. The combination of excessive intake of unhealthy food and reduced physical activity seemingly contributed to the severity of the problem of obesity in the Saudi society [9].

The habit of overeating during social occasions has also been reported which agrees with a previous study [12]. The Saudi culture often enables weight gain through serving calorie-dense foods during these occasions. The common tradition is to serve Arabic coffee with dates or something sweet such as sugary desserts, chocolates or other savouries [25]. Most of these social gatherings take place during evenings and nighttime with meals typically served at late hours. A significant evidence base suggests that excessive nighttime eating increases the risk of obesity and adverse health effects [26, 27]. Difficulties in changing eating habits were reported as one major barrier in culturally different parts of the world [28]. The relationship between the individual`s mental state and controlling weight has also been highlighted in this study. Stressed people are more prone to weight gain as reported by some authors [29]. Low morale related to work pressure or due to experiencing depressive symptoms upon beginning a diet is thought to hinder weight-loss efforts. It is necessary for those who seek weight loss to initially focus on their mental wellbeing. In this context, social support is considered key to weight loss success.

Not having enough time due to busy work schedules, childcare responsibilities mentioned by some women, expensive gyms, and unfavourable weather conditions in Qassim have also been mentioned as common hindrances to adherence to regular exercise. Apparently, some of these barriers are directly related to the individual`s ability to manage time, while others related to the uncontrollable physical environment. A previous study which collected data from three big cities in Saudi Arabia revealed significantly lower levels of physical activity among adolescents with obesity compared to non-obese [30]. Simple personal approaches could be followed to promote physical activity such as incorporating exercise into the individual`s daily routine, having a walk with the family, using stairs instead of elevators, etc. However, the implementation of community-wide interventions for promoting physical activity has gained substantial attention worldwide over individual-oriented approaches [31]. Evidence suggests three major sets of solutions to overcome barriers to physical activities in a community: informational strategies for enhancing knowledge and attitudes towards advantages of physical activity; behavioral strategies for equipping the public with a range of skills needed to support and maintain behavior change; and environmental strategies for providing safe and accessible facilities for exercise [32].

Despite the barriers mentioned, some important motivators were described by study participants. These motivators could play a significant role as predictors of success of the weight loss programs. For instance, an internal motivation which is often stated as the initial trigger for weight loss has been recognised as a predictor of effective weight loss in the literature [33]. Highly motivated people are more likely to adhere to their goals and maintain long-

term weight loss.

Awareness about one's ill-health is another strong motivator for weight loss. A previous study supports this finding and indicates that strict doctor's instructions and firm personal decisions taken following a serious health event can motivate people to start on a weight loss plan [34]. Furthermore, evidence shows that higher degrees of weight loss and maintenance were observed among people driven by their medical condition [35]. Dissatisfaction with one's body size and desire to improve physical shape are common motivators. Interestingly, research on the relationship between motivation and physical activity has shown that people who are motivated to follow a physical fitness program in order to enhance their body image rather than to enhance their health are more likely to suffer from lower self-esteem, higher level of problematic eating, and greater social anxiety [36, 37].

Social support is yet another important element of almost all weight loss programs. The natural network surrounding an individual made up of family members, and friends represent the main source of social support. In the present study social support has been recognised as a motivator, while the lack of it was identified as a barrier. Similar findings were shown in the literature [38]. Receiving a higher level of support from a spouse, friend or other family member has helped some participants to initiate and continue their weight loss program. A previous study comparing the differences between a group of individuals who have effectively controlled their weight and another group who failed to maintain their weight loss showed a greater social support to the former group [33]. Furthermore, social support was found to be associated with fewer drops out from weight loss programs [39]. On the other hand, lack of social support has been recognised as a barrier in this study. Similar results were found in the literature indicating the negative impact that results from lack of social support on both physical activity and adopting a healthy diet [40].

This study has some limitations. While interviews produced rich data about barriers and motivators to weight loss, yet it lacked in-depth inquiry into the participants` past attempts to lose weight. Another limitation was lack of data validation. Verification of data analysis either through respondent verification or peer review is one way to safeguard against the subjectivity and bias in qualitative research. However, it is time-consuming and was not performed in this study.

In conclusion, effective weight loss depends on multiple factors. This study showed that concerns about the impact of weight on health and wellbeing, and the desire to have an ideal body image were the main motivators, while the barriers included low nutrition awareness, social overeating at social occasions and lack of exercise facilities. It is critical to understand the context within which those factors operate A deeper understanding of the factors motivating or demotivating people with overweight or obesity to lose weight in Saudi Arabia will help devise targeted interventions to reduce the burden of obesity and its complications at both individual and public levels. Future research is necessary to test the feasibility and effectiveness of such interventions. Also, more studies are needed using the qualitative approach to explore the factors determining the success of weight loss programs at the community level, particularly from the perspective of health planners in Saudi Arabia.

Acknowledgment

The authors gratefully acknowledge Qassim University, represented by the Deanship of Scientific Research, on the material support for this research under the number (1736 med 2016 1 12 S) during the academic year 1438 AH/2016 AD. The authors also are extremely grateful to all participants who took part in this study.

References

1. World Health Organization. Obesity and overweight. 2015. http://www.who.int/mediacentre/factsheets/fs311/en/

2. Carter MJ. Michael J. Carter Male Obesity: A Qualitative Study of Clinical Attitudes and Perspectives [Internet]. 2008. Available from: http://scholarworks.smith.edu/theses Recommended

3. Lang A, Froelicher ES. Management of overweight and obesity in adults: Behavioral intervention for long-term weight loss and maintenance. Eur J Cardiovasc Nurs. 2006;5(2):102–14. https://doi.org/10.1016/j.ejcnurse.2005.11.002 PMid:16406709

4. World Health Organization, 2000. Obesity: preventing and managing the global epidemic (No. 894). World Health Organization, 2000.

5. Oster G, Edelsberg J, O'Sullivan AK, Thompson D. The clinical and economic burden of obesity in a managed care setting. Am J Manag Care. 2000;6(6):681–9. PMid:10977477

6. Finkelstein EA, Trogdon JG, Cohen JW, Dietz W. Annual medical spending attributable to obesity: Payer-and service-specific estimates. Health affairs. 2009; 28(5):w822-w831. https://doi.org/10.1377/hlthaff.28.5.w822 PMid:19635784

7. Klem ML, Wing RR, McGuire MT, Seagle HM, Hill JO. A descriptive study of individuals successful at long-term maintenance of substantial weight loss. Am J Clin Nutr. 1997; 66(2), pp.239-246. PMid:9250100

8. ALNohair S. Obesity in gulf countries. Int J Health Sci (Qassim) 2014; 8(1):79–83. https://doi.org/10.12816/0006074

9. Memish ZA, El Bcheraoui C, Tuffaha M, Robinson M, Daoud F, Jaber S, et al. Obesity and associated factors--Kingdom of Saudi Arabia, 2013. Prev Chronic Dis. 2014;11:E174. https://doi.org/10.5888/pcd11.140236 PMid:25299980 PMCid:PMC4193060

10. Duffey KJ, Popkin BM. Energy density, portion size, and eating

occasions: Contributions to increased energy intake in the United States, 1977-2006. PLoS Med. 2011; 8(6), p.e1001050. https://doi.org/10.1371/journal.pmed.1001050 PMid:21738451 PMCid:PMC3125292

11. DeNicola E, Aburizaiza OS, Siddique A, Khwaja H, Carpenter DO. Obesity and public health in the Kingdom of Saudi Arabia. Rev Environ Health. 2015; 30(3):191-205. https://doi.org/10.1515/reveh-2015-0008 PMid:26351801

12. Alqout O, Reynolds F. Experiences of obesity among Saudi Arabian women contemplating bariatric surgery: an interpretative phenomenological analysis. J Health Psychol. 2014; 19(5):664-677. https://doi.org/10.1177/1359105313476977 PMid:23479306

13. El Mouzan MI, Al Herbish AS, Al Salloum A, Al Omar A, Qurachi MM. Regional variation in prevalence of overweight and obesity in Saudi children and adolescents. Saudi J Gastroenterol. 2012; 18(2):129. https://doi.org/10.4103/1319-3767.93818 PMid:22421719 PMCid:PMC3326974

14. Beaglehole R, Bonita R, Horton R, Adams C, Alleyne G, Asaria P, et al. Priority actions for the non-communicable disease crisis. Lancet 2011;377(9775):1438–47. https://doi.org/10.1016/S0140-6736(11)60393-0

15. Ali HI, Bernsen RM, Baynouna LM. Barriers to Weight Management among Emirati Women: A Qualitative Investigation of Health Professionals' Perspectives. Int Q Community Health Educ. 2008;29(2):143–59. https://doi.org/10.2190/IQ.29.2.d PMid:19546089

16. Bandura A. Social foundation of thought and action: A social-cognitive view. Englewood Cliffs, 1986.

17. Ali HI, Baynouna LM, Bernsen RM. Barriers and facilitators of weight management: perspectives of Arab women at risk for type 2 diabetes. Health & social care in the community. 2010;18(2):219-28. https://doi.org/10.1111/j.1365-2524.2009.00896.x PMid:20059569

18. Boehm G, Bracharz N, Schoberberger R. Evaluation of the sustainability of the Public Health Program "slim without diet (Schlank ohne Diet)." Wien Klin Wochenschr. 2011;123(13–14):415–21. https://doi.org/10.1007/s00508-011-1594-3 PMid:21739208

19. Khan AR, Al-Abdul Lateef ZN, Al Aithan MA, Bu-Khamseen MA, Al Ibrahim I, Khan SA. Factors contributing to non-compliance among diabetics attending primary health centers in the Al Hasa district of Saudi Arabia. J Fam Community Med. 2012;19(1):26–32. https://doi.org/10.4103/2230-8229.94008 PMid:22518355 PMCid:PMC3326767

20. Grave RD, Suppini A, Calugi S, Marchesini G. Factors Associated with Attrition in Weight Loss Programs. Int J Behav Consult Ther. 2006;2(3):341–53. https://doi.org/10.1037/h0100788

21. Wing RR. Behavioral interventions for obesity: recognizing our progress and future challenges. Obes Res. 2003;11 Suppl(October):3S–6S. https://doi.org/10.1038/oby.2003.219 PMid:14569033

22. Amin TT, Al-Sultan AI, Ali A. Overweight and obesity and their relation to dietary habits and socio-demographic characteristics among male primary school children in Al-Hassa, Kingdom of Saudi Arabia. Eur J Nutr. 2008;47(6):310–8. https://doi.org/10.1007/s00394-008-0727-6 PMid:18677544

23. Mahfouz AA, Abdelmoneim I, Khan MY, Daffalla AA, Diab MM, Al-Gelban KS, et al. Obesity and related behaviors among adolescent school boys in Abha City, Southwestern Saudi Arabia. J Trop Pediatr. 2008;54(2):120–4. https://doi.org/10.1093/tropej/fmm089 PMid:18039676

24. Sabra A. Obesity among female intermediate nursing students of health science collage in Dammam city, Saudi Arabia: prevalence and associated factors. Can J Clin Nutr. 2014; 2(1):29–45. https://doi.org/10.14206/canad.j.clin.nutr.2014.01.04

25. Al-Othaimeen AL, Al-Nozha M, Osman AK. Obesity: an emerging problem in Saudi Arabia. Analysis of data from the National Nutrition Survey. Eastern Mediterranean health journal. 2007; 441–8. PMid:17684864

26. Katayose Y, Tasaki M, Ogata H, Nakata Y, Tokuyama K, Satoh M. Metabolic rate and fuel utilization during sleep assessed by whole-body indirect calorimetry. Metabolism. 2009; 58(7):920–6. https://doi.org/10.1016/j.metabol.2009.02.025 PMid:19394978

27. Kinsey AW, Ormsbee MJ. The health impact of nighttime eating: Old and new perspectives. Nutrients. 2015; 7(4):2648–62. https://doi.org/10.3390/nu7042648 PMid:25859885 PMCid:PMC4425165

28. Hammarström A, Wiklund AF, Lindahl B, Larsson C, Ahlgren C. 2014. Experiences of barriers and facilitators to weight-loss in a diet intervention-a qualitative study of women in Northern Sweden. BMC women's health. 2014; 14(1):59. https://doi.org/10.1186/1472-6874-14-59 PMid:24739099 PMCid:PMC3998240

29. Block JP, He Y, Zaslavsky AM, Ding L, Ayanian JZ. Psychosocial stress and change in weight among US adults. American journal of epidemiology. 2009;170(2):181-92. https://doi.org/10.1093/aje/kwp104 PMid:19465744 PMCid:PMC2727271

30. Al-Hazzaa HM, Abahussain NA, Al-Sobayel HI, Qahwaji DM, Alsulaiman NA, Musaiger AO. 2014. Prevalence of overweight, obesity, and abdominal obesity among urban Saudi adolescents: Gender and regional variations. Journal of health, population, and nutrition. 2014; 32(4):634. PMid:25895197 PMCid:PMC4438694

31. Charter TT. The Toronto Charter for Physical Activity : A Global Call for Action. 2010;(May):1–6.

32. Kahn EB, Ramsey LT, Brownson RC, Heath GW, Howze EH, Powell KE, et al. The Effectiveness of Interventions A Systematic Review. Am J Prev Med. 2002;22(2):73–107. https://doi.org/10.1016/S0749-3797(02)00434-8

33. Elfhag K, Rössner S. Who succeeds in maintaining weight loss? A conceptual review of factors associated with weight loss maintenance and weight regain. Obes Rev. 2005;6(1):67–85. https://doi.org/10.1111/j.1467-789X.2005.00170.x PMid:15655039

34. Teixeira PJ, Silva MN, Mata J, Palmeira AL, Markland D. Weight Control. Int J Behav Nutr Phys Act. 2012;9(1):22. https://doi.org/10.1186/1479-5868-9-22 PMid:22385818 PMCid:PMC3312817

35. Gorin AA, Phelan S, Hill JO, Wing RR. Medical triggers are associated with better short- and long-term weight loss outcomes. Prev Med (Baltim). 2004;39(3):612–6. https://doi.org/10.1016/j.ypmed.2004.02.026 PMid:15313102

36. Thome JL, Espelage DL. Obligatory exercise and eating pathology in college females: Replication and development of a structural model. Eat Behav. 2007;8(3):334–49. https://doi.org/10.1016/j.eatbeh.2006.11.009 PMid:17606231

37. Crawford S, Eklund RC. Social Physique Anxiety, Reasons for Exercise, and Attitudes Toward Exercise Settings. J Sport Exerc Psychol. 1994;16:70–82. https://doi.org/10.1123/jsep.16.1.70

38. Tessaro I, Rye S, Parker L, Trangsrud K, Mangone C, McCrone S, et al. Cookin'Up Health: Developing a Nutrition Intervention for a Rural Appalachian Population. Health Promot Pract. 2006;7(2):252. https://doi.org/10.1177/1524839905278887 PMid:16585148

39. Verheijden MW, Bakx JC, van Weel C, Koelen M, van Staveren W. Role of social support in lifestyle-focused weight management interventions. Eur J Clin Nutr. 2005;59(Suppl 1):S179–86. https://doi.org/10.1038/sj.ejcn.1602194 PMid:16052189

40. Andajani-Sutjahjo S, Ball K, Warren N, Inglis V, Crawford D. Perceived personal, social and environmental barriers to weight maintenance among young women: A community survey. Int J Behav Nutr Phys Act. 2004;1:15. https://doi.org/10.1186/1479-5868-1-15 PMid:15462679 PMCid:PMC524367

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):1036-1041.
https://doi.org/10.3889/oamjms.2017.214
eISSN: 1857-9655
Public Health

Public Health Profile of Road Traffic Accidents in Kosovo 2010-2015

Naser Ramadani[1,2], Valbona Zhjeqi[1,2*], Merita Berisha[1,2], Rina Hoxha[1,2], Ilir Begolli[1,2], Drita Salihu[1,2], Pranvera Krasniqi[2]

[1]Faculty of Medicine, Hasan Prishtina University, Prishtina 10000, Kosovo; [2]National Institute of Public Health of Kosova, Pristina 10000, Kosovo

Citation: Ramadani N, Zhjeqi V, Berisha M, Hoxha R, Begolli I, Salihu D, Krasniqi P. Public Health Profile of Road Traffic Accidents in Kosovo 2010-2015. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):1036-1041. https://doi.org/10.3889/oamjms.2017.214

Keywords: Road traffic accidents; Public health problem; traffic injuries; traffic mortality; Kosovo.

***Correspondence:** Valbona Zhjeqi. Faculty of Medicine, Hasan Prishtina University, Prishtina 10000, Kosovo; National Institute of Public Health of Kosova, Pristina 10000, Kosovo. E-mail: valbona_zhjeqi@yahoo.com

Received: 12-Jul-2017; **Revised:** 06-Sep-2017; **Accepted:** 18-Oct-2017; **Online first:** 13-Dec-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

AIM: To determine the characteristics of the Socio-medical profile of road traffic accidents in Kosovo, between 2010 and 2015 year.

STUDY DESIGN: Retrospective study.

METHODS: A descriptive method based on the database of road traffic accidents from the National Police of Kosovo.

RESULTS: In Kosovo for the period 2010-2015, on average, the yearly number of road traffic accidents is 18437 with mortality rate 7.4 per 100000 and lethality of 1.5%. The highest number of fatal cases are drivers and above 19 years old with more than 80%. Among injured significantly highest percentage is among passengers for all years and above 19 years old. Road traffic accident with a vehicle occurs most frequently, with approximately over 70%, mostly on dry road 72.9% and clear weather 71.1%. The driver is the contributing factors of road traffic accidents on average 99.3% whereas climatic conditions only 0.5%, with over 50% of crashes occurring in urban road 56.2%, mostly during Monday 16.0% and in the afternoon rush hours between 14.00-18.00 with 31.0%.

CONCLUSIONS: There is a slight decrease in the mortality rate of 0.1‰ and lethality rate of 0.1% each year, whereas there is an increase of 21.5‰ for traumatism rate for each year.

Introduction

Road traffic accidents are the major public health problem, in both developed and developing countries. Based on WHO report, the overall global road traffic fatality rate is 17.4 per 100000. Low-income countries have the highest annual road traffic fatality rates, 24.1 per 100000, middle-income countries 18.4 while the rate in high-income countries is lowest, at 9.2 per 100000 [1]. Traffic mortality, traumatism, absenteeism, and disabilities have an impact not only each victim but also on their families and wider society at the national level of pandemic proportions with medical, psychological, economic and quality of life consequences. According to WHO report road traffic injuries are the eighth leading cause of death globally, and the leading cause of death for young people aged 15–29 years. Globally, more than a million people die annually. Approximately, 90% of the world's fatalities on the roads occur in low and middle-income countries [1]. The rapid increase in road traffic crashes in low and middle-income countries has driven an overall global increase in deaths and injuries due to the rapid rate of motorization and lack of prioritization for several years of safety strategies, disordered urbanization which causes financial costs up to 3–5 % of their gross national product whereas consequences of these road traffic accidents cost up billions of dollars due to the cost of treatment, rehabilitation lost productivity and incident investigation [1].

The annual cost of road traffic accident in Australia, in 2003, was about 2.3% of the Gross Domestic Product (GDP) [2]. For the same year, in

Barcelona total costs of road traffic accidents were 367 million euro with (89.8%) direct costs equalled euro 329 million, including property damage costs, insurance administration costs, and hospital costs [3]. In Iran, traffic accident costs were US$2.2 million in 2007 [4] while in Kuwait cost per traffic fatality is more than the US $500,000 [5].

In Kosovo, a country in transition with the improper and non-strict implementation of road security measures, inappropriate land use planning, an increase of the number of cars and particularly imported old cars from the second-hand European Union market increases the risk for traffic insecurity together with lack of proper road infrastructure and urban spatial planning, characteristic for developing countries.

Public health importance of road traffic accidents stands because they are largely preventable with traffic security cost-beneficial and cost-effective measures. Consequences are not only health related but also economic and social too.

This study aims to determine socio-medical characteristics of road traffic accidents in Kosovo, between 2010 and 2015 by exploring trend in road traffic accidents, fatal and non-fatal accidents, type, contributing factors, the site, superficial road conditions, time, day of the week and climatic condition of occurrence.

Methods

For this study, a retrospective study of road traffic accidents in the period from January 2010 to December 2015 was conducted. As descriptive study based on a database of road traffic accidents of Kosovo National Police and demographic data (2010 - 2015) from Statistical Agency of Kosovo, therefore we did not need any ethical approval since data are anonymous, presented by gender and age group.

The information provided by the National Police of Kosovo included the 2010-2015 databases, in Excel format. This information allowed the analysis of the following variables: number of accidents, type, contributing factors, the day of the week, time of occurrence, climatic conditions, and road users involved by age, the place, superficial road conditions, distributions of the accidents. A limitation of the database is the lack of disaggregated age data. From the database, incidence, mortality and lethality indicators are calculated. The incidence was determined by the number of new cases of road traffic accidents deaths and injuries that occurred during 2010-2015. Mortality was calculated by dividing the number of deaths among the total susceptible population, according to the Kosovo Statistical Agency per 100.000 inhabitants whereas lethality by dividing the number of deaths on the number of injured persons by road traffic accidents per 100. The statistical analysis reported frequencies, percentages, trends and chi-square statistical significance test. For the study purpose statistical program Excel, 2016 was used.

Results

According to the Statistics of the National Police of Kosovo, for the period 2010-2015, there have been 110622 road traffic accidents at the national level. Regarding mortality, between the years 2010-2015, road traffic accidents have caused 826 deaths nationwide. The year 2010 had the highest number of deaths in the country 175. For the period 2010-2015, 54809 injured people are recorded, with an average yearly number of road traffic accidents 18437, annually average of dead persons 138, injured 9135 and average mortality of 7.4 per 100.000, average traffic traumatism 497.6 per 100.000 and average lethality of 1.5%.

Table 1: Traffic accidents and related health indicators, Kosovo 2010-2015

Year	Population	Accidents	Ib	Iv	Dead	Injuried	Mt per 100000	Tr per 100000	Le per 100
2010	2207000	18030	100.0	-	175	7730	7.9	350.2	2.3
2011	1739825	18888	104.8	104.8	157	8321	9.0	478.3	1.9
2012	1793000	19754	109.6	104.6	121	8561	6.7	477.5	1.4
2013	1820631	19928	110.5	100.9	117	9813	6.4	539.0	1.2
2014	1804944	16300	90.4	81.8	127	9713	7.0	538.1	1.3
2015	1771604	17722	98.3	108.7	129	10671	7.3	602.3	1.2
Average		18437	-	-	138	9135	7.4	497.6	1.5

The basic index shows an increase of road traffic accidents for period 2010-2013 and in 2014 show decrease of 9.6%, for 2015 decrease of 1.7%. Verig index show similarly increases for the same period 2010-2013, and for 2014 there is a decrease of 18.6% and 8.7% for 2015. According to trend analysis, there is a slight decrease of the mortality rate of 0.1 ‰ and lethality rate of 0.1% each year whereas there is an increase of 21.5‰ for traumatism rate for each year (Table 1).

Table 2, shows trend and statistical significance for the period 2010-2015. For death cases, the trend is decreasing on average for nine new cases each year and for injuries increase of 575.2 new cases, and for victims in general also, increase for 566 each year. For years there is statistical significance for $p<0.00$ for death cases with the highest number recorded on 2010 and for injuries highest number for 2015. We can notice that while the number of accidents is falling, the number of fatal cases is decreasing and the number of victims and

injuries increasing (Table 2).

Regarding age group, highest percentage is among victims with above 19 years with more than 80% during period 2010-2015 and least percentage among adolescents 13-18 years old but there is no statistical significance (Table 3).

Table 2: Victims of traffic accidents, Kosovo 2010-2015

Year	Dead		Injured		Victims	
	N	%	N	%	N	%
2010	175	21.2	7730	14.1	7905	14.2
2011	157	19.0	8321	15.2	8478	15.2
2012	121	14.6	8561	15.6	8682	15.6
2013	117	14.2	9813	17.9	9930	17.8
2014	127	15.4	9713	17.7	9840	17.7
2015	129	15.6	10671	19.5	10800	19.4
Total	826	100.0	54809	100.0	55635	100.0
Trend	y = 170.07 -9.2571x		y = 7121.5+575.23x		y = 7291.6+565.97x	
X2, FD=5	0.0017		0.000		0.000	

Among injured according to their involvement in traffic, highest percentage is among passengers for all years and the smallest percentage is among pedestrians, the statistically significant difference (p=0.0001). Age group, similarly above 19 years old are mostly injured for entire period with a statistically significant difference (p = 0.000) (Table 3).

Table 3: Dead and injured according to their involvement in traffic accidents and age-group, Kosovo 2010-2015

Year	Dead persons						X^2-test
	Drivers		Passenger		Pedestrian		FD=10
	N	%	N	%	N	%	8.789
2010	61	34.9	54	30.9	60	34.3	
2011	57	36.3	55	35.0	45	28.7	
2012	44	36.4	35	28.9	42	34.7	
2013	37	31.6	47	40.2	33	28.2	
2014	49	38.6	39	30.7	39	30.7	
2015	48	37.2	33	25.6	48	37.2	p=0.552
Year	Dead persons according age-group						X^2-test
	0-12		13-18		19 +		FD=10
	N	%	N	%	N	%	4.589
2010	17	9.7	8	4.6	150	85.7	
2011	15	9.6	10	6.4	132	84.1	
2012	11	9.1	5	4.1	105	86.8	
2013	13	11.1	9	7.7	95	81.2	
2014	8	6.3	7	5.5	112	88.2	
2015	10	7.8	6	4.7	113	87.6	p=0.917
Year	The injured						X^2-test
	Drivers		Passenger		Pedestrian		FD=10
	N	%	N	%	N	%	33.825
2010	2936	38.0	3741	48.4	1053	13.6	
2011	3222	38.7	4072	48.9	1027	12.3	
2012	3309	38.7	4223	49.3	1029	12.0	
2013	3757	38.3	4814	49.1	1242	12.7	
2014	3675	37.8	4822	49.6	1216	12.5	
2015	4099	38.4	5396	50.6	1176	11.0	0.0001
Year	The injured according to age group						X^2-test
	0-12		13-18		19 +		FD=10
	N	%	N	%	N	%	134.889
2010	655	8.5	436	5.6	6639	85.9	
2011	627	7.5	701	8.4	6993	84.0	
2012	630	7.4	644	7.5	7287	85.1	0.000
2013	753	7.7	601	6.1	8459	86.2	
2014	783	8.1	586	6.0	8344	85.9	
2015	906	8.5	525	4.9	9240	86.6	

Road traffic accidents are caused by three main factors, human, motor vehicles, and environmental conditions. In Kosovo for the period 2010-2015, an accident with a vehicle is most frequent, with approximately over 70% during the entire period, followed by vehicle to vehicle with average 8%. Regarding contributing factors, the driver is responsible for an average of 99.3% for entire period 2010-2015 whereas climatic conditions only 0.5% and technical vehicle condition 0.1%. Among superficial road conditions in road traffic accidents, dry road recorded mostly with average 72.9% for the entire period, followed by wet road with 14.2%, snow 3.3% and icy road only 1.2%. As far the climatic conditions, most accidents happened in clear weather with 71.1%, cloudy 8.5%, fog 1.0% with snow 4.3%. Most crashes occur in the afternoon rush hours between 14.00-18.00 with 31.0%, followed by 10.00-14.00 with 27.9% and followed by 18.00-22:00 with 18.9%. Over 50% of crashes occurred in the urban road with an average of 56.2% followed by national road with 24.3% and rural road with 9.5%. Most of these crashes take place during Monday with an average of 16.0%, followed with 15.0% for Tuesday, Wednesday, Friday and at least on Sundays with 10.8%. There is no statistically significant difference between years and all above-analyzed modalities (Table 4).

Table 4: Traffic accidents characteristics, Kosovo 2010-2015

		Year												
		2010		2011		2012		2013		2014		2015		
	Accidents	N	%	N	%	N	%	N	%	N	%	N	%	X^2 test
		18030	100.0	18888	100.0	19754	100.0	19928	100.0	16300	100.0	17722	100.0	
Accident Type	Accident with a vehicle	12407	68.9	13526	71.6	14469	73.2	14053	70.5	11437	70.2	12717	71.8	
	Vehicle - Vehicle	1496	8.2	1217	6.4	1288	6.5	1407	7.1	1516	9.3	1637	9.7	
	Vehicle - Train	1007	5.6	1157	6.1	1001	5.1	1441	7.2	696	4.3	815	4.6	X2=3415.
	Vehicle - Bike	962	5.3	904	4.8	881	4.5	1073	5.4	1074	6.6	1031	5.8	051
	Other	874	4.8	821	4.3	921	4.7	892	4.5	782	4.8	754	4.3	DF=05
	Vehicle - Pedestrian	951	5.3	909	4.8	822	4.2	676	3.4	440	2.7	438	2.5	p=0
	Vehicle - Bus - Minibus	204	1.1	192	1.0	210	1.1	232	1.1	168	1.0	190	1.1	
Contributing factors	Man Factor (Driver)	17575	97.9	18788	99.5	19549	99.0	19911	99.9	16252	99.7	17662	99.7	
	Pedestrian	12	0.1	8	0.0	7	0.0	3	0.0	7	0.0	7	0.0	X2=983.3
	Technical vehicle condition	17	0.1	18	0.1	7	0.0	3	0.0	9	0.1	11	0.1	09
	Road infrastructure	14	0.1	8	0.0	6	0.0	0	0.0	3	0.0	4	0.0	DF=20
	Climatic conditions	332	1.8	59	0.3	179	0.9	3	0.0	5	0.0	2	0.0	p=0
Road superficial conditions	Dry	12215	68.0	13634	72.2	12743	64.5	14752	74.0	12066	78.9	14146	79.8	
	Wet	2589	14.4	2261	12.0	1932	9.8	3481	17.5	2827	17.3	2531	14.3	X2=7107
	Slippery	115	0.6	24	0.1	38	0.2	6	0.0	21	0.1	24	0.1	276
	Snow	660	3.7	437	2.3	1645	8.3	300	1.5	240	1.5	403	2.3	DF=30
	Icy	191	1.1	209	1.1	448	2.3	125	0.6	66	0.4	281	1.6	p=0
	Damaged road	2	0.0	10	0.1	0	0.0	3	0.0	3	0.0	3	0.0	
Climatic conditions	Clear	11885	66.1	13339	70.6	12696	64.3	14333	71.9	12279	75.3	13871	78.3	
	The Cloudy	1703	9.5	1184	6.3	1335	6.8	2046	10.3	1780	10.9	1309	7.4	X2=7629.
	Rainy	1326	7.4	1368	7.2	915	4.6	1583	7.9	1443	8.9	926	5.2	0626
	With snow	830	4.6	592	3.1	1864	9.4	479	2.4	380	2.3	691	3.9	DF=25
	Fog	66	0.3	80	0.4	17	0.1	246	1.2	114	0.7	625	3.5	p=0
Time	22.00-02.00	1188	6.6	1178	6.2	1268	6.4	1286	6.5	1037	6.4	1206	6.8	
	02.00-06.00	411	2.3	390	2.1	471	2.4	525	2.6	440	2.7	619	3.5	X2=122.8
	06.00-10.00	2533	14.1	2626	13.9	2683	13.6	2767	13.9	2166	13.3	2411	13.6	64
	10.00-14.00	4999	27.8	5326	28.2	5541	28.0	5637	28.3	4542	27.9	4833	27.3	DF=25
	14.00-18.00	5473	30.4	5820	30.8	6149	31.1	5230	31.3	5172	31.7	5478	30.9	p=0
	18.00-22.00	3395	18.9	3552	18.8	3643	18.4	3483	17.5	2945	18.1	3177	17.9	
Place of accident	Nationale road	4726	26.3	4217	22.3	4639	23.5	4886	24.5	3932	24.1	4406	24.9	
	Regionale road	1492	8.3	1204	6.4	1406	7.1	1491	7.5	1321	8.1	1328	7.5	X2=2170.
	Urban road	9788	54.4	10661	56.4	11392	57.7	11147	55.9	9201	56.4	9936	56.1	461
	Highway	0	0.0	0	0.0	0	0.0	0	0.0	248	1.5	167	0.9	DF=25
	Rural	1751	9.7	1831	9.7	2012	10.2	2073	10.4	1279	7.8	1625	9.2	p=0
Days	Monday	2875	16.0	2996	15.9	3238	16.4	3295	16.5	2501	15.3	2849	16.1	
	Tuesday	2701	15.0	2875	15.2	2799	14.2	3058	15.3	2466	15.1	2615	14.8	
	Wednesday	2709	15.0	2842	15.0	2834	14.3	2959	14.8	2456	15.1	2596	14.6	X2=97.49
	Thursday	2515	14.0	2712	14.4	3002	15.2	2735	13.7	2360	14.5	2615	14.8	6
	Friday	2672	14.8	2804	14.8	3029	15.3	2925	14.7	2481	15.2	2630	14.8	DF=30
	Saturday	2719	15.1	2779	14.7	2770	14.0	2909	14.6	2242	13.8	2394	13.5	p=0
	Sunday	1824	10.1	2084	11.0	2082	10.5	2073	10.4	1794	11.0	2023	11.4	

According to Health For All database, for SEE-countries for 2011, and Kosovo Police data for Kosovo, Kosovo has the highest number of road traffic accidents with injuries, 478.3 per 100 000 (Fig. 1).

Discussion

Road traffic accidents are among main epidemiologic problems and public health issues in developed and in developing countries. Road traffic accidents as a global challenge are on the global

agenda through Sustainable Development Goals SDG 3 and 11 which aim to half the number of global deaths and injuries from road traffic accidents by 2020 and provide access to safe, affordable, accessible and sustainable transport systems and improving road safety by 2030.

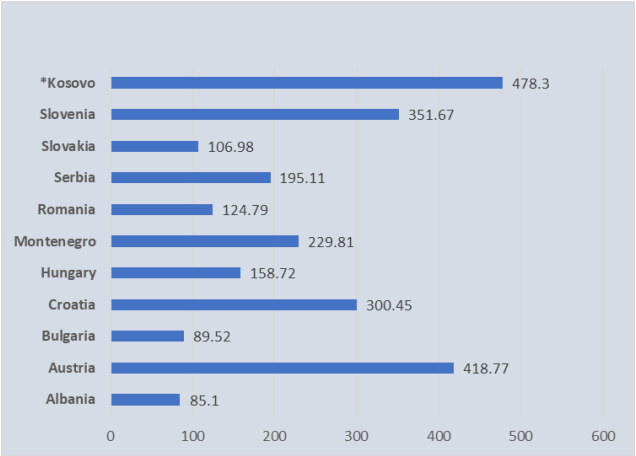


Figure 1: Road traffic accidents with injury per 100 000, SEE countries, 2011

Public health importance of road traffic accidents besides that are largely preventable, are also health consequences as death, disability, quality of life, economic burden with direct and indirect losses for the victims and their families.

It is estimated that every day in the world 3287 people dies in-car accidents [6]. According to the World Health Organization's Report-2015 on the situation of road safety, the traffic accident mortality rate in Italy is 6.1 per 100,000, Austria 5.4, France 5.1 less than Kosovo mortality rate 6.4 per 100,000 inhabitants, which is the smaller rate in comparison with Hungary and Serbia with 7.7 and Bulgaria 8.3 per 100 000 inhabitants. The highest mortality rate in the region is recorded in Bosnia and Hercegovina with 17.7 and Albania with 15.1 per 100,000 inhabitants. In Iran, road traffic accidents are the reason for 25% of unnatural deaths [4]. In Rumania injuries caused by road traffic accidents represent an important morbidity and mortality risk factor. The incidence of road traffic injuries was 30.61 for 100,000 inhabitants, whereas the mortality rate 10.28 deaths for 100,000 inhabitants [7].

In Kosovo for the period 2010-2015, on average yearly number of road traffic accidents is 18437, dead persons 138, injured 9135, mortality rate 7.4 per 100000 and lethality of 1.5%. In our study the most frequent age of fatal cases and victims in general in traffic crashes was over 19 years old that is in line with many other studies like one in Brazil [8], similarly mostly affected in road traffic accidents in Africa are young and especially young men. People in the 20-54 age group accounted for 71.8% of all those fatal cases annually [9]. The young population between ages of 20 and 34 is the age group mostly affected, and since is economically active, consequences overcome a person with wider implication on economic losses of their families also [10].

Highest fatal cases according to their involvement in traffic, is among drivers. Regarding contributing factors, the driver is responsible on average of 99.3% for entire period 2010-2015 whereas climatic conditions only 0.5% and technical vehicle condition 0.1%. Similar results were found in different studies, in the United Arab Emirates, driving behaviour are more prevailing contributing factors, and vehicle safety is least [11]. In South Africa, most of the casualties of road traffic accidents were drivers (46%) [10]. In Taiwan, human factor respectively psychosocial influences are responsible up 3 to 4% of accidents [12]. In Lasi driver's error was identified as the main contributing factor in about two-thirds of all road traffic accidents [7].

In a report from Africa, more passengers were killed than any other road user in the years 2010-2012, accounting for 43.8% of all fatalities in 2010, 51.1% in 2011 and 43% in 2012 and the least affected road users are drivers, at 26.4%, 25.7% and 28.5% of all fatalities in 2010, 2011 and 2012 with the most prevalent death risk factors disregarding traffic rules (45%) and driver rushing (31%) [13]. While in Africa passengers are the most affected road users in crashes, in Kosovo mostly fatal cases are among drivers. In Peru, the majority of the fatalities were pedestrians (61%) [14], and in Basel similarly, for injuries, the majority of the fatalities (84.8%) occurred among pedestrians [15].

In Kosovo for the period 2010-2015, an accident with a vehicle is most frequent with about over 70% during the entire period, followed by vehicle to vehicle with average 8%. Similarly, in a study conducted in Iran, most collisions were vehicle-vehicle crashes 52.3% [16]. While in Kosovo over 50% of crashes occurred in the urban road, in Iran most fatal injuries (61.4%) occurred on outer-city roads and only 27.4% occurred on inner-city roads [16]. In Kosovo majority of accidents happen on Fridays and Saturdays similarly with other studies [9, 10, 14, 18, 19), while Sunday is the day with the lowest accident rate, similar with Peru [14].

In Kosovo most crashes occur in the afternoon rushing hours between 14.00-18.00hrs with 31.0%, similarly, with other studies, road traffic accidents occur mainly between two in the afternoon and eight at night [9, 10, 14], followed by 10.00-14.00 hrs with 27.9%. In Saudi Arabia, the most frequent time was during the rush period of noon to 3 pm [17], while in other studies during early evening hours [18, 19].

Among superficial road conditions in road

traffic accidents, the dry road was recorded mostly with average 72.9%, mostly in clear weather with 71.1% similar to another study [17].

A large number of vehicles contribute to air pollution which has an impact on increasing burden of chronic respiratory diseases as asthma and emphysema. Policy interventions in many countries as in the example in Rumania have reduced road traffic crashes, in a short period [7]. Safety belts are shown to be helpful as road safety measures, since 1976 after enforcement of the law on safety belts, 31% less injured persons, four times fewer head injuries, three times less minor injuries and five times less severe injuries were found [15]. In Verona after low enforcement on the seat belt, a significant reduction of injured/accidents ratio was recorded 29%, head trauma for 50.3% [20]. In line with Decade of Action for Road Safety (2011–2020) aiming to stabilise and reducing the increasing trend of road traffic fatalities and saving an estimated 5 million lives over the period, in Kosovo several interventions were taken to maintain citizens' life and wellbeing. Road safety is associated with legal, institutional, technical and financial support with enforcement of new traffic Law no. 05 / L-088, with new rules for all participants in traffic to increase safety in road traffic, traffic flow and environmental protection [21].

For the period 2010-2015, there is a slight decrease in the mortality rate of 0.1‰ and lethality rate of 0.1% each year whereas there is an increase of 21.5‰ for traumatism rate for each year. A Higher number of fatal cases are drivers and above 19 years with more than 80%. Among injured significantly highest percentage is among passengers for all years and above 19 years old. Traffic accident with a vehicle is mostly too happened with approximately over 70%, mostly on dry road 72.9% and clear weather 71.1%. The driver is contributing factors of road traffic accidents on average 99.3% whereas climatic conditions only 0.5%, with over 50% of crashes occurred in urban road 56.2% mostly during Monday 16.00% and in the afternoon rushing hours between 14.00-18.00hrs with 31.0%. A joint effort from health, education and police sectors should compile a public health strategy and action plan to increase awareness and traffic culture focused on human risk factors.

In conclusion, there is a slight decrease in the mortality rate of 0.1‰ and lethality rate of 0.1% each year, whereas there is an increase of 21.5‰ for traumatism rate for each year. Limitation of the study was disaggregated data on excel database which was a barrier for depth calculation and analyse.

Acknowledgment

To the Statistics Department of the National Police of Kosovo for access to the information they collect.

References

1. WHO. Global status report on road safety 2015. Geneva, Switzerland, 2015. http://who.int/violence_injury_prevention/road_safety_status/2015/en

2. Jadaan KS. Road traffic accidents in Kuwait: an economic dimension. Accid Anal Prev. 1990;22(4):399-401. https://doi.org/10.1016/0001-4575(90)90055-P

3. Connelly LB, Supangan R. The economic costs of road traffic crashes Australia, states and territories. Accid Anal Prev. 2006;38(6):1087-93. https://doi.org/10.1016/j.aap.2006.04.015 PMid:16797462

4. García-Altés A, Pérez K. The economic cost of road traffic crashes in an urban setting. Inj Prev. 2007;13(1):65-8. https://doi.org/10.1136/ip.2006.012732 PMid:17296693 PMCid:PMC2610566

5. Hejazi R, Shamsudin MN, Radam A, Rahim KA, Ibrahim ZZ, Yazdani S. Estimation of traffic accident costs: a prompted model. Int J Inj Contr Saf Promot. 2013;20(2):152-7. https://doi.org/10.1080/17457300.2012.720578 PMid:23025243

6. http://asirt.org/initiatives/informing-road-users/road-safety-facts/road-crash-statistics

7. Manole M, Duma O. The dynamics of risk factors in road traffic accidents in Iaşi county. Rev Med Chir Soc Med Nat Iasi. 2005;109(3):633-7. PMid:16607763

8. Al Marzooqi AH, Badi M, El Jack A. Road road traffic accidents in Dubai, 2002-2008. Asia Pac J Public Health. 2010;22(3 Suppl):31S-39S. https://doi.org/10.1177/1010539510372834 PMid:20566531

9. Bakhtiyari M, Delpisheh A, Monfared AB, Kazemi-Galougahi MH, Mehmandar MR, Riahi M, Salehi M, Mansournia MA. The road traffic crashes as a neglected public health concern; an observational study of Iranian population. Traffic Inj Prev. 2015;16(1):36-41. https://doi.org/10.1080/15389588.2014.898182 PMid:24761776

10. Olukoga A. Pattern of road traffic accidents in Durban municipality, South Africa. West Afr J Med. 2008;27(4):234-7. PMid:19469402

11. Hassan MN, Hawas YE, Maraqa MA. A holistic approach for assessing traffic safety in the United Arab Emirates. Accid Anal Prev. 2012;45:554-64. https://doi.org/10.1016/j.aap.2011.09.009 PMid:22269542

12. Ding SL, Wang JD, Chen KT. Epidemiologic analysis of 845 cases of pedestrian traffic injuries. Zhonghua Yi Xue za Zhi. Chinese Medical Journal Free China Ed. 1994, 53 (6 Suppl B): 16-22.

13. Motor Vehicle Accident Fund. Annual Road Crash & Claims Report 2012.

14. Choquehuanca-Vilca V, Cárdenas-García F, Collazos-Carhuay J, Mendoza-Valladolid W. Epidemiological profile of road road traffic accidents in Peru, 2005-2009. Rev Peru Med Exp Salud Publica. 2010;27(2):162-9. https://doi.org/10.1590/S1726-46342010000200002 PMid:21072466

15. Hell K. Injuries through road traffic accidents to car occupants with and without the obligation to wear safety belts. Unfallchirurgie. 1980;6(1):28-34. https://doi.org/10.1007/BF02589447 PMid:7210261

16. Heydari ST, Hoseinzadeh A, Ghaffarpasand F, Hedjazi A, Zarenezhad M, Moafian G, Aghabeigi MR, Foroutan A, Sarikhani Y, Peymani P, Ahmadi SM, Joulaei H, Dehghankhalili M, Lankarani KB. Epidemiological characteristics of fatal road traffic accidents in

Fars province, Iran: a community-based survey. Public Health. 2013;127(8):704-9. https://doi.org/10.1016/j.puhe.2013.05.003 PMid:23871394

17. Nofal FH, Saeed AA, Anokute CC. Aetiological factors contributing to road road traffic accidents in Riyadh City, Saudi Arabia. J R Soc Health. 1996;116(5):304-11. https://doi.org/10.1177/146642409611600508 PMid:8936950

18. Durić P, Miladinov-Mikov M. Epidemiological characteristics of road traffic injuries in AP Vojvodina. Med Pregl. 2009;62(1-2):17-22. https://doi.org/10.2298/MPNS0902017D PMid:19514595

19. Caixeta CR, Minamisava R, Oliveira LM, Brasil VV. Traffic injuries among youth in Goiania, Goias State. Cien Saude Colet. 2010;15(4):2075-84. https://doi.org/10.1590/S1413-81232010000400021 PMid:20694329

20. Campello C, Preite G, Poli A, Zuppichini F, Marigo M. Effects of seat belt legislation on injuries of road traffic accidents. Epidemiol Prev. 1996;20(4):313-7. PMid:9044894

21. Kosovo Police Annual Report, 2013. http://www.kosovopolice.com/repository/docs/raporti_anglisht_2013.pdf

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):1042-1046.
https://doi.org/10.3889/oamjms.2017.195
eISSN: 1857-9655
Review Article

Subclinical Hypothyroidism – Whether and When To Start Treatment?

Milena Cojić[1,2*], Ljiljana Cvejanov-Kezunović[3,4]

[1]Primary Health Center Podgorica, Trg Nikole Kovačevića 6 , Podgorica 81000, Montenegro; [2]Medical Faculty Podgorica, Univercity of Montenegro - Family Medicine, Kruševac bb, Podgorica, Montenegro; [3]JZU Dom Zdravlja Podgorica - ZS Nova Varos, Podgorica 20000, Montenegro; [4]Medical Faculty - Podgorica - Occupational Medicine, Krusevac bb, Podgorica 20000, Montenegro

Citation: Cojić M, Cvejanov-Kezunović L. Subclinical Hypothyroidism – Whether and When To Start Treatment? Open Access Maced J Med Sci. 2017 Dec 15; 5(7):1042-1046. https://doi.org/10.3889/oamjms.2017.195

Keywords: Subclinical hypothyroidism; Substitution therapy; effects.

***Correspondence:** Milena Cojić. Primary Health Center Podgorica, Trg Nikole Kovačevića 6, Podgorica 81000, Montenegro; 2Medical Faculty Podgorica, University of Montenegro - Family Medicine, Kruševac bb, Podgorica, Montenegro. E-mail: milenarovcanin@yahoo.com

Received: 13-Aug-2017; **Revised:** 20-Sep-2017; **Accepted:** 24-Sep-2017; **Online first:** 26-Oct-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Subclinical hypothyroidism represents a state with increased values of thyroid stimulating hormone (TSH) and normal values of thyroxine (T4) and triiodothyronine (T3). The disorder is asymptomatic, and the diagnosis is made based on the results of laboratory findings when the level of TSH reaches values above 4.0 mU/l. It is still subject to debate whether patients with subclinical hypothyroidism are at increased risk of cardiovascular disease, neuropsychiatric and neuromuscular disorders. Studies have shown that the appearance of general symptoms and complications are more common in patients whose values of TSH are above 10 mU/l. Therefore, the initiation of therapy with levothyroxine, which is the foundation of substitution therapy, is advised in patients whose TSH is >10 mU/l. As for patients whose values of TSH are from 4.0 to 10.0 mU/l and who make up 90% of the patients with subclinical hypothyroidism, further research is needed to determine the effects of the disorder and levothyroxine therapy on the health. Until then, the introduction of the substitution therapy in patients with TSH which is <10 mU/l should be considered in the case of the presence of general symptoms, anti-thyroid antibodies, increased lipids and other risk factors, goitre, pregnancy, ovarian dysfunction and infertility.

Introduction

Subclinical hypothyroidism (SH) is a very common disorder in the general population, especially among middle-aged and elderly patients. It represents a state with increased values of thyroid stimulating hormone (TSH) and normal values of thyroxine (T4) and triiodothyronine (T3) [1]. In most cases, patients with SH have no symptoms that would indicate this disorder, so diagnosis is made based on laboratory findings [2]. As the values of thyroid hormone are normal, increased level of TSH represents a compensatory mechanism that stimulates the thyroid gland to produce sufficient amounts of thyroid hormones. The disorder can eventually progress to overt hypothyroidism (OH) which is characterised by increased values of TSH but reduced values of thyroid hormones [3]. Since the SH is asymptomatic disorder in which the values of thyroid hormones are normal, and it may be a prelude to a clinically manifest disease of the thyroid gland, the question is whether it should be treated. This paper aims to summarize the available data on the influence of this disorder on the health of patients, as well as data on the effects of treatment to answer the question whether SH should be treated and if so when to start the treatment.

Subclinical hypothyroidism and clinical significance

Clinically OH and decreased production of the thyroid hormones is associated with an increased cardiovascular risk [4], but what about the SH where

the values of the thyroid hormones are still normal?

Values of TSH above 4.0 mU/l represent increased levels and by their increase the SH can be divided into a mild form (values from 4.0-10.0 mU/l) and a more severe form (values >10.0 mU/l). As long as the values of this hormone are over the limit, levels of thyroid hormones are not sufficient to provide the euthyroid state. If it weren't so, we would expect the value of TSH, whose half-life is 1h, to drop to normal values as soon as T4 and T3, whose half-life is seven days and 1 day, reach normal values. However, this does not happen in SH because the values of TSH remain increased even when T4 and T3 reach normal values [3].

SH is usually asymptomatic, but in some patients may still appear symptoms that would indicate hypothyroidism. In the US Colorado Thyroid Disease Prevalence Study, which included 20,862 examinees, patients with SH more frequently reported symptoms compared to euthyroid examinees but less frequently than patients with OH. The most common symptoms were dry skin, poor memory, slower thinking, weakness and muscle cramps, swollen face with periorbital oedema, fatigue, hoarseness, deep voice and constipation. The same study showed that in the majority of patients (74%) with SH the levels of TSH were between 5.1-10.0 mU/l, while in 26% were above ten mU/l [5]. This means that most of these patients have mainly a slight disturbance of the function of the thyroid gland which is the case in 90% of patients with SH in general population [6].

Although the appearance of these symptoms could mean that this disorder could have consequences for the health of patients, there is still no evidence for possible harmful effects on individual's health. Besides the ability to progress to clinically OH, some studies have shown that SH could be associated with increased risk of cardiovascular disease (CVD), mood disorders and cognitive dysfunction as well as impaired neuromuscular function [2, 3, 6, 7].

Subclinical hypothyroidism and cardiovascular system

Thyroid hormones exert a direct influence on the heart and blood vessels. The deficit of these hormones leads to functional disorders of the CVS, so changes in cardiac frequency, cardiac output and systemic vascular resistance are closely related to the thyroid status [8, 9].

In SH there is a disruption of the systolic and diastolic function of the left ventricle. In the blood vessels, there are also changes in the form of increased vascular resistance, increased arterial stiffness and endothelial dysfunction [6].

Thyroid hormones also influence the lipid status. Many studies have shown that patients with SH have increased the level of total cholesterol, as well as low-density lipoprotein (LDL) about the euthyroid patients [5, 10]. Despite these results, a clear connection between lipids and SH has not been established because some studies have shown that the lipid profiles of patients with SH were not significantly different compared to euthyroid patients [11]. However, the lipid profile was more impaired in patients whose TSH is >10 mU/l and in smokers [12]. Patients with SH are also believed to be at increased risk of atherosclerosis. That is shown in Rotterdam study that examined the connection between the atherosclerotic process and SH in 1,149 women aged over 55 years. In this study, patients with TSH >4 mU/l had an increased risk of atherosclerosis and occurrence of myocardial infarction [13].

Disturbed blood coagulation is also seen in patients with SH. The values of some coagulation factors are increased, and the whole fibrinolytic activity is decreased which might result in increased blood coagulation [12]. Bearing in mind the potential influence on the structure and function of the CV system and lipid status, Rodondi et al. examined 2,730 patients between 70 and 79 years, of whom 338 had SH, to investigate their risk of CV morbidity and mortality. They have shown that in patients with TSH levels ≥ 7, there was an increased risk of chronic heart failure in comparison to other euthyroid patients, but there was no increased risk of other CV events and mortality. In examinees whose TSH values were between 4.5 and 6.9 mU/l an increased risk of CV morbidity and mortality has not been observed compared to euthyroid examinees [14]. The same author's analysis of 11 prospective studies that included 55,287 examinees showed that increase in levels of TSH increases the risk of CV events and CV mortality, especially among those whose TSH is >10 mU/l. The disadvantage in interpreting these results lies in the fact that some of these studies involved patients with the prior existence of CV disease [15, 16].

Due to the extreme heterogeneity of the studies, we cannot make accurate conclusions about the influence of SH on CV system, although we can conclude that there is no evidence that a mild form of SH (TSH values are from 4.0 to 10.0 mU/l) may have consequences for patient's CV system.

Subclinical hypothyroidism, mood and cognitive functions

Some, but not all studies have shown the connection between anxiety and depressive disorders

with SH [17, 18]. In middle-aged patients with SH was observed the more frequent occurrence of depression and the occurrence of severe forms of depressive disorders compared to euthyroid examinees.

Although the deficit of thyroid hormones leads to disorder of affective and cognitive functions, the influence of SH on these functions is not yet fully understood. Given that in most cases the SH is caused by autoimmune process and associated with an increased titer of antibodies to thyroid peroxidase (TPO) and thyroglobulin, it should be noted that the presence of these antibodies can cause cerebral dysfunction known as Hashimoto encephalopathy [8].

Subclinical hypothyroidism and neuromuscular function

The exact mechanism that would be placed by disorders of neuromuscular function is not fully understood, but it is thought that disorder in glycogen lysis, expression of heavy chains of myosin and the mitochondrial activity could be the reason for the appearance of these symptoms in patients with SH. One study examined 12 patients with SH who complained of neuromuscular ailments during rest and exercise. The amount of created lactates and pyruvates in skeletal muscles during exercise were significantly higher in patients with SH in comparison to the control group. Based on the results, it can be concluded that energy metabolism of muscles may be disturbed in patients with SH [12].

Subclinical hypothyroidism and the progression to clinical hypothyroidism

SH is a disorder that occurs more frequently in women, the elderly and in areas where there is an increased intake of iodine. Prevalence rate ranges from 4 to 10% in the adult population, and if there is an increased intake of iodine, it is up to 24% [12, 19]. In 80% of patients with SH, there is an increased titer of anti-thyroid antibodies, which means that in most cases an autoimmune process is present [7].

The clinical course of SH can move in the direction of development of OH, as well as in the direction of normalisation of values of TSH. One of the prospective studies followed the clinical course in 82 women who had increased values of TSH and showed that after a period of 10 years, 28% of them developed OH (TSH > 20 mU/l and decreased levels of free T4), 68% of them still had a subclinical disorder, while 4% of them reached the normalization of TSH levels [20].

Diez et al. examined the natural course of SH in 107 patients and have shown that patients with mild SH disorder (TSH levels from 5.0 to 9.9 mU/l) have more chances to have values of TSH normalised compared to patients whose TSH is > 10.0 mU/l. It was also shown that the value of TSH was the most important prognostic factor for the outcome of SH [21].

The effects of the treatment of patients with subclinical hypothyroidism

Studies that dealt with the effects of therapy often researched its influence on the disorder of the lipid profile of patients, as a possible significant risk for future CV disease. In the report of the working group of the United States for the prevention (U.S. Preventive Services Task Force - USPSTF), seven studies examined the influence of treatment of SH on the values of lipids. Six of them showed that the treatment of SH does not lead to improvement in lipid parameters [16].

On the other hand, an analysis of 13 studies and a total of 247 examinees showed different results. All patients had a disorder of TSH levels, no matter if they had spontaneous SH or OH with an insufficient dose of levothyroxine to normalise the value of TSH. The mean value of TSH at the beginning was 10.8 mU/l, and after the treatment period (12 weeks to 3 months) it was 2.6 mU/l. In 11 out of 13 studies there has been a decrease in the value of total cholesterol (-0.20 mmol/L (-7.9 mg/dL, or 5%)) and 7 out of 9 there has been a decrease in the value of LDL (-0.26 mmol/L (-10 mg/dL)). Changes in values of HDL and triglycerides were not statistically significant. However, most of these studies included small samples, most of them were not randomised and did not include a control group [22].

Since it is known that thyroid hormones perform a substantial effect on the heart, some studies have examined the influence of levothyroxine therapy on the structure and function of the heart in patients with SH. One such, double-blind and placebo-controlled study was conducted by Monzani et al. and it showed that patients with SH had a disorder in systolic and diastolic function of the left ventricle and that the levothyroxine therapy led to complete regression of it [23].

Great retrospective study based on data from the Danish National Patient Registry has examined the influence of levothyroxine therapy in patients with SH on the risk of myocardial infarction, as well as cardiovascular and total mortality. No effect was seen on the risk of myocardial infraction or the CV mortality.

As for total mortality, the results showed that patients younger than 65 years could have some marginal benefit [24].

Substitution therapy had different effects regarding cognitive functions which may be because SH is not a disorder that leads to global cognitive dysfunction and its numerous domains, but the more subtle changes in specific domains such as memory and executive functions of the brain [25].

As for mood disorders, although there is evidence that in patients with affective disorders often occurs SH, there is no evidence that substitution therapy has favourable effects [8, 26].

Levothyroxine therapy can lead to a reduction and the complete disappearance of the present antibodies in hypothyroid patients with Hashimoto thyroiditis. Still, there is no clear evidence that the early initiation of therapy might affect the spontaneous course of SH [1, 12, 27].

The negative effects of therapy

The most common negative consequence of levothyroxine therapy is the occurrence of subclinical hyperthyroidism and in some cases occurrence of iatrogenic hyperthyroidism [2, 27, 28]. Between 10% and 33% of patients who take levothyroxine have values of TSH below normal, and in more than half of these patients, TSH is less than 0.1 m/L [27].

Other adverse effects are related to the effect of levothyroxine on the bones and heart. Studies that examined the effect of TSH suppression by levothyroxine on bone density did not provide specific conclusions whether the therapy could lead to an increased risk of osteoporosis [26, 27]. As for the effects on the heart, subclinical hyperthyroidism in people older than 60 years is associated with increased 10-year risk of developing atrial fibrillation [26].

Whether and when to start treatment?

SH is considered to be a mild disorder which can have consequences for the health and introduction of substitution therapy may be beneficial in some patients. Most of these patients have values of TSH from 4.0 to 10.0 mU/l and minimal metabolic and physiological disorders. Therefore, starting substitution therapy in these patients isn't usually justified. They should be rather monitored and controlled every 6 to 12 months [7, 12, 27]. Therapy should be considered if there are present antibodies on TPO, general symptoms that are suggestive of hypothyroidism, increased values of total and LDL cholesterol, nodular or diffuse enlargement of the thyroid gland, pregnancy or ovulatory dysfunction with infertility [12, 17].

The results of many studies have shown that treatment with substitution therapy should be initiated in patients with TSH values above ten mU/l because these patients are at increased risk of developing health disorders (dyslipidemia, CV events, psychiatric, neuromuscular disorders, and occurrence of general symptoms). Although there isn't enough evidence that levothyroxine therapy could lower the values of total and LDL cholesterol, it's beneficial effects cannot be excluded. Clinicians are advised to decide on a case-by-case basis, particularly if patients are smokers and have other risk factors for cardiovascular disease (hypertension, insulin resistance and diabetes, renal failure, etc.) [27, 29].

High values of TSH also increase the risk of developing clinically OH. There is no evidence that treatment will postpone the progression of the disorder, but perhaps it could help coping with symptoms [30]. One of the strongest reasons why the treatment is proposed when TSH reaches values above 10.0 mU/l is that the favourable results of substitution therapy are more obvious in these patients [2, 6, 7, 12, 29, 30].

Treatment is initiated with levothyroxine. Usual doses 25-75 mg/day are sufficient to normalise levels of TSH in patients with SH. Doses should be carefully titrated until TSH reaches value between 1 and 2-3 mU/l in younger and middle-aged patients. When the optimum values are reached, TSH should be controlled every 6-12 months [12].

References

1. Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, Cobin RH et al. Subclinical thyroid disease scientific review and guidelines for diagnosis and management. JAMA. 2004; 291(2): 228-38. https://doi.org/10.1001/jama.291.2.228 PMid:14722150

2. Gillett M. Subclinical hypothyroidism: subclinical thyroid disease: scientific review and guidelines for diagnosis and management. The Clinical Biochemist Reviews. 2004; 25(3): 191-94. PMCid:PMC1880829

3. McDermott MT, Ridgway EC. Subclinical hypothyroidism is mild thyroid failure and should be treated. J Clin Endocrinol Metab. 2001; 86(10): 4585–90. https://doi.org/10.1210/jcem.86.10.7959 PMid:11600507

4. Pesic M, Antic S, Kocic R, Radojkovic D, Radenkovic S. Cardiovascular risk factors in patients with subclinical hypothyroidism. (srb. Kardiovaskularni faktori rizika kod bolesnika sa supklinčkom hipotireozom.) Vojnosanit Pregl. 2007; 64(11): 749-52. https://doi.org/10.2298/VSP0711749P PMid:18050969

5. Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado thyroid disease prevalence study. Arch Intern Med. 2000; 160: 526-34. https://doi.org/10.1001/archinte.160.4.526 PMid:10695693

6. Pearce S, Brabant G, Duntas LH, Monzani F, Peeters RP, Razvi S et al. 2013 ETA Guideline: management of subclinical hypothyroidism. Eur Thyroid J. 2013; 2: 215–28. https://doi.org/10.1159/000356507 PMid:24783053 PMCid:PMC3923601

7. Fatourechi V. Subclinical Hypothyroidism: An update for primary care physicians. Mayo Clin Proc. 2009; 84(1): 65–71. https://doi.org/10.4065/84.1.65 PMid:19121255 PMCid:PMC2664572

8. Zarković M. The deficit of thyroid hormones - effect on cardiovascular and cognitive functions. (srb. Deficit tiroidnih hormona – uticaj na kardiovaskularne i kognitivne funkcije.) Medical Gazette Special hospital for thyroid gland diseases and metabolic diseases Zlatibor (srb. Medicinski glasnik Specijalna bolnica za bolesti štitaste žlezde i bolesti metabolizma Zlatibor). 2007; 12 (21): 47-53.

9. Klein I, Ojamaa K. Thyroid hormone and cardiovascular System. N Engl J Med. 2001; 344: 501-9. https://doi.org/10.1056/NEJM200102153440707 PMid:11172193

10. Rodondi N, Aujesky D, Vittinghoff E, Cornuz J, Bauer D. Subclinical hypothyroidism and the risk of coronary heart disease: a meta-analysis. Arch Intern Med. 2005;165: 2460-66. https://doi.org/10.1001/archinte.165.21.2460 PMid:16314541

11. Caraccio N, Ferrannini E, Monzani F. Lipoprotein profile in subclinical hypothyroidism: response to levothyroxine replacement, a randomized placebo-controlled study. J Clin Endocrinol Metab. 2002; 87: 1533–38. https://doi.org/10.1210/jcem.87.4.8378 PMid:11932277

12. Biondi B, Cooper DS. The clinical significance of subclinical thyroid dysfunction. Endocrine Reviews. 2008; 29(1):76–131. https://doi.org/10.1210/er.2006-0043 PMid:17991805

13. Hak EA, Pols HAP, Visser TJ, Drexhage HA, Hofman A, Witteman JCM. Subclinical hypothyroidism is an independent risk factor for atherosclerosis and myocardial infarction in elderly women: the Rotterdam study. Ann Intern Med. 2000;132: 270-78. https://doi.org/10.7326/0003-4819-132-4-200002150-00004 PMid:10681281

14. Rodondi N, Newman AB, Vittinghoff E, Rekeneire N, Satterfield S, Harris TB et al. Subclinical hypothyroidism and the risk of heart failure, other cardiovascular events, and death. Arch Intern Med. 2005; 165(21): 2460-6. https://doi.org/10.1001/archinte.165.21.2460 PMid:16314541

15. Rodondi N, den Elzen WPJ, Bauer DC, Cappola AR, Razvi S, Walsh JP et al. Subclinical hypothyroidism and the risk of coronary heart disease and mortality. JAMA. 2010; 304 (12): 1365-74. https://doi.org/10.1001/jama.2010.1361 PMid:20858880 PMCid:PMC3923470

16. Rugge JB, Bougatsos C, Chou R. Screening for and treatment of thyroid dysfunction: an evidence review for the U.S. Preventive Services Task Force. Evidence synthesis no. 118. AHRQ publication no. 15-05217-EF-1. Rockville, MD: Agency for Healthcare Research and Quality, 2014.

17. Cooper DS. Subclinical hypothyroidism. N Engl J Med. 2001;345(4):260-65. https://doi.org/10.1056/NEJM200107263450406 PMid:11474665

18. Joffe RT, Pearce EN, Hennessey JV, Ryan JJ, Stern RA. Subclinical hypothyroidism, mood, and cognition in the elderly: A Review. Int J Geriatr Psychiatry. 2013;28(2):111–18. https://doi.org/10.1002/gps.3796 PMid:22410877 PMCid:PMC3488161

19. Vanderpump M. The epidemiology of thyroid disease. Br Med Bull. 2011; 99 (1): 39-51. https://doi.org/10.1093/bmb/ldr030 PMid:21893493

20. Huber G, Staub J, Meier C, Mitrache C, Guglielmetti M, Huber P et al. Prospective study of the spontaneous course of subclinical hypothyroidism: prognostic value of thyrotropin, thyroid reserve, and thyroid antibodies. J Clin Endocrinol Metab. 2002; 87:3221-6. https://doi.org/10.1210/jcem.87.7.8678 PMid:12107228

21. Diez JJ, Iglesias P. Spontaneous Subclinical Hypothyroidism in Patients Older than 55 Years: An Analysis of Natural Course and Risk Factors for the Development of Overt Thyroid Failure. J Clin Endocrinol Metab. 2004; 89: 4890–97. https://doi.org/10.1210/jc.2003-032061 PMid:15472181

22. Danese M, Ladenson PW, Meinert CL, Powe NR. Effect of thyroxine therapy on serum lipoproteins in patients with mild thyroid failure: a quantitative review of the literature. J Clin Endocrinol Metab. 2000; 85: 2993–3001. https://doi.org/10.1210/jc.85.9.2993

23. Monzani F, Di Bello V, Caraccio N, Bertini A, Giorgi D, Giusti C et al. Effect of levothyroxine on cardiac function and structure in subclinical hypothyroidism: a double blind, placebo-controlled Study. J Clin Endocrinol Metab. 2001; 86: 1110–5. https://doi.org/10.1210/jcem.86.3.7291 PMid:11238494

24. Andersen MN, Olsen AMS, Madsen JC, Faber J, Torp-Pedersen C, Gislason GH et al. Levothyroxine substitution in patients with subclinical hypothyroidism and the risk of myocardial infarction and mortality. PloS one. 2015; 10(6):e0129793. https://doi.org/10.1371/journal.pone.0129793 PMid:26069971 PMCid:PMC4466400

25. Samuels MH. Cognitive function in subclinical hypothyroidism. J Clin Endocrinol Metab. 2010; 95(8): 3611–3. https://doi.org/10.1210/jc.2010-1242 PMid:20685889

26. Weetman AP. Hypothyroidism: screening and subclinical disease. BMJ. 1997; 314: 1175-8. https://doi.org/10.1136/bmj.314.7088.1175 PMid:9146393 PMCid:PMC2126522

27. Chu JW, Crapo LM. The treatment of subclinical hypothyroidism is seldom necessary. J Clin Endocrinol Metab. 2001; 86(10): 4591-9. https://doi.org/10.1210/jcem.86.10.7961 PMid:11600508

28. Adlin V. Subclinical hypothyroidism: deciding when to treat. Am Fam Physician. 1998; 57(4): 776-80. PMid:9491000

29. Biondi B, Wartofsky L. Treatment with thyroid hormone. Endocrine Reviews. 2014; 35: 433–512. https://doi.org/10.1210/er.2013-1083 PMid:24433025

30. Col NF, Surks MI, Daniels GH. Subclinical thyroid disease. JAMA. 2004; 291: 239-43. https://doi.org/10.1001/jama.291.2.239 PMid:14722151

ID Design 2012/DOOEL Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2017 Dec 15; 5(7):1047-1048.
https://doi.org/10.3889/oamjms.2017.188
eISSN: 1857-9655
Letter to the Editor

Endoscopic Removal of a Giant Complicated Hyperplastic Gastric Polyp

Zaim Gashi[1], Aida Ferri Polloshka[1], Arber Veliu[1], Fisnik Kurshumliu[2], Elton Bahtiri[3,4*]

[1]Clinic of Gastroenterology, University Clinical Center of Kosovo, Prishtina, Kosovo; [2]Institute of Pathology, University Clinical Center of Kosovo, Prishtina, Kosovo; [3]Department of Pharmacology, Faculty of Medicine, University of Prishtina, Prishtina, Kosovo; [4]Clinic of Endocrinology, University Clinical Center of Kosovo, Prishtina, Kosovo

Citation: Gashi Z, Ferri Polloshka A, Veliu A, Kurshumliu F, Bahtiri E. Z. Endoscopic Removal of a Giant Complicated Hyperplastic Gastric Polyp. Open Access Maced J Med Sci. 2017 Dec 15; 5(7):1047-1048. https://doi.org/10.3889/oamjms.2017.188

Keywords: Hyperplastic polyps; Endoscopic removal; Histological examination.

***Correspondence:** Elton Bahtiri, Department of Pharmacology, Faculty of Medicine, University of Prishtina, Republic of Kosovo. Telephone: +377 44 218 692; Email: elton.bahtiri@uni-pr.edu

Received: 17-Jun-2017; **Revised:** 20-Sep-2017; **Accepted:** 29-Sep-2017; **Online first:** 16-Oct-2017

Funding: This research did not receive any financial support.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

The patient, a 40-year-old male, was referred to our clinic with intermittent nausea, vomiting and symptomatic anemia for 4 months. Notable hematological indices were low hemoglobin levels of 9.6 g/dl and hematocrit levels of 35.8%, while after receiving two units of concentrated red blood cells, at discharge; they achieved levels of 15.2 g/dl and 42.3%, respectively. Esophagogastroduodenoscopy revealed a 3 cm antral pedunculated polyp, prolapsing into pylorus thus causing intermittent pyloric obstruction and anemia. Histological examination revealed a hyperplastic polyp without evidences of malignancy. No atrophy, metaplasia, dysplastic changes or Helicobacter pylori infection were detected in samples taken from the antrum and the corpus; however, the examination provided evidence for gastritis. Follow-up endoscopy was provided after 12 weeks to see polypectomy site after a course of Pantoprazole administration, and to define symptom-free time after polypectomy. Endoscopic removal of complicated gastric polyps should be considered at the time of initial diagnostic endoscopy. Endoscopic resection of polyps enables to determine the exact histopathologic type as well as to effectively treat symptomatic gastric outlet obstruction and anemia.

Dear Sir,

Hyperplastic polyps represent the most common type of gastric polyps [1]. They are characterised by proliferation of foveolar cells with variable amounts of edematous stroma [1]. When hyperplastic gastric polyps occur in the antrum, they may prolapse into the pyloric channel, consequently causing gastric outlet obstruction and chronic blood loss leading to iron deficiency anaemia [2].

The patient, a 40-year-old male, was referred to our clinic with intermittent nausea, vomiting and symptomatic anaemia for four months. Notable haematological indices were low haemoglobin levels of 9.6 g/dl and hematocrit levels of 35.8%, while after receiving two units of concentrated red blood cells, at discharge; they achieved levels of 15.2 g/dl and 42.3%, respectively. Esophagogastroduodenoscopy revealed a 3 cm antral pedunculated polyp, prolapsing into pylorus thus causing intermittent pyloric obstruction and anaemia. Submucosal injection of the saline-epinephrine solution [3] in the basis of pedunculus and endo-loop placement was performed before the polyp was removed (Fig. 1). Rapid urease test for Helicobacter pylori performed on endoscopically taken tissue samples resulted negative. Histological examination revealed a hyperplastic polyp without evidence of malignancy. No atrophy, metaplasia, dysplastic changes or Helicobacter pylori infection were detected in samples taken from the antrum and the corpus; however, the examination provided evidence for gastritis. Histologically, hyperplastic polyp had corkscrew appearance characterised by marked elongation of the pits with branching and cystic dilatation of foveolae (Fig. 2A, 2B).

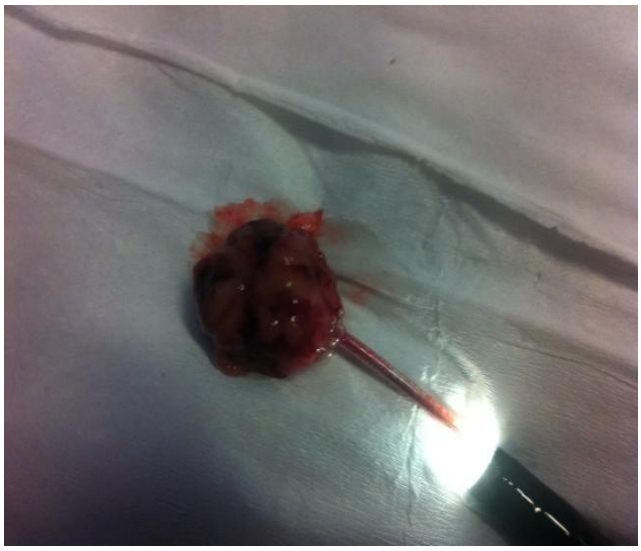
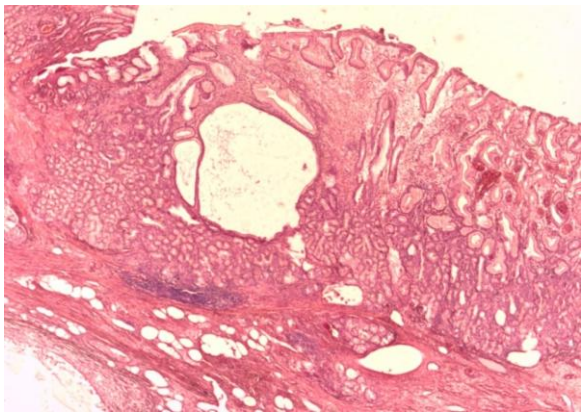


Figure 1: Gastric polyp after removal

Follow-up endoscopy was provided after 12 weeks to see polypectomy site after a course of Pantoprazole administration, and to define symptom-free time after polypectomy.

A)



B)

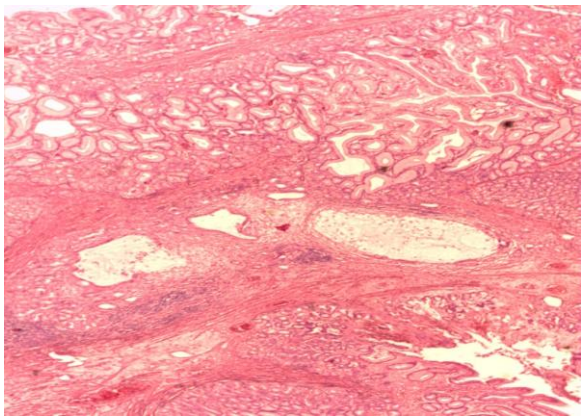


Figure 2: A) Irregular and dilated mucosal foveolae, lined by columnar epithelial cells are set in inflamed lamina propria (A), Hematoxylin and Eosin stain, 5x magnification). B) Smooth muscle fascicles traverse between the dilated glands, similar to what is observed in Peutz-Jeghers polyp (B), Hematoxylin and Eosin stain, 10x magnification)

Although most of these polyps are small (< 20 mm), large polyps may be encountered at endoscopy. The risk for complications is higher if the polyps exceed 20 mm in size [1]. Gencosmanoglu R. et al. [4] reported a similar case of a patient, in whom esophagogastroduodenoscopy revealed a prepyloric polyp causing intermittent gastric obstruction. Up to 80% of hyperplastic gastric polyps have been found to regress after eradication of H. pylori before endoscopic removal [5].

Endoscopic removal of complicated gastric polyps should be considered at the time of initial diagnostic endoscopy. Endoscopic resection of polyps enables to determine the exact histopathologic type as well as to effectively treat symptomatic gastric outlet obstruction and anaemia.

Ethics Committee Approval

The study protocol was approved by a local ethical committee of University Clinical Center of Kosovo.

Informed Consent

The participant gave written informed consent.

References

1. Jain R, Chetty R. Gastric hyperplastic polyps: a review. Dig Dis Sci. 2009; 54:1839-46. https://doi.org/10.1007/s10620-008-0572-8 PMid:19037727

2. Gencosmanoglu R, Sen-Oran E, Kurtkaya-Yapicier O, Tozun N. Antral hyperplastic polyp causing intermittent gastric outlet obstruction: case report. BMC Gastroenterol. 2003; 3:16. https://doi.org/10.1186/1471-230X-3-16 PMid:12831404 PMCid:PMC166166

3. Kosai NR, Gendeh HS, Norfaezan AR, Razman J, Sutton PA, Das S. Prolapsing Gastric Polyp Causing Intermittent Gastric Outlet Obstruction. Int Surg. 2015; 100:1148-52. https://doi.org/10.9738/INTSURG-D-14-00205.1 PMid:25578789 PMCid:PMC4587521

4. Uraoka T, Saito Y, Yamamoto K, Fujii T. Submucosal injection solution for gastrointestinal tract endoscopic mucosal resection and endoscopic submucosal dissection. Drug Des Devel Ther. 2009; 2:131-8. PMid:19920900 PMCid:PMC2761197

5. Ohkusa T, Takashimizu I, Fujiki K, et al. Disappearance of hyperplastic polyps in the stomach after eradication of Helicobacter pylori. A randomized, clinical trial. Ann Intern Med. 1998; 129:712-5. https://doi.org/10.7326/0003-4819-129-9-199811010-00006 PMid:9841603