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Medical Informatics

How to Verify Plagiarism of the Paper Written in Macedonian and Translated in Foreign Language?

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Key words: medical science; misconduct; plagiarism; Republic of Macedonia.

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

Abstract

AIM: The aim of this study was to show how to verify plagiarism of the paper written in Macedonian and translated in foreign language.

MATERIAL AND METHODS: Original article “Ethics in Medical Research Involving Human Subjects”, written in Macedonian, was submitted as an assay-2 for the subject Ethics and published by Ilina Stefanovska, PhD candidate from the Iustinianus Primus Faculty of Law, Ss Cyril and Methodius University of Skopje (UKIM), Skopje, Republic of Macedonia in Fabruary, 2013. Suspected article for plagiarism was published by Prof. Dr. Gordana Panova from the Faculty of Medical Sciences, University Goce Delchev, Shtip, Republic of Macedonia in English with the identical title and identical content in International scientific on-line journal "SCIENCE & TECHNOLOGIES", Publisher "Union of Scientists - Stara Zagora".

RESULTS: Original document (written in Macedonian) was translated with Google Translator; suspected article (published in English pdf file) was converted into Word document, and compared both documents with several programs for plagiarism detection. It was found that both documents are identical in 71%, 78% and 82%, respectively, depending on the computer program used for plagiarism detection. It was obvious that original paper was entirely plagiarised by Prof. Dr. Gordana Panova, including six references from the original paper.

CONCLUSION: Plagiarism of the original papers written in Macedonian and translated in other languages can be verified after computerised translation in other languages. Later on, original and translated documents can be compared with available software for plagiarism detection.

Introduction

Plagiarism of the papers is one of the biggest problems in publication ethics worldwide. Simple definition of plagiarism given in the Merriam-Webster Dictionary is: the act of using another person's words or ideas without giving credit to that person or the act of plagiarizing something [1].

Committee on Publication Ethics (COPE) provides advice to editors and publishers on all aspects of publication ethics and, in particular, how to handle cases of research and publication misconduct. It also provides a forum for its members to discuss individual cases. COPE does not investigate individual cases but encourages editors to ensure that cases are investigated by the appropriate authorities (usually a research institution or employer) [2].

COPE published flowcharts on what to do if you suspect plagiarism: (a) suspected plagiarism in a submitted manuscript [3] and (b) suspected plagiarism in a published manuscript [4].

If plagiarism was documented, then retraction of the plagiarised paper is mandatory. Retraction is a mechanism for correcting the literature and alerting readers to publications that contain such seriously flawed or erroneous data that their findings and conclusions cannot be relied upon. Unreliable data may result from honest error or from research misconduct. Retractions are also used to alert readers to cases of redundant publication (i.e. when authors present the same data in several publications), plagiarism, and failure to disclose a major competing interest likely to influence interpretations or

recommendations. The main purpose of retractions is to correct the literature and ensure its integrity rather than to punish authors who misbehave [5].

System for plagiarism detection and analysis was developed and installed from Ministry of Education and Science, Republic of Macedonia. This system provides an easy and efficient way of detecting documents that contain plagiarised parts from already published and presented documents. The system's goal is to provide an easy and intuitive interface for uploading documents. The students and researchers/scientists can easily upload their homework, bachelor's thesis, master's thesis, doctoral thesis (dissertation) and other published papers and documents. The system also provides a mechanism for comparing the uploaded documents with all the other documents that are already present in the system and to measure their originality, i.e. to detect if any of the content is already published [6]. Unfortunately the system is robust, language restricted, and closed database is limited to the documents deposited in the local website only.

The aim of this study was to show how to verify plagiarism of the paper written in Macedonian and translated in English or other languages.

Material and Methods

Original article “Ethics in Medical Research Involving Human Subjects”, written in Macedonian, was submitted as an assay-2 for the subject Ethics and published by Ilina Stefanovska, PhD candidate from the Iustinianus Primus Faculty of Law, Ss Cyril and Methodius University of Skopje (UKIM), Skopje, Republic of Macedonia in Fabruary, 2013. The assay was published and is publicly available at the website of Institute of Immunobiology and Human Genetics at the Faculty of Medicine, UKIM, Skopje, Republic of Macedonia [7].

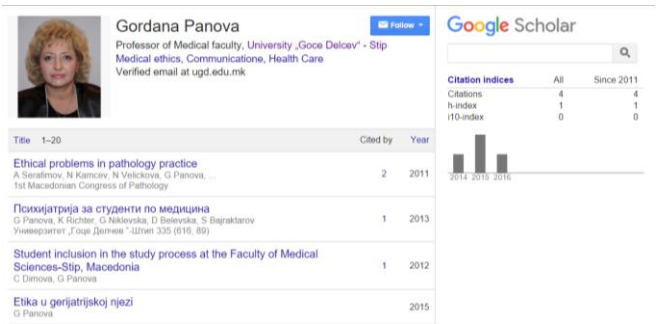


Figure 1: Google Scholar user profile of Prof. Dr. Gordana Panova from the Faculty of Medical Sciences, University Goce Delchev, Shtip, Republic of Macedonia

Two years later (in 2015 year) Prof. Dr. Gordana Panova from the Faculty of Medical Sciences, University Goce Delchev, Shtip, Republic of Macedonia (Figure 1) published a paper in English with the identical title and identical content in International scientific on-line journal "SCIENCE & TECHNOLOGIES", Publisher "Union of Scientists - Stara Zagora" [8, 9].

In order to compare two papers and verify the plagiarism, the original paper written in Macedonian [7] was translated in English by Google translator. Plagiarised paper, published in English [9], was converted to Word document. And finally, both Word document were compared with several softwares for checking plagiarism (Copyscape [10], WCopyfind [11], and Plagiarism Checker [12]).

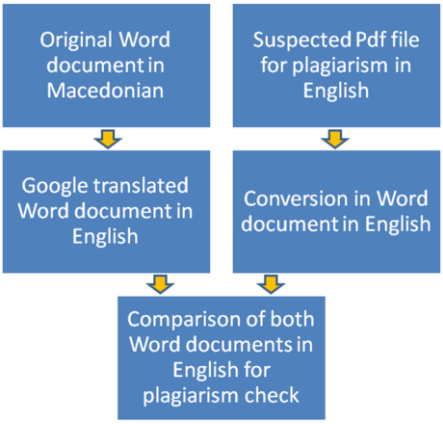


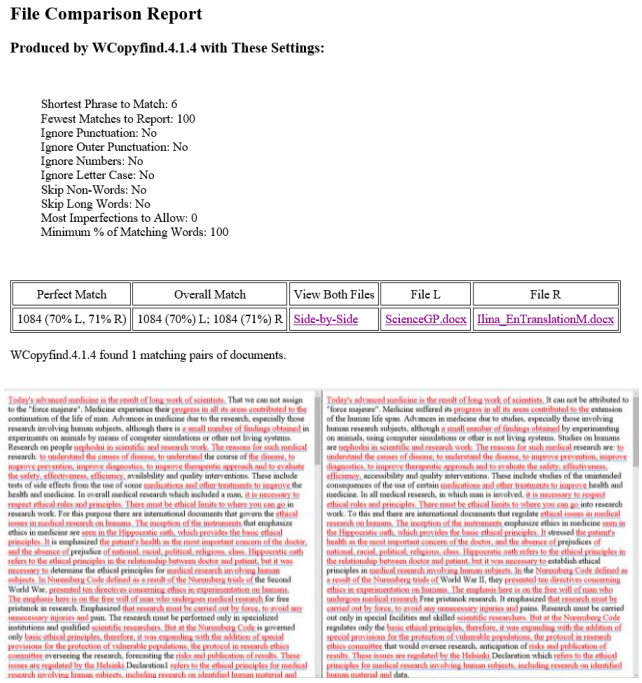
Figure 2: Several steps for checking plagiarism of the paper written in Macedonian and suspected paper translated in English

Results

An original and suspected paper were compared with Copyscape program and was found that they have almost identical number of words (1547 words in the original paper and 1574 words in suspected paper or Item 2). It was found that 1232 words were matched or 78% of the words were matched (Fig. 3).

Figure 3: Comparison of original and suspected articles as a Word document with the Copyscape program. Suspected paper (Item 2, on the right) contains 1574 words and was 78% matched

Comparison of the two papers with the WCopyfind program has shown that overall match was 71% between original and suspected paper. Identical sentences in both documents were labelled with red (Fig. 4).



File Comparison Report

Produced by WCopyfind.4.1.4 with These Settings:

Shortest Phrase to Match: 6
Fewest Matches to Report: 100
Ignore Punctuation: No
Ignore Outer Punctuation: No
Ignore Numbers: No
Ignore Letter Case: No
Skip Non-Words: No
Skip Long Words: No
Most Imperfections to Allow: 0
Minimum % of Matching Words: 100

Perfect Match	Overall Match	View Both Files	File L	File R
1084 (70% L, 71% R)	1084 (70%) L; 1084 (71%) R	Side-by-Side	ScienceGP.docx	Ilina_EnTranslationM.docx

WCopyfind.4.1.4 found 1 matching pairs of documents.

Figure 4: Comparison of original and suspected articles as a Word document with the WCopyfind program. Overall match (labelled with red) was 71% between original and suspected paper

The results of the comparison of the original and suspected papers analyzed with Plagiarism Checker program are shown in Fig. 5. Target (suspected) document was 82% duplicate with the Source (original) document. Identical sentences are labeled in red.

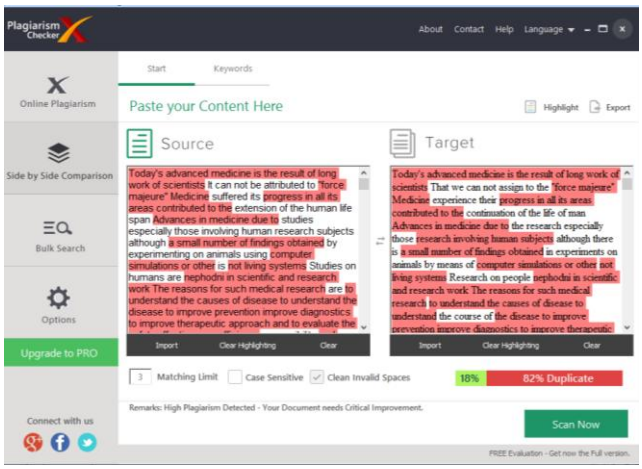


Figure 7: Comparison of original and suspected articles as a Word document with the Plagiarism Checker program. Target (suspected) document was 82% duplicate with the Source (original) document

Discussion

In this paper I presented how to verify plagiarism of document written in Macedonian and translated in English language. I translated original document written in Macedonian with Google Translator, converted plagiarised pdf file into Word document, and compared both documents with several programs for plagiarism detection. I found that both documents are identical in 71%, 78% and 82%, respectively, depending on the computer program I used for plagiarism detection.

It was obvious that original paper [7] was entirely plagiarised by Prof. Dr. Gordana Panova [9], including six references from the original paper. This is not isolated case, but rather serial case of plagiarism of Prof. Dr. Gordana Panova. Four retractions in Društvo defektologa Vojvodine, Novi Sad, Serbia from the same author were published [13, 14] and were reported in Retraction Watch [15].

Common characteristics of four retracted papers [16-19] from Prof. Dr. Gordana Panova are: original papers are parts or entire texts of students; original papers are written in Macedonian; plagiarised papers are translated in English; plagiarised papers are presented at the conferences outside Republic of Macedonia; plagiarised papers are published as a Book of Abstracts or Book of Papers without reviews (conference papers).

Research and publication misconduct may not be an isolated incident. In many cases, when serious misconduct comes to light, investigation of the researcher's earlier work reveals further problems. Therefore, when a researcher is found to have committed serious misconduct (such as data fabrication, falsification or plagiarism) the institution should review all the individual's publications, including those published before the proven misconduct took place. In such cases, it may be necessary to alert previous employers to enable them to review work carried out by the discredited researcher when working at their institution, to determine the reliability of publications arising from that work [20].

After this verification of plagiarised paper by Prof. Dr. Gordana Panova, I expect two appropriate reactions, one from the Editor-in-Chief of the International scientific on-line journal "SCIENCE & TECHNOLOGIES", Publisher "Union of Scientists - Stara Zagora", and another one from the Dean of the Faculty of Medical Sciences, University Goce Delchev, Shtip, Republic of Macedonia.

Editor-in-Chief of the International scientific on-line journal "SCIENCE & TECHNOLOGIES" should follow the Retraction guidelines from the Committee on Publication Ethics [5]. In short, he/she will publish a letter for retraction of the plagiarized paper [for examples of this see reference 21] and

label all pages of the published paper with the watermark "Retracted" [for examples of this see reference 22].

Dean of the Faculty of Medical Sciences, University Goce Delchev, Shtip, Republic of Macedonia should create Independent Body of experienced professors, from the fields of medicine and ethics, with the task to analyse all published papers of Prof. Dr. Gordana Panova for possible plagiarism and/or other misconducts [3-5, 20].

This comparison of the original paper written in Macedonian with suspected paper translated into foreign languages can be modified with back translation. Namely, suspected paper written in any language can be translated and compared with the original paper written in the identical language. I suppose that by using these methods, detection of plagiarized papers in Macedonia will be possible and percentage of plagiarized papers will increase very much. It is also necessary to support programmers to implement these possibilities of translation and back translations into the actual computer programs fro plagiarism detection.

In conclusion, plagiarism of the original papers written in Macedonian and translated in other languages can be verified after computerised translation in other languages. Later on, original and translated documents can be compared with available software for plagiarism detection.

Acknowledgement

I would like to thank Prof. Dr. Vladimir Trajkovski, Editor-in-Chief of the Journal for Special Education and Rehabilitation, for the information about the possible plagiarism of the paper “Ethics in Medical Research Involving Human Subjects” from the PhD candidate Ilina Stefanovska (Legal Studies).

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Basic Science

The Study of Cellular and Molecular Physiological Characteristics of Sperm in Men Living in the Aral Sea Region

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Key words: Aral Sea; ASF; RNA; DNA; H1; H2A; H3; H4.

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Abstract

BACKGROUND: Extreme environmental situation in the Aral crisis has caused a massive chemical pollution of the territory for decades with high doses of pesticides, herbicides. Discharge of industrial waste into the rivers that feed the Aral Sea has lead to the development of various pathological processes in the human body, as well as disruption of reproductive function in young men.

AIM: To evaluate the performance of molecular cellular changes in the sperm of men under the influence of dust and salt aerosols in Aral Sea region.

MATERIAL AND METHODS: Clinical and laboratory studies were conducted in men 5 settlements (Aralsk-city, v. Aiteke-Bi, v. Zhalagash, v. Zhusaly, v. Shieli). We have studied male ejaculate obtained after 4-5 days of abstinence, and placed it in a warm tube with a glass stopper. On the investigation proceeded ejaculate within 20-30 minutes after its preparation, during which time he was subjected to liquefaction. Isolation and quantification of ASF, RNA, DNA, and determining the fraction of histones in sperm was performed by the method of Markusheva and Savina.

RESULTS: It was found that the value of ASF in the semen of men living in the zone of ecological disaster higher compared with the values of parameters in men living in the area of environmental crisis, and this trend is observed in all age groups. The study of circulating extracellular DNA and RNA in the sperm of men registered their decline with a corresponding increase of acid precursors that can be attributed to the degradation of nucleic acids under the influence of negative factors in the complex area of ecological trouble. Also, according to a study in men residing in the areas of environmental catastrophe at the age of 18-29 years, found an increased content of the H1 histone H2A lower total fraction, H3, H4 - and a sharp increase in histone H2B content - histones.

CONCLUSIONS: Men living in environmentally disadvantaged areas of Kyzylorda region under the influence of dust and salt aerosols and other toxicants leads to disruption of the reproductive function in men.

Introduction

The Aral Sea crisis is recognized as one of the global environmental problems of our time. Extreme ecological situation in the Aral crisis caused a massive chemical pollution of the territory for decades with high doses of pesticides, herbicides; discharge of industrial waste into the rivers that feed the Aral Sea. Due to the drying of the Aral Sea occurred arid areas, climate change and soil salinization, breach of public water supply.

Existing environmental trouble in the region is reflected in the health of the population in almost all areas of the Aral Sea region is marked increase in the number of diseases of the endocrine, nervous, digestive and urinary systems [1]. Numerous studies conducted by scientists of Kazakhstan shows that the state of health of the population in recent decades, the Aral Sea region continues to deteriorate [2].

Previous studies on a range of health, social and environmental problems of the Aral Sea region is mainly devoted to the study of the sanitary-epidemiological situation and the health of the population only in the zone of ecological disaster - the Aral and Kazalinsk areas. Most research in this area relate to aspects of child health and reproductive health of women [3-5].

The problem of male infertility in recent years is of particular medical and social importance due to the increased incidence of sexual organs, increases in congenital malformations caused by environmental pollution, extensive and uncontrolled use of drugs [6-8] and has not been studied in men of reproductive age living in the area ecological catastrophes and ecological crisis of Kyzylorda region.

Therefore, the purpose of work is to study the negative impact of environmental factors on the stages of spermatogenesis in men of reproductive age living in the zone of ecological disaster and environmental crisis Kyzylorda region.

Material and Methods

The studies were conducted in the Laboratory of the Department of Molecular Biology and Medical Genetics, KSMU.

Clinical and laboratory studies were conducted in men 5 settlements (Aralsk-city, v. Aiteke-Bi, v. Zhalagash, v. Zhusaly, v. Shieli). They were surveyed 1,010 people the same age group (18-49 years). The criterion for inclusion is the stay of an adult in the Aral Sea area is not less than 5 years, employment in occupations with the hazard no more than 2 classes.

We have studied male ejaculate obtained after 4-5 days of abstinence, and placed it in a warm tube with a glass stopper. On the investigation proceeded ejaculate within 20-30 minutes after its preparation, during which time he was subjected to liquefaction. Macroscopic and microscopic examination of the semen was performed by the method of V. Dolgov. Isolation and quantification of ASF, RNA, DNA, and determining the fraction of histones in sperm was performed by the method of L. Markusheva and M.I. Savina [7].

Morphological studies of ejaculate of surveyed persons living in the Aral Sea region held on a light microscope MICROS model MC 20 equipped with a digital camera eyepiece OSMOSIS 14000KPA. Molecular and cellular studies were performed on a digital spectrophotometer UV-VIS PD-303UV.

Results

Freshly ejaculate initially evaluated by external characteristics, our findings showed that, in all the surveyed men living in the areas of environmental disasters of Kyzylorda region, there are changes in the integral characteristics of the ejaculate. So, almost all the investigated areas there is a high percentage of individuals with a transparent color ejaculate in all age groups (21 to 42%), indicating a reduced amount of sperm in the ejaculate.

Ejaculate healthy male should have a characteristic odor; no smell indicates a lack of secretion of the prostate. The presence or absence of odor was observed in the surveyed people in all 5 areas. The maximum number of persons to the lack of odor ejaculate was noted in Aiteke Bi in Aralsk (37.4% and 41.7%).

Also, the persons surveyed all 5 groups observed some increased ability to ejaculate liquefaction, according to the WHO the duration of thinning in men ejaculate normally ranges from 15 to 30 minutes, averaging 29.7 minutes. The highest percentage of people over time, liquefaction of the ejaculate to 2 minutes was observed in living in Aralsk (61.6%) and up to 5 minutes in Aiteke-Bi (39.2%), among the highest number of men with liquefaction time less than 15 minutes noted in Zhusaly (36%) (Table 1).

Table 1: Time ejaculate liquefaction of the surveyed persons

Groups	Liquefaction time. min			
	2 min	5 min	15 min	15-30 min
18-29 years old				
Norm According to the World Health Organization	-	-		100
v. Ayteke Bi	48.3%	16.7%	35%	-
Aralsk- city	52.5%	17%	30.5%	-
v. Zhusaly	35%	28%	37%	-
v. Zhalagash	27.4%	28.6%	42%	-
v. Shieli	38.2%	30.8%	31%	-
30-39 years old				
Norm According to the World Health Organization	-	-		100
v. Ayteke Bi	30.2%	59.8%	10%	-
Aralsk- city	49.6%	24.4%	26%	-
v. Zhusaly	33%	18%	49%	-
v. Zhalagash	26%	51.3%	22.7%	-
v. Shieli	33.6%	29.4%	37%	-
40-49 years old				
Norm According to the World Health Organization	-	-		100
v. Ayteke Bi	41.3%	39.2%	19.5%	-
Aralsk- city	61.6%	25.2%	13.2%	-
v. Zhusaly	35.7%	28.3%	36%	-
v. Zhalagash	37.1%	29.9%	33%	-
v. Shieli	49.2%	26.4%	24.4%	-

According to the regulations, sperm count below 60 million in 1 ml shows a decrease in fertility ejaculate. According to the literature found that healthy male sperm count in 1 ml of ejaculate varies between 70 million in 1 ml and above. In all five groups surveyed persons of all ages observed low sperm count, that is, less than 70 million in 1 ml of ejaculate. A comparative analysis of not prolific ejaculate had been done in all age groups of surveyed men. It was found that relatively high values of not prolific semen found in the age group 18-29 years in all five districts of Kyzylorda region, the highest percentage observed in the zone of ecological disaster - in Aralsk (84.7%).

In addition, from the results of research showed the maximum increase in the number of mobile forms of sperm in men v. Shieli, the maximum amount of sperm ejaculate with reduced fertility

observed in v. Zhusaly and with significantly reduced fertility in v. Aiteke Bi. Fixed forms of spermatozoa observed in Aralsk and v. Aiteke Bi.

The study examined all men living in the Kyzylorda region, there are deviations from the norm WHO morphophysiological characteristics of sperm.

In assessing the pathological forms of sperm attach special importance to the changes of the head, which for the fertility of an ejaculate are more important than changes in the intermediate portion and the tail. In the area of environmental disaster (v. Aiteke Bi and c. Aralsk) of the men surveyed, depending on the age of the following changes are observed: deformation of the head and the body of sperm abnormality found in men aged 18 to 29 years (up to 26.0% and 18 %, respectively). When comparing the pathological forms of sperm in men living in the zone of ecological crisis, a high percentage of abnormal deformation of the head and the body of sperm were observed in men aged 18-39 years in villages Zhalagash and Shieli.

Analysis of clinical and laboratory research showed that men living in ecologically unfavorable conditions Kyzylorda region there are persistent violations of spermatogenesis. Of particular note is that a violation of the morphological and physiological characteristics of sperm is most significant in young adults. The picture of these disorders manifested itself in the form of increasing the number of fixed sperm cells, the appearance of atypical forms of the deformation of the head and the doubling of the axoneme.

We performed molecular genetic studies of acid-soluble fractions (ASF), extracellular nucleic acids (RNA, DNA) and histone-similar fractions (H1, H2A, H3, H4) in the ejaculate of men in the zone of ecological disaster Kyzylorda region (Table 2 and 3).

Table 2: Indicators of extracellular nucleic acids in semen of men (M ± m)

	Years	ASF, conventional units	RNA, conventional units	DNA, conventional units
The zone of ecological disaster (v. Ayteke Bi Aralsk-city)	18-29 years	0.72 ± 0.13	0.73 ± 0.12	0.49 ± 0.058
	30-39 years	0.68 ± 0.087	0.88 ± 0.082	0.57 ± 0.04
	40-49 years	0.63 ± 0.09	0.76 ± 0.094	0.46 ± 0.063
The zone of ecological crisis (v. Zhosaly, v.Zhalagash, v.Shieli)	18-29 years	0.58 ± 0.4	3.13 ± 2.61**	0.42 ± 0.034*
	30-39 years	0.63 ± 0.05*	0.7 ± 0.053**	0.56 ± 0.054
	40-49 years	0.46 ± 0.06*	0.6 ± 0.048	0.69 ± 0.09*

Note: reliability, compared with groups of different ecological zones, p <0.001, ** - significant in comparison with groups of different ecological zones, p <0.001

It was found that the value of ASF in the semen of men living in the zone of ecological disaster higher compared with the values of parameters in men living in the area of environmental crisis, and this trend is observed in all age groups. The study of circulating extracellular DNA and RNA in the sperm of men registered their decline with a corresponding increase of acid precursors that can be attributed to the degradation of nucleic acids under the influence of negative factors in the complex area of ecological trouble. Also, according to a study in men residing in the areas of environmental catastrophe at the age of 18-29 years, found an increased content of the H1 histone H2A lower total fraction, H3, H4 - and a sharp increase in histone H2B content - histones.

Men living in zones of ecological crisis of all age groups showed an increased content of total fraction of H2A, H3, H4 and H2B histones.

Table 3: Indicators of histones in sperm in men (M ± m)

	Years	H_1. ml/l	H_2A,H_3,H_4, ml/l	H_2B, ml/l
The zone of ecological disaster (v. Ayteke Bi Aralsk-city)	18-29 years	0.83 ± 0.18*	0.81 ± 0.032	1.59 ± 0.084*
	30-39 years	0.69 ± 0.062	0.51 ± 0.059*	0.83 ± 0.07*
	40-49 years	0.74 ± 0.036	0.71 ± 0.091	1.062 ± 0.074
The zone of ecological crisis (v. Zhosaly, v. Zhalagash, v. Shieli)	18-29 years	0.60 ± 0.035	0.61 ± 0.030	0.61 ± 0.029
	30-39 years	0.54 ± 0.046	0.61 ± 0.039	0.66 ± 0.039
	40-49 years	0.56 ± 0.050	0.68 ± 0.036	0.72 ± 0.046

Note: - reliability, compared with groups of different ecological zones, p <0.001, ** - significant in comparison with groups of different ecological zones, p <0.001

Discussion

When comparing the results of the men living in the zones of ecological disaster and environmental crisis in all age groups may be noted the high content of H1 histone-similar factions in individuals living in v. Zhusaly, v. Zhalagash and v. Shieli.

According to a study in the sperm of men who live in the zones of ecological disaster, there is a different dynamics of the studied parameters in different areas, which determines the extent of metabolic abnormalities in sperm.

Thus, the violation of morphological parameters of spermatogenesis was observed in all the surveyed people in the zone of ecological crisis and environmental disasters. These changes lead to the development of pathological processes at the molecular and cellular level. This indicates a change of extracellular nucleic acids and histones in the semen of the surveyed men living in environmentally disadvantaged areas of Kyzylorda region. In this regard, we can assume that the dust and salt aerosols and ecotoxicants adversely affect the reproductive health of the local male population. Our assumption is confirmed by the results of other domestic researchers. In recent years, the world in general and in particular in the Aral Sea region, much attention is paid to the impact on the human body of heavy metals, especially lead [9]. The destruction of natural ecosystems, degradation of flora and fauna and as a consequence of the unfavorable ecological situation caused substantial harm to the health of the population [10]. The deterioration of the environment impact on health status [2].

Pathology affects the reproductive system

disorders of the immune system and the biochemical, pathophysiological changes.

The problem of male infertility in recent years is of particular medical and social importance all over the world.

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Basic Science

Expression of OCT-4 and SOX-2 in Bone Marrow-Derived Human Mesenchymal Stem Cells during Osteogenic Differentiation

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Key words: pluripotency markers; human mesenchymal stem cells; osteodifferentiation.

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Abstract

AIM: Determine the levels of expression of pluripotency genes OCT-4 and SOX-2 before and after osteogenic differentiation of human mesenchymal stem cells (hMSCs).

METHODS: Human MSCs were derived from the bone marrow and differentiated into osteoblasts. The analyses were performed on days 0 and 14 of the cell culture. *In vitro* differentiation was evaluated due to bone markers – alkaline phosphatase (AP) activity and the messenger RNA (mRNA) expression of AP and bone sialoprotein (BSP). The OCT-4 and SOX-2 expression was evaluated at mRNA level by real-time qPCR and at protein level by immunocytochemistry.

RESULTS: In vitro cultures on day 14 showed an increase in AP activity and upregulation of AP and BSP gene expression. OCT-4 and SOX-2 in undifferentiated hMSCs on day 0 is detectable and very low compared to tumor cell lines as a positive control. Immunocytochemistry detected OCT-4 in the cell nuclei prior (day 0) and post differentiation (day 14). On the same time points, cultures were negative for SOX-2 protein.

CONCLUSION: Messenger RNA for pluripotency markers OCT-4 and SOX-2 isolated from hMSCs was less present, while OCT-4 protein was detected in cell nuclei prior and post differentiation into osteoblast lineage.

Introduction

Molecular mechanisms regulating proliferation and multipotency of MSCs have not been well understood. In contrast, the molecular basis of proliferation and multipotency of embryonic stem cells (ESCs) is in the focus of research for a long time and it is described in detail. Expression of the three transcription factors, OCT-4, SOX-2 and NANOG, is essential for the major properties of stem cells, self-renewal and pluripotency [1, 2].

OCT-4 belongs to the family of Pou-domain transcriptional factors, and it is found in developing embryos, developing endoderm as well as developing neurectoderm [3–5]. OCT-4 together with SOX-2 up-regulates the expression of *NANOG* [6]. OCT-4 is a dose-depended pluripotency regulator that controls lineage commitment of ESCs [7]. *OCT-4* gene is expressed in tumor cells as well, but has not been found in differentiated tissues [8–10].

OCT-4 expression has been confirmed in bone marrow (protein and mRNA level) [11], dental pulp [12], heart, liver [13] and adipose tissue-derived stem cells [14].

SOX-2 is a member of the SRY-related HMG-box (SOX) transcription factor family with a diverse role in stem cell potency and maintenance, embryonic development and cancer [15–18]. It is closely co-regulated alongside core pluripotency factors OCT-4 and NANOG in ESCs, embryonic carcinoma cells (ECCs) and induced pluripotent stem cells (iPSCs)

[18–20]. Recently, SOX-2 has been implicated in the maintenance and differentiation of adult stem cells. *SOX-2* expression has been reported in bone marrow, neuronal tissues and sensory epithelia [11, 21].

It has been speculated that the same set of transcription factors plays an important role in the maintenance of multipotency and self-renewal of adult stem cells. Adult stem cells are present within the tissues with the purpose to repopulate them after injury or physiological loss. Human MSCs derived from bone marrow are progenitors that can differentiate into cells of multilineage, including osteogenic, chondrogenic, adipogenic and myogenic lineages [22–24]. Human MSCs, like human ESCs, are depended on fibroblast growth factor (FGF) to maintain self-renewal and pluripotency [25]. FGF inhibits differentiation by bone morphogenetic protein (BMP) signaling inhibition and sustain expression of OCT-4, SOX-2 and NANOG pluripotency associated genes [26]. Upon differentiation expression of pluripotency transcriptional factors should be downregulated [27]. However, there is a controversy among different research groups regarding the expression of pluripotency regulators in adult tissues [28]. Human MSCs exhibit heterogeneous characteristics with regard to morphology, proliferation rate and secreted factors as a consequence of variable gene expression pattern. Difference in gene expression is dependent of their intrinsic heterogeneity and culture conditions [29]. In particular, *ex vivo* expansion of hMSCs is used for cell therapy and tissue engineering. Therefore it is an important to characterize cells cultured *in vitro* for the expression of genes responsible for self-renewal ability in order to predict better their behavior.

In this study, we evaluated the expression of *OCT-4* and *SOX-2,* in cultured bone marrow-derived mesenchymal stem cells of human origin. There are no universal stem cell markers that are expressed in all types of stem cells [30]. For that reason it is crucial to determine specific stem cell markers for cells that can be used for clinical research, such are hMSCs. By determining the stem cells markers expression we would be able to estimate the quality of stem cells that must be associated with cell therapy efficacy. Therefore, we have examined changes of stem cell markers expression during osteodifferentiation of hMSCs. Here we report effects of osteodifferentiation on OCT-4 and SOX-2 mRNA and protein expression.

Materials and Methods

Appropriate regulatory approval from Medical Ethics Committee of Clinic for Traumatology Zagreb was taken before initiating the experiments.

Human mesenchymal stem cells, cell lines and culture conditions

The hMSCs were isolated and propagated using previously described methods (Romanov et al. 2005). Two human cancer cell lines were used in the experiment: glioma cell line A1235 and liver cell cancer line HepG2. The cells were kindly provided by Prof. M. Matulić, University of Zagreb, The Faculty of Science (Croatia). Both cell lines were cultured in a medium which contained Dulbecco's Modification of Eagle's Medium (Sigma-Aldrich) supplemented with 10% FBS (Gibco) and penicillin (100 U/ml)/streptomycin (100 µg/ml) (Sigma-Aldrich) at 37°C in a humidified atmosphere containing 5% CO_2.

In vitro differentiation of hMSCs into osteogenic lineage

Three or four passages of BMSCs were adjusted to a concentration of 47 500 cells/well in a 6-well plate (Corning) in proliferation medium DMEM supplemented with 10% FBS, penicillin/streptomycin (P/S) and 10 ng/ml FGF2. The cells were cultured in a humidified incubator (37°C, 5% CO_2) with renewal of the culture medium every 3 days. The cells were tested for their known ability to differentiate into osteogenic lineages by growing them in osteogenic induction medium when the plates were 100% confluent. Culture media was then replaced with osteogenic induction medium (αMEM 10% FBS + P/S + 50 µg/ml ascorbic acid + 4 mM β-glycerophosphate + 1 µM dexamethasone) after 24 hours and cells were maintained 14 more days under differentiating conditions. All the osteogenic induction factors were purchased from Sigma-Aldrich. Medium was replaced every 2 days. Process of differentiation was held for 14 days, and day 0 was marked as the day when differentiation medium was added.

Determination and quantification of alkaline phosphatase (ALP) activity

After osteogenic differentiation, cells were washed in PBS, briefly fixed with citrate-acetone-formaldehyde fixative and ALP expression was assessed using the ALP staining kit (86R, Sigma-Aldrich) according to the manufacturer's instructions. The results were recorded using a scanner. The alkaline phosphatase activity was assessed 14 days after osteogenic induction. Cell layers were twice washed in PBS and the activity was measured by using p-nitrophenyl phosphate (Sigma-Aldrich) as a substrate. Briefly, cells were incubated with p-nitrophenyl phosphate in substrate buffer (50 mM glycin, 1mM $MgCl_2$, pH 10.5) at room temperature for 10 min. Supernatant was mixed with 1 ml 1M NaOH and absorbance at 405 nm was recorded [32]. Each experiment was done in triplicates and repeated at least three times.

RNA extraction and real-time quantitative PCR

Total RNA was collected from 14 days old osteoblast cultures as well as from undifferentiated hMSCs and two human cancer cell lines using TRIzol reagent (Invitrogen Life Technologies) according to manufacturer's instructions. Briefly, cell layers were washed with PBS and scrapped and homogenized in 1 ml TRIzol. RNA was treated with DNase I (Invitrogen) to remove genomic DNA and 3 μg of total RNA was reverse transcribed to cDNA using Superscript III First-Strand Synthesis System for RT-PCR (Invitrogen) according to the manufacturer's instructions. Reverse transcription was performed in thermomixer (Eppendorf) at following conditions: 10 minutes at 20°C, 1 hour at 42°C, 5 minutes at 99°C and 5 minutes at 5°C. Gene expression levels were determined by real time-qPCR using Power SYBR Green Mastermix (Applied Biosystems). Primers (Sigma-Aldrich) used to determine gene expression levels were presented in Table 1. The PCR reaction conditions were as follows: 10 minutes at 95°C for 1 cycle, 15 seconds at 95°C and 1 minute at 60°C for 40 cycles. Quantitative RT-PCR was performed on 7500 Fast PCR system (Applied Biosystems). Expression levels were normalized to *β-actin*. Relative expression of target genes was calculated using the ΔΔCt method. Stem cell markers expression in differentiated cultures (day 14) was normalized to 1 as well as *BSP* and *AP* expression in 14 days old hMSCs cultures (day 14).

Table 1: Primers used for quantitative real-time PCR

Primers	forward	reverse
human POU5F1	5'-GATCACCCTGGGTATACAC-3'	5'-GCTTTGCATATCTCCTGAAG-3'
human SOX2	5'-ATAATAACAATCATCATCGGCGG-3'	5'-AAAAAGAGAGAGGCAAACTG-3'
human IBSP	5'-GGAGACTTCAAATGAAGGAG-3'	5'-CAGAAAGTGTGGTATTCTCAG-3'
human ALPL	5'-TCTTCACATTTGGTGGATAC-3'	5'-ATGGAGACATTCTCTCGTTC-3'
human ACTB	5'-GACGACATGGAGAAAATCTG-3'	5'-ATGATCTGGGTCATCTTCTC-3'

Immunocytochemistry

Cells were seeded in 35 mm Petri dishes. Medium was removed, cell layers were rinsed in ice cold PBS, fixed in 4% paraformaldehyde for 15 min and washed in PBS. Cells were permeabilized with 0.25% Triton-X-100 (Packard) and blocked with 1% bovine serum albumin (BSA) in PBS for 30 min. Used primary antibodies were ab19857 rabbit polyclonal anti-Oct4 (Abcam) diluted 1:100, ab97959 rabbit polyclonal anti-Sox2 (Abcam) diluted 1:100 in 1% BSA and ab21624 rabbit polyclonal anti-Nanog (Abcam) diluted 1:100. All antibodies were applied for 1 hour at room temperature. Secondary antibody (Alexa Fluor® 488 Donkey Anti-Rabbit IgG (H+L), Invitrogen) were applied for 1 hour at room temperature in a dark room. Cells were further counterstained for 1 min with Hoechst solution (1μg/ml) and imaged with CCD camera on Olympus BX51.

Statistical analysis

A statistical analysis of the data was performed using a one-way ANOVA analysis of variance followed by a Duncan test using the *STATISTICA 12* program (StatSoft, Inc.,). Statistical significance was set at $p < 0.05$.

Results

Bone marrow-derived human mesenchymal stem cells were propagated *in vitro* and osteogenic differentiation was induced. The level of differentiation was assessed by analysis of bone markers. Early marker of osteoblast differentiation is the activity of alkaline phosphatase (AP). The AP activity was evaluated before and after osteogenic differentiation by cytochemical staining. The quantitative method where p-nitrophenil phosphate was used as a substrate, showed 6-fold increase in AP activity on day 14 compared to undifferentiated culture (Figure 1A).

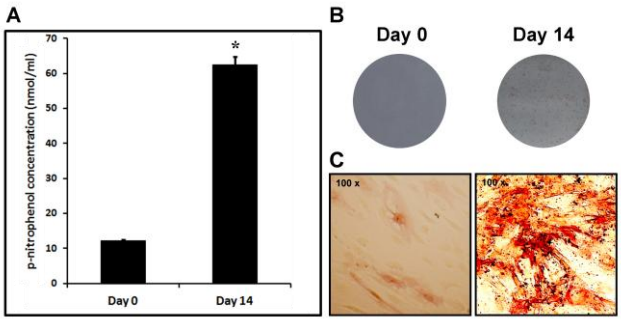

*Figure 1: High alkaline phosphatase activity is an indication of successful differentiation of hMSCs into osteoblast after 14 days of osteoinduction. The alkaline phosphatase (AP) activity was measured by using p-nitrophenyl phosphate as a substrate. This quantitative method has shown higher alkaline phosphatase activity in differentiated hMSC cultures (Day 14) than in undifferentiated cultures (Day 0) (A). This result has been confirmed by AP cytochemical staining (B, C). Red dye deposits indicate sites of alkaline phosphatase activity in hMSC cultures on day 14 and just a few undifferentiated hMSCs were red. The data are expressed as the means + SD of triplicate determination (*P<0.05).*

These findings are in coherence with results obtained from the cytochemical staining of day 0 and day 14 cultures (1B, 1C). Further analysis of differentiation included quantitative analysis of mRNA levels by real-time qPCR. We analyzed expression of two genes, alkaline phosphatase (AP) as an early marker of osteoblast differentiation and bone sialoprotein (BSP) as an intermediate to late marker of osteoblast differentiation with the peak of expression on day 14. The 5-fold increase of AP mRNA and 10-fold increase of BSP mRNA confirmed that MSCs differentiated into osteoblast lineage after 14 days of osteogenic induction (Figure 2).

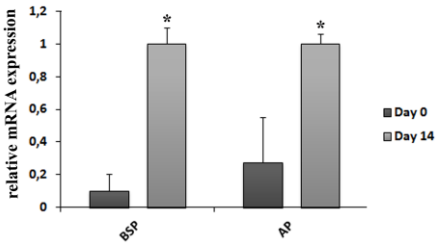

*Figure 2: Relative mRNA expressions of osteogenic markers detected by real-time quantitative PCR on day 0 and day 14. The relative gene expression of BSP and AP was analyzed by ΔΔ cycle threshold method and the values were normalized to β-actin expression. Those values were then normalized to hMSCs after 14 day of osteoinduction. Data are represented as an average of three independent patient samples and error bars represent mean value + SD (*P<0.05). Abbreviations: BSP, bone sialoprotein; AP, alkaline phosphatase; hMSCs, human Mesenchymal Stem Cells*

To estimate the expression of pluripotency genes, we analyzed *OCT-4* and *SOX-2* mRNA levels by real-time qPCR prior and post differentiation of human MSCs. To confirm data on protein level, we performed immunostaining for OCT-4 and SOX-2. Data were compared with positive controls, tumor cell lines positive for OCT4/SOX2 expression, glioma cell line A1235 and liver cell cancer line HepG2.

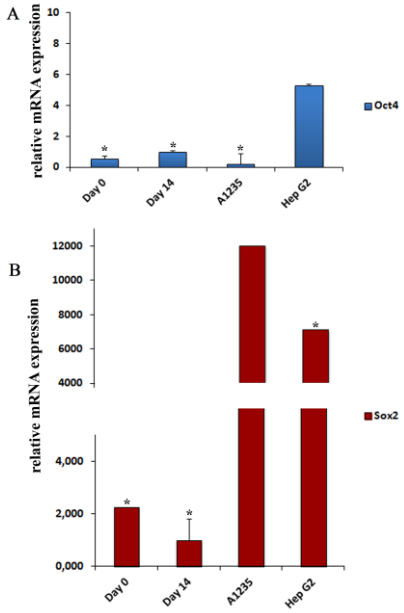

*Figure 3: Expression levels of OCT-4 and SOX-2 genes in hMSCs after 14 days of osteoinduction, undifferentiated hMSCs and two human cancer cell lines A1235 and HepG2 detected by real-time qPCR. OCT-4 is expressed in undifferentiated hMSC (Day 0) but expression of OC-T4 gene does not go down after osteoinduction on day 14. Relative mRNA expression of OCT-4 in Hep G2 cell line was 9.9 x more expressed than in undifferentiated hMSCs (A). Transcription factor SOX-2 is expressed in undifferentiated hMSCs and its expression goes down after osteoinduction on day 14. Relative mRNA expression of SOX-2 in Hep G2 cell line was 5321.9 x more expressed than in undifferentiated hMSCs while in glioblastoma A1235 cell line was 3169.8 x respectively (B). The quantity of gene expression was normalized to β-actin to determine the quantitative differences. Data are represented as an average of three independent patient samples and error bars represent mean value + SD. In the columns marked with asterisk * the mean values are significantly different then control (Hep G2) (A) or A1235 and Hep G2 (B), respectively, according to Duncan test (*P<0.05)*

Expression of *OCT-4* mRNA was detected prior the differentiation and it unexpectedly increased 2-fold after differentiation. However, the levels of expression are very low when compared to OCT-4 positive tumor cell line HepG2 (Figure 3A). To confirm our findings on protein level we performed immunostaining and protein OCT-4 was detected in cultures before and after differentiation (Figure 4A). OCT-4 signal was localized in the cell nuclei as expected for transcription factor (Figure 5).

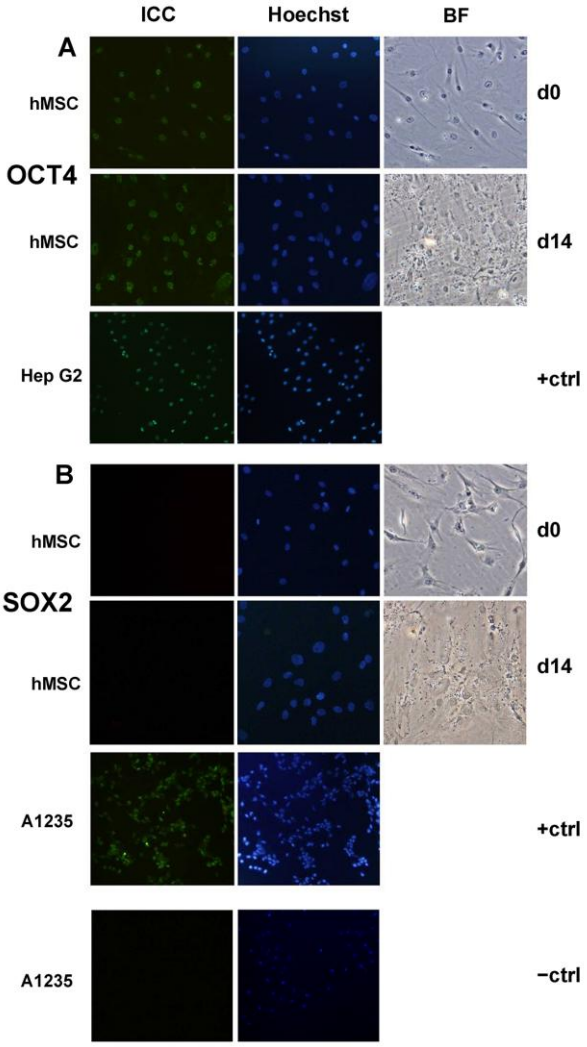

Figure 4: Immunostaining for OCT-4 did not show any changes in OCT-4 protein level in osteo-differentiated hMSC cultures compared to undifferentiated. Osteoblast and undifferentiated hMSCs were both positive for OCT-4 expression and had a nuclear localization of OCT-4 (A). Immunofluorescence staining for SOX-2 did not confirm levels of SOX-2 protein in osteo-differentiated hMSCs (Day 14) nor in undifferentiated hMSCs (Day 0) (B). Liver cell cancer line HepG2 has been used as positive control for OCT-4 expression. Glioblastoma A1235 cell line has been used as positive control for SOX-2 expression and for no primary antibody control (-ctrl) as well. Nuclei were stained with Hoechst (200x, CCD camera on Olympus BX51). Abbreviations: ICC, immunocytochemistry; BF, bright field; d0, the day when differentiation medium was added; d14, 14th day of osteogenic differentiation

Expression of *SOX-2* mRNA was also detected in undifferentiated MSCs and after

differentiation at day 14 was downregulated. Overall expression of *SOX-2* was extremely low when compared to positive control cell lines A1235 and HepG2. At protein level, SOX-2 was undetectable before and after differentiation. Therefore, findings at protein level did not reflect findings on mRNA level.

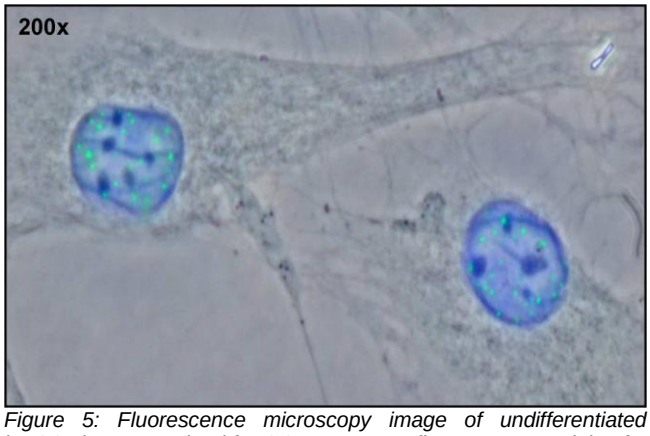

Figure 5: Fluorescence microscopy image of undifferentiated hMSCs immunostained for OCT-4. Immunofluorescence staining for Hoechst (blue) and OCT-4 (green) indicates a nuclear localization of OCT-4 protein (20x, CCD camera on Olympus BX51)

Discussion

Human mesenchymal stem cells (hMSCs) provide an excellent source of multipotent progenitor cells that are present in bone marrow as well as in most of connective tissues. Due to their proliferation and differentiation capacity, they are able to differentiate toward multiple mesodermal lineages [13]. In this work, we have examined hMSCs osteogenic potential. Human MSCs were differentiated into osteoblasts by standardized method where hMSCs are treated with osteogenic inductors, ascorbic acid, β-glycerophosphate and dexamethasone [33]. Here, we have investigated and compared the change of osteogenic markers expression and the alteration of stem cell markers expression OCT-4 and SOX-2 during osteogenic differentiation of hMSCs.

We have followed the change of early and late osteogenic markers. Alkaline phosphatase was used as an early osteogenic marker and bone sialoprotein as late marker. Our results showed that on day 14 all observed osteogenic markers are highly expressed and hMSCs were differentiating towards osteoblast lineage. As previously described [34] we have also observed a few distinct alkaline phosphatase positive cells on day 0. In this report, we have investigated weather two stem cell markers, OCT-4 and SOX-2, known to regulate potency and self-renewal of embryonic stem cells, were expressed in hMSCs and how their expression changed after osteoinduction. OCT-4 and SOX-2 mRNA expression was examined at mRNA level by qPCR and at protein level by immunocytochemistry. Real-time qPCR revealed that OCT-4 and SOX-2 mRNAs are expressed in hMSCs and their expression is very low when compared with tumor cell lines used as a positive control. After differentiation the level of OCT-4 increased and the level of SOX-2 decreased. Immunocytochemical staining revealed that OCT4 protein is expressed in all hMSCs and stays expressed on day 14, localized in the nuclei. In contrast to previous observations [11], SOX2 have not been detected neither in hMSCs on day 0 nor in osteoblasts on day 14.

We demonstrate presence of stem cell markers OCT-4 and SOX-2 in human mesenchymal stem cells at mRNA level. Immunocytochemical analysis revealed OCT4 expression in nuclei but not in cytoplasm as previously has been demonstrated [34). In past decade, many controversial results have been obtained regarding the expression and the role of stem cell transcriptional factors in adult stem cells. OCT-4 and SOX-2 were undoubtedly confirmed in some types of adult progenitor and multipotent stem cells [34–37]. They are expressed in tumor cells [38–40] and might be responsible for cancer stem cells resistance to chemotherapy [41]. Previous reports showed inconsistent results in the expression of stem cell markers in adult stem cells. Beltrami et al., as well as some other groups have confirmed OCT-4 and SOX-2 and NANOG stem cell markers expression in mesenchymal stem cells [11, 13, 34] but Pierantozzi et al. have not detected OCT-4 and NANOG in adult hMSCs [24]. Little is known about the role of OCT-4 and SOX-2 in adult stem cells. *OCT-4* gene knockdown promotes differentiation thereby that transcriptional factor play an important role in stem cell self-renewal [42]. We find that hMSCs express low levels of OCT-4, what is crucial for multipotency and self-renewal of these adult stem cells. It has been previously reported that increased expression of *OCT-4* causes increased differentiation efficiency towards osteoblasts [43].

Immunohistochemical staining did not confirm OCT4 protein *in vivo*, in bone marrow sections. There is a possibility that *ex vivo* expansion of human mesenchymal stem cells causes activation of endogenous *OCT-4* gene expression. Therefore, OCT-4 might play an important role for maintaining potency in somatic stem cells [28] including hMSC. Synthesis and degradation of OCT-4 protein is very rapid process and alteration of *OCT-4* transactivation potential in response to extracellular signals is reversible [44]. This kinetic is not completely understood yet, but might explain why very high OCT-4 expression is present in osteoblasts on day 14. Relative amounts of OCT-4 protein are essential for proper differentiation and early development of an organism because it determines developmental directions. Post-translational modification of OCT-4 by sumoylation can enhance OCT-4 stability, DNA binding and transactivation [45]. OCT-4 is ubiquitinated by Wwp2 and degradated during differentiation of embryonal carcinoma cell line but

does not affect OCT-4 protein level in embryonic stem cells [46]. These post-translational modifications are important for OCT-4 stability, activity and degradation, so these mechanisms may maintain its quantity during differentiation of hMSCs.

SOX2 is a transcription factor co-expressed with OCT-4. It has been identified a crucial player in the maintenance and differentiation of adult stem cells such as in neural stem cells. Seo et al. showed that SOX-2 maintains self-renewal and proliferation of the osteoblast precursors [47]. SOX-2 inactivation in cultured primary osteoblasts has been shown to cause exhaustion of their proliferative ability and senescence [48]. Our findings support the existence of progenitors that express SOX-2 among the population of hMSCs, however the levels detected by qPCR may be under the detection limit of immunocytochemistry or posttransciptional regulation may be responsible for absence of protein. Downregulation of SOX-2 after the differentiation is consistent with suggested role of SOX-2.

Despite the fact that probably there is no universal stem-cell marker which could be applied to all types of stem cells [30], it is important to define stem cell markers specific for clinically relevant cell types such as MSCs. These markers could be used for the evaluation of stem cell differentiation potential. In this study, since hMSCs have well defined osteogenic markers, we have tried to define some stem cell markers in these cells. We have evaluated two potential candidates, OCT-4 and SOX-2. It is crucial to further examine their function in hMSCs as well as in osteoblasts. It is important to be able to evaluate the quality of stem cells, because the stem cell potential is certainly associated with the potential results of cell therapy.

One of the biggest problems in cell therapy is a potential malignant transformation of stem cells. It is well known that the stem and tumor cells in some aspects of their biology are very similar. During the process of the tumor stem cell dedifferentiation, re-expression of some stem cell markers occurs [40] and very often OCT-4 levels are dramatically increased in cancer cells [49]. Potentially, by monitoring the expression of OCT-4 gene in stem cells we could predict the behavior of cells in terms of malignant transformation. This is another reason why it is necessary to investigate the role of pluripotency genes in somatic stem cells.

In conclusion, bone marrow-derived mesenchymal stem cells that are expanded in culture in the presence of FGF2 clearly express low levels pluripotency markers OCT-4 and SOX-2 mRNA. Protein OCT-4 is detected in cell nuclei before and after differentiation while SOX-2 is not detected. Expression of OCT-4 is not desirable property of cells that are going to be applied in the clinical setting. We conclude that culture conditions do have an effect on stem cell marker expression and it is important to standardize cultivation conditions in order to better predict cell behavior. It is necessary to clarify better the role and mechanism of action of core pluripotency genes in mesenchymal stem cells following their cultivation *in vitro*.

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Basic Science

Role of Cardiac Myocytes Heart Fatty Acid Binding Protein Depletion (H-FABP) in Early Myocardial Infarction in Human Heart (Autopsy Study)

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Key words: detection myocardial infarction; H-FABP; immunohistochemistry; MI; sudden cardiac death.

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Abstract

BACKGROUND: Many immunohistochemical markers have been used in the postmortem detection of early myocardial infarction.

AIM: In the present study we examined the role of Heart-type fatty acid binding protein (H-FABP), in the detection of early myocardial infarction.

MATERIAL AND METHODS: We obtained samples from 40 human autopsy hearts with/ without histopathological signs of ischemia.

RESULTS: All cases of definite and probable myocardial infarction showed a well-defined area of H-FABP depletion. All of the control cases showed strong H-FABP expression, except two markedly autolysed myocardial samples that showed affected antigenicity.

CONCLUSION: Thus, we suggest H-FABP as being one of the valuable tools facing the problem of postmortem detection of early myocardial infarction/ischemia, but not in autolysis.

Introduction

Myocardial infarction (MI) is the most frequent diagnosis made in the majority of sudden deaths subjected to clinical and medico legal autopsies [1]. The problem is to recognize an infarct that has occurred within a very short time [2].

It is impossible to get the histological diagnosis of early myocardial infarction as a cause of sudden death in routine H&E stained sections, as it is unlikely to find special changes for myocardial infarction aged less than 6 hours [3, 4].

Thus, in the current autopsy practice, there is a great need for more sensitive diagnostic methods for the postmortem diagnosis of early myocardial damage [5].

Also, we need further studies on the postmortem stability of immunohistochemical markers and the regularity of their expression alteration in different postmortem intervals to define the role of autolysis [6].

Heart-type fatty acid binding protein (H-FABP) is a low molecular weight protein (14-15kDa), which is abundant in the cytoplasm of myocardial cells. As myocardial cell membrane is damaged by ischemia, H-FABP leaks to the extracellular space and enters the blood circulation very easily and quickly due to its small size and water solubility [7].

Therefore, loss of H-FABP in myocardial ischemic cells could occur as early as 15 minutes, and as the ischemia intervals prolonged, the depletion areas increase gradually in myocardial cells. The deletion areas of H-FABP reach a peak after four hours of myocardial ischemia [8].

The primary results suggest that H-FABP staining can detect very early ischemic damages in human myocardium [8, 9].

In the present study we examined the role of Heart-type fatty acid binding protein (H-FABP), in the detection of early myocardial infarction.

Material and Methods

Tissues

The study included 40 hearts from autopsy cases; with/without evidence of coronary artery disease (CAD). CAD cases included advanced or complicated lesions.

Tissue sections, from the myocardia and the corresponding coronary artery supply were obtained. They were fixed in 10% neutral-buffered formalin and embedded in paraffin. H&E stained sections are obtained and examined histologically.

Cases were grouped according to the histological findings as GI, GII, and GIII according to the Lodge Patch histological criteria for MI [10]:

1. Group I: includes 15 hearts represented cases showing signs of old ischaemia (fibrosis, granulation tissue and established infarction)..
2. Group II: includes 18 cases represented cases showing either signs of recent ischemia (coagulative necrosis, contraction band necrosis, etc.) or with evidence of advanced or complicated CAD (severe stenosis, complicated atheroma or recent thrombus).
3. Group III: includes 7 cases showing no signs of ischemia, two of them showed severe autolytic changes which were selected to assess the expression of H-FABP in autolysis.

Death/Autopsy time ranged between 12-24 hours in all examined cases, except for the two autolysed control cases where death/ autopsy time exceeded 60 hours.

Finally, we obtained 40 sections of myocardial tissue from the 40 cases for immunohistochemical staining by H-FABP.

Immunohistochemistry

Immunohistochemical staining with H-FAB is performed using a standard avidin–biotin–peroxidase system.

Approximately 4 μm thick histologic sections were deparaffinated in xylene and alcohol, rehydrated in distilled water for 5 minutes, and then washed in PBS for 5 minutes. To reveal the antigens, they were pre-treated by using the proteolytic enzyme proteinase K and then washed in PBS for 5 minutes.

The primary monoclonal antibody to H-FABP (Aviscera Bioscience, USA) was diluted to a concentration of the antibody 1:250 (the optimal concentration supplied by the manufacturer) and incubated for 60 minutes at 37°C, then washed in PBS for 5 minutes.

Secondary antibody was applied for 60 minutes (DAKO, Denmark), followed by rinsing with PBS. The reaction was visualized with DAB chromogen (DAKO, Denmark). The slides were counterstained with diluted hematoxylin.

Each slide has its built in negative and positive controls for H-FABP. Areas with definitive signs of ischaemia by hematoxylin and eosin (H&E) acted as negative internal control, whereas non-ischemic regions of myocardium are expected to be positive internal control.

Microscopic Evaluation

The amount and extent of depletion of the cell marker is recorded. Loss of myocardial staining of H-FABP in the three groups was recorded. The obtained results were scored from (0) to (-3) according to the percent of myocardial fibers showing depletion of H-FABP staining: Score 0: no loss of H-FABP staining, Score -1: loss in less than 25% of fibers, Score -2: loss in 25-50% of fibers, Score -3: loss in >50% of fibers [11].

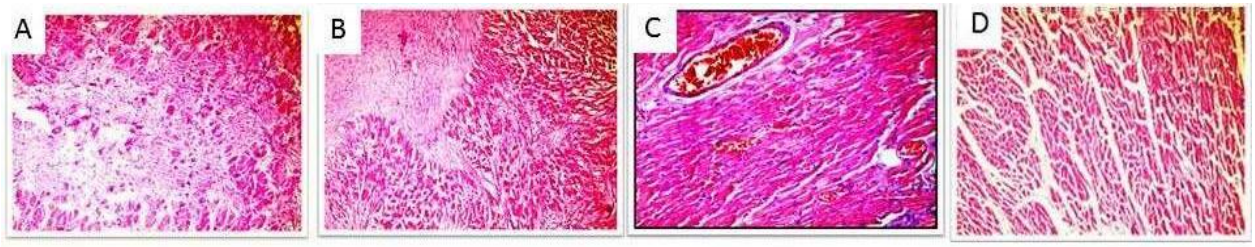
Results

The results are summarized in the Table 1.

Table: immune reactivity of H-FABP in all study groups. Data are presented as number (percent)

		H-FABP Score	Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Group III	0	5	71.4	71.4	71.4
		-2	1	14.3	14.3	85.7
		-3	1	14.3	14.3	100.0
		Total	7	100	100	
	Group II	-1	2	11.1	11.1	11.1
		-2	7	38.9	38.9	50.0
		-3	9	50.0	50.0	100.0
		Total	18	100.0	100.0	
	Group I	-1	2	13.3	13.3	13.3
		-2	6	40.0	40.0	53.3
		-3	7	46.7	46.7	100.0
		Total	15	100	100	

Myocardial ischemia with Hematoxylin and eosin stain (x 200) is shown in Figure 1, and H-FABP immunostaining with hematoxylin counterstain is shown in Figure 2.



A) coagulative necrosis and granulation tissue formation, sign of recent ischemia.

B) myocardial fibrosis denoting old ischemic changes.

C) congestion, a probable sign of ischemia .

D) fragmentation, a probable sign of ischemia.

Figure 1: Myocardial ischemia with Hematoxylin and eosin stain (x 200)

Group I [Figure 1B]; all cases showed variable degrees of H-FABP depletion. 7 out of 15 cases showed near total loss of cytoplasmic brown staining (score -3) [Figure 2D], 6 cases showed focal areas with loss of cytoplasmic brown staining (-2), and 2 cases showed minimal loss of staining (score -1) in myocardial areas surrounding the known ischemic areas.

Group II [Figures 1A, 1C and 1D]; all cases showed variable degrees of H-FABP depletion. 9 out of 18 cases showed near total loss of cytoplasmic brown cytoplasmic staining (score -3), 7 cases showed focal areas of loss of cytoplasmic brown staining (-2) [Figure 2C], and 2 cases showed minimal loss in staining (score -1) [Figure 2B].

Group III; 5 of the selected 7 cases showed strong brown cytoplasmic staining with no loss (score 0), denoting the integrity of the myocardium [Figure 2A]. The remaining two cases were selected with advanced autolytic changes to assess the efficacy of the H-FABP on autolysis. One of them showed near total loss (score -3) [Figure 2E] and the other showed wide areas of loss (score -2).

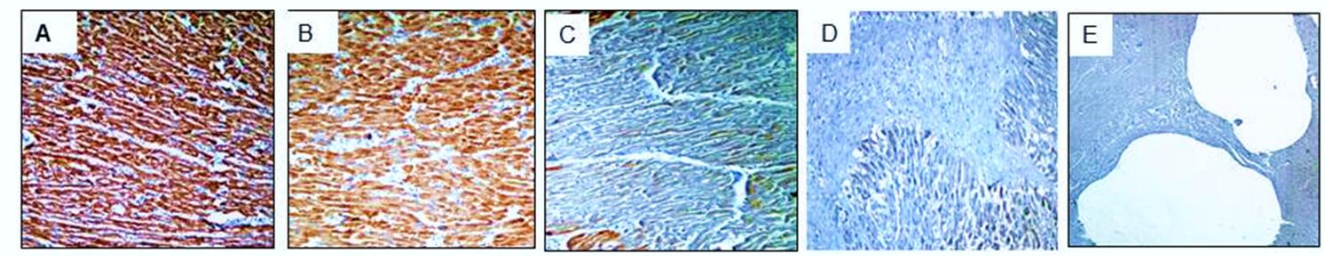
Discussion

Histopathological examination of the heart must be performed as a part of routine autopsy as to clarify the cause of death, especially in non-traumatic deaths in which the autopsy didn't reveal any confessant non-cardiac cause of death [12].

Many studies have proved the usefulness of variable immunohistochemical markers (especially Troponin, myoglobin, C5a, S100a1 and desmin) in the diagnosis of early myocardial injury has been promoted because most of them can be visible very early after the beginning of the symptoms (chest pain and angina attacks) [13-16].

However, very few studies have indicated the usefulness of H-FABP in cardiology for detection of acute myocardial infarction (AMI) in both human and animal models [7, 8].

On studying H-FABP immunostaining, all group I cases with old myocardial infarction, showed H-FABP depletion in the infarcted areas.



A) diffuse brown staining with no depletion of H-FABP (score 0) , (x 100).

B) minimal H-FABP depletion (score -1), (x 100).

C) clear decrease in staining with some positivity remaining (score -2), (x100).

D) diffuse depletion of H-FABP (score -3)

E) near total depletion of H-FABP in normal autolysed heart (score -3) (x40).

Figure 2: H-FABP immunostaining with hematoxylin counterstain

These results are consistent with the results obtained by Meng et al., 2006, who recognized H-FABP depletion in infarcted area in the human myocardia [9]. They are also comparable to the results given by Ming et al 2004, where all 7 cases with definite infarcted area, showed significant or total loss of H-FABP in fibrous tissue (confirmative infarcted area) [8].

We also noticed variable degrees of H-FABP depletion in the myocardial fibers surrounding the infarcted areas that appeared normal in the H&E stained slides. This was rather more remarkable than the results of Ming et al., 2004 who declared that these apparently normal cardiomyocytes were brown mixed with some faintly immunostained cardiomyocytes [8].

An example from this group was a heart with left ventricular concentric hypertrophy. Microscopic examination revealed severe stenosis in his left circumflex artery and minimal foci of myocardial fibrosis in the lateral wall of left ventricle. H-FABP staining of the section selected from and around the fibrotic foci revealed wider areas of ischemia - loss (-2) - around fibrotic foci denoting more pronounced ischemia, which could only be detected by the antibody, giving the impression of its high sensitivity.

Selected group II cases, with probable signs of ischaemia had coronary stenosis and their complication showed loss of cytoplasmic staining by various degrees ranging from minimal depletion (-1) and up to near total depletion (-3). Meng et al, 2006, in their study, noticed H-FABP depletion in areas of suspected early myocardial infarction that showed normal H&E staining [9].

Worth mentioning, one of group II cases belonging to a male in his 5th decade of life. He died in custody half an hour after exposure to a homicidal attempt by strangulation by another prisoner. On autopsy, there were only bruises on neck side. Examination of the heart revealed no gross myocardial abnormalities. Microscopic examination revealed severe stenosis of the left anterior descending coronary artery with rupture in arterial wall atheromatous plaque complicated by recent thrombus formation. Myocardium showed only congestion and an area of fragmentation by H&E staining. However; immunohistochemical examination of the myocardium revealed areas of H-FABP depletion (score -2) documenting early ischemic changes.

Acute coronary syndrome found in this case led to death with the subsequent myocardial ischemia only detected by Immunohistochemical staining [6].

On examining group III cases - control positive group- five out of the seven cases showed diffuse cytoplasmic H-FABP immunoreactivity exhibited in the cardiac myocytes (score 0). In comparison with the study performed by Meng et al., 2006, cases with no signs of ischemia, showed weak, but diffuse staining [8].

The remaining two cases that showed depletion in this group were two autolysed hearts from two putrefied female cadavers, with no evidence of ischemia. Estimated time of death exceeded 60 hours. Both cases showed H-FABP depletion, which reflects that H-FABP immunoreactivity in this study was markedly affected by autolysis.

This is contradictory to the results recorded by Ming and Meng, 2004. They stated that the efficacy of H-FABP for detection of ischemic cardiomyocyte lesions was not reduced by autolysis, even in cases where death autopsy time extended as long as 60 hours after death on human hearts [8].

We can summarize that H-FABP may be a useful marker in the early detection of AMI as well as the ongoing ischemia in areas surrounding well established infarctions in human hearts which can't be detected using routine H&E stain, as well as the ongoing ischemia in areas surrounding well established infarctions.

However; it is not the marker of choice that can be used in the detection of early myocardial ischemia in advanced autolytic changes - despite its sensitivity generally. Other antibodies that can withstand autolysis as troponin, myoglobin and desmin can do that role instead [5, 11, 17].

Many factors are needed for well-established confident diagnosis besides the immunohisto-chemistry. We should correlate with the history, clinical data and case circumstances and their insufficiency may act as an obstacle in achieving satisfactory results that we are aiming at .In forensic practice, we can't give opinion depending on single factor, there are many factors should be put together to get out at the end by reliable confident decision, and every case in our practice has its unique characteristics, circumstances and findings that make it an indivisible standalone entity.

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Basic Science

Susceptibility of Urinary Tract Bacteria to Newer Antimicrobial Drugs

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Key words: Antibiotic; Urinary tract infection; uropathogens.

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Abstract

Urinary tract infections (UTIs) are among the commonest types of bacterial infections. The antibiotic treatment for UTIs is associated with important medical and economic implications. Many different microorganisms can cause UTIs though the most common pathogens are E. coli and members of family Enterobacteriaceae. The knowledge of etiology and antibiotic resistance pattern of the organisms causing urinary tract infection is essential. The present study was undertaken to evaluate trends of antibiotic susceptibility of commonly isolated uropathogens using newer antimicrobial agents, prulifloxacin, fosfomycin (FOM) and doripenem. We conclude that maintaining a record of culture results and the antibiogram may help clinicians to determine the empirical and/or specific treatment based on the antibiogram of the isolate for better therapeutic outcome.

Introduction

Urinary tract infection (UTI) has long been recognized as one of the commonest bacterial infection [1] which is prevalent in both community and health care settings. Symptomatic infections are common and are associated with morbidity and rarely mortality. However asymptomatic infections are more common. Any demarcation regarding its prevalence in specific age group is not been reported. It may infect individuals from different age groups and from both the sexes [2]. It occurs as both complicated and uncomplicated infection, uncomplicated one is common in healthy adults and non-pregnant females whereas complicated UTI is frequently associated with structural and functional abnormalities [3]. Though the sufficient treatment options facilitate the management of the disease but increasing resistance towards various antimicrobial agents leads to numerous problems. Multi-drug resistant (MDR) uro-pathogens have leaded to increase in proportion of UTIs and because of limited treatment options for these MDR strains; it has become matter of serious concern and posing a financial burden too [4]. This study has been undertaken to evaluate the susceptibility of uro-pathogens to newer antimicrobial agents, prulifloxacin, fosfomycin (FOM) and doripenem.

Prulifloxacin is an oral fluoroquinolone specifically a lipophilic prodrug of ulifloxacin. It has broad spectrum antimicrobial activity against gram positive and gram negative bacteria as well [5]. The in vitro activity of prulifloxacin is generally greater than that of ciprofloxacin and other flouoroquinolones against isolates of Gram-negative bacteria, including *E. coli, Klebsiella* spp., *Proteus, Providencia* and *Morganella* spp., *Pseudomonas aeruginosa, Moraxella catarrhalis* and *Haemophilus* spp. Against Gram-positive bacteria, such as *Streptococcus* spp., *S. aureus, Enterococcus* spp. and coagulase-negative staphylococci [6], the in vitro activity of ulifloxacin is generally similar to or greater than that of ciprofloxacin, but lower than that of moxifloxacin [7]. FOM has shown promising *in vitro* activity against

MDR uropathogens, however sufficient clinical data regarding its antimicrobial susceptibility behavior is not available. It is a phosphonic acid derivative and is a naturally occurring antibiotic. It acts primarily by interfering with bacterial peptidoglycan synthesis and hence disrupting cell wall synthesis [8]. It has been approved by Food and Drug Administration for the treatment of uncomplicated UTIs in women [9]. FOM represents its own class of antibiotics and no other member of this class is currently approved by regulatory agencies worldwide. It has got broad spectrum activity against both gram positive and gram negative bacteria. It can be administered orally with a convenient dosing schedule, including single dose therapy for uncomplicated cystitis. Doripenem a 1beta-methylcarbapenem, is a broad spectrum antibiotic which has been approved for intra-abdominal infections and complicated urinary tract infections [10]. The spectrum of activity of doripenem has been established *in vitro* and *in vivo* for gram negative, gram positive and anaerobic micro organisms. Compared with the other carbapenems, doripenem has a higher threshold for selection of non susceptible mutants in vitro, and it seems that high level resistance may require the coexistence of more than one resistance mechanism [11].

Material and Methods

Samples

This was a retrospective study with an observation period of eighteen months (May 2012 to October 2013) during which all the urine samples received in the department of Microbiology of Dr. Harvansh Sigh Judge Institute of Dental Sciences & Hospital were screened for the presence of bacteria. In this period of eighteen months the total number of samples screened was 259. Qualitative urine cultures were performed in CLED agar plates. Plates were incubated at 37^0C for 18-24 h. Identification of the bacterial isolates was done using conventional biochemical methods [12].

Antibiotic susceptibility testing

Besides the antibiotics viz amoxicillin, nitrofurantoin, ciprofloxacin, nalidixic acid, augmentin, amikacin, ceftazidime, cefotaxime, imepenem and sulbactam which are tested in routine; the sensitivity pattern of all the *E. coli* isolates was also tested for doripenem, fosfomycin and prulifloxacin. The Clinical and Laboratory Standard Institute (CLSI) criterion was used for the interpretation of the antimicrobial susceptibility [13].

Result and Discussion

Of all the samples received in microbiology department during the study period of eighteen months 30.8% (80) turned out to be *E. coli.* The susceptibility pattern of the eighty E. coli isolates tested for various antibiotics is described in Figure 1.

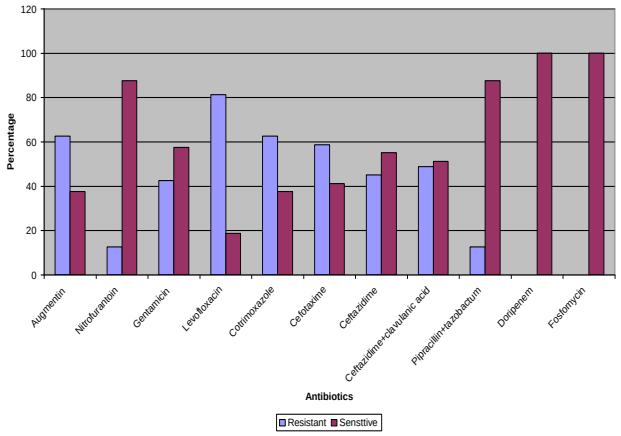

Figure 1: Antibogram of E. coli isolates.

Out of eighty E. coli isolates fifteen (18.75%) were sensitive to prulifloxacin whereas number of isolates that were resistant to prulifloxacin were 65 (81.75%). If we look at the susceptibility of the E. coli isolates towards fluoroquinolones, this study showed that the different members of floroquinolones showed similar behavior. The finding is supported by another study by Carmignani et al. [14] who reported that there was no statistical significant difference in the clinical and microbiological parameters of prulifloxacin and ciprofloxacin. Other studies [6, 7] have shown that the ulifloxacin MICs and minimum bactericidal concentrations tend to be equal or even lower compared with ciprofloxacin, while they are generally lower compared with levofloxacin, for most gram-negative pathogens including *P. aeruginosa.* The strains of E. coli were altogether sensitive to doripenem and fosfomycin as our study reported 100% susceptibility to both these drugs. Our study was supported by another study by Maraki [15] which indicated acivity of fosfomycin against a considerable percentage of urinary isolates that simultaneously exhibited high rates of antimicrobial drug resistance to the conventionally used antimicrobial agents for the treatment of UTIs . The excellent activity of fosfomycin against E. coli has been reported by similar susceptibility findings [16] indicating that the drug is a valuable therapeutic option for urinary tract infections. In vitro activity of doripenem and other antimicrobial agents was evaluated against Gram-negative bacilli recently isolated in Brazilian study [17] and these studies supported our finding of 100 percent susceptibility of uropathogens to doripenem. Hence

like other newer drugs, doripenem exhibited excellent urinary bactericidal activity and appears to be a good alternative in the empirical treatment of UTI and pyelonephritis. This study highlighted that susceptibility pattern is necessary to obtain sensitivity reports before start of antibiotic treatment in cases of suspected UTI. The knowledge of antimicrobial pattern of routinely isolated uropathogens may provide guidance to clinicians regarding the empirical treatment of UTI when therapy must be started before laboratory reports are available

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Basic Science

The Validity of Body Adiposity Indices in Predicting Metabolic Syndrome and Its Components among Egyptian Women

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Key words: Body adiposity; metabolic syndrome; women; validity.

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Abstract

AIM: To assess the associations between the body adiposity indices and risk of metabolic syndrome (MS) and its components in Egyptian women and to evaluate their predictive power.

MATERIALS AND METHODS: This was a cross-sectional analysis performed on 180 Egyptian women aged between 25-35 years. They were 90 women with MS diagnosed by International Diabetes Federation (IDF) and 90 healthy age matched controls. Body adiposity index (BAI), body mass index (BMI), waist to hip ratio (WHR) and waist to height ratio (WHtR) were calculated and serum samples were analyzed for metabolic parameters. Receiver operating characteristic curves (ROC) was used to determine the discriminatory capacity of BAI, WHR WHtR and BMI for MS.

RESULTS: Area under the curve (AUC) was highest for BIA, followed by WHR, WHtR and then BMI. All adiposity indices were significantly correlated with metabolic components and BAI had the highest correlation coefficients compared to other indices.

CONCLUSION: BAI is a practical predictor for MS and has satisfactory diagnostic accuracy for diagnosing MS among Egyptian women and can be used in addition to WHR, WHtR and BMI for identifying MS in the field studies.

Introduction

Obesity has become one of the most important public health problems. The increase in prevalence of obesity involves an increase in the prevalence of several obesity-related diseases [1–3]. Several studies show the relation between th e adipose tissue accumulation and the incidence of adverse metabolic events and, also, with a higher risk for developing metabolic diseases [4-10]. The metabolic syndrome (MS) is a set of interrelated risk factors such as hypertension, dyslipidemia, obesity and high blood glucose.

Insulin resistance together with central/abdominal or visceral obesity has been proposed as the key factors in the development of the MS. Several authors have tested the correlations between the indices of adiposity and several health outcomes [11-13]. There is no universally agreed definition for MS. Despite the use of the same index for central obesity assessment, the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) [14] and International Diabetes Federation (IDF) [15] differed in the waist circumference (WC) cut-off points. ATPIII proposed WC more than or equals 88 cm for women, whereas IDF proposed WC cut-off points based on population estimates. Body mass index (BMI), WC, and waist to hip ratio has all been tested for their relation to MS, but with no consistent results across the globe. The prevalence also varies by ethnicity. In the National Health and Nutrition Examination Survey III (NHANES III) [16], the age-adjusted prevalence was 30–40%higher in p eople of Mexican–American origin than in persons of White and African–American origin.

The highest prevalence is found in the Middle East region, where more than every third person

above the age of 20 fulfils the criteria for having the metabolic syndrome. The syndrome is common and has a rising prevalence worldwide, relating largely to a complex interplay of rapid nutritional alterations, sedentary lifestyle and socioeconomic evolution, increasing affluence, rural-to urban migration, leading to obesity.

This study aims to evaluate the predictive power of adiposity indices as predictors for MS in the sample of Egyptian women.

Material and Methods

The present cross-sectional study was carried out on 90 obese Egyptian women (aged 25-35 years old) recruited from obesity clinic National Research Centre with MS having the presence of 3 or more criteria/parameters according to the International Diabetes Federation (IDF) and 90 healthy controls without or even have a single parameter of metabolic syndrome. A written informed consent was obtained from each study subject.

IDF criteria are: central obesity (defined as waist circumference >80 cm). If BMI is > 30 kg/m^2, central obesity can be assumed and waist circumference does not need to be measured, BMI has no relation with central obesity measured by WC, as there are some bodies have BMI > 30 Kg/m and WC less than 88 cm, triglycerides >150 mg/dL or specific treatment for this lipid abnormality, HDL-cholesterol < 50 mg/dL and blood pressure >130/85 mmHg or treatment of previously diagnosed hypertension, fasting plasma glucose >100 mg/dL or previously diagnosed type 2 diabetes. When central obesity plus two of the four previous criteria are met, a diagnosis of metabolic syndrome can be made [17]. BMI was calculated as weight (kg) divided by height (m) squared (kg/m^2). BAI was calculated using the equation ((hip circumference)/((height) 1.5)-18) [18].

WC and hip circumference (HC) were measured using inelastic tape at the level midway between the lateral lower rib margin and iliac crest as well as at the levels of trochanters. WHR was calculated as WC divided by HC and WHtR was calculated as WC divided by height in centimeters.

Serum analysis

Blood samples were collected after a 12-h overnight fast and stored at < 80°C until analyzed. An Olympus AU400 automatic analyzer (Olympus Corporation, Tokyo, Japan) was used to measure serum total cholesterol (TC), High Density Lipoprotein cholesterol (HDL-C), triglycerides (TG) and low Density Lipoprotein cholesterol (LDL-C) was calculated. Fasting blood glucose (FBG) was measured with commercial kits (Roche Diagnostics, Indianapolis, IN, USA) and fasting blood insulin (FBI) were determined with the Phadebas Insulin Test (Pharmacia, Uppsala, Sweden) using a radioimmunosorbent technique. The insulin sensitivity was then calculated using Homeostasis Model Assessment (HOMA-IR) according to the following formula: HOMA-IR = FBG (mmol/L) ×FBI (μU/ml)/22.5.

Table 1: Age, anthropometric, clinical and biochemical indices characteristics of the study participants

Characteristics	MS	Non-MS
Age	28.42 ± 2.45	29.42 ± 3.56
BMI (kg/m^2)	30.42 ± 3.70	23.51 ± 2.91 ***
Waist circumference (cm)	106.42 ± 10.00	99.46 ± 17.395 ***
Hip circumference (cm)	125.22 ± 12.89	119.01 ± 10.78 ***
WHR	0.89 ± 0.05	0.80 ± 0.13 ***
WHtR	0.72 ± 0.01	0.59 ± 0.01 ***
BAI (kg/m^2)	35.75 ± 4.22	25.65 ± 3.31 ***
Systolic BP (mmHg	120.00 ± 12.6	115.71 ± 18.44 ***
Diastolic BP (mmHg)	94.54 ± 6.87	73.83 ± 10.43 ***
FBG (mg/dl)	112.52 ± 8. 22	90.52 ± 5.22 ***
FBI (μU/ml)	18.20 ± 0.85	8.20 ± 0.65 ***
HOMA-IR	6.88 ± 1.29	2.3 ± .99 ***
Triglycerides (mg/dl)	145.65 ± 30.61	100.41 ± 33.29 ***
Total cholesterol (mg/ dl)	174.71 ± 30.81	124.71 ± 23.81 ***
LDL-C (mg/dl)	163.71 ± 35.890	112.61 ± 29.381 ***
HDL-C (mg/dl)	45.44 ± 15.217	47.86 ± 14.560 ***

BMI: body mass index; WHR: waist to hip ratio; WHtR: waist to height ratio; BAI: body adiposity Index; FBG: fasting blood glucose; FBI: fasting blood insulin; HOMA-IR: homeostasis model assessment of Insulin Resistance; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. *** Significant at p-value < 0.001.

Statistical Analyses

All the data were tested for their normal distribution (Kolmogorov–Smirnov test). Results are expressed as means and standard deviations (SD). Student t test for unpaired data was used to evaluate differences in anthropometric and biochemical characteristics between cases and controls.

The existence of significant bivariate correlations between parameters such as BAI, BMI, WHR, WHtR and biochemical parameters and metabolic risk factors was ascertained by determining correlation coefficients. Receiver operating characteristic curves were used to determine discriminatory capacity of BAI, WHR, WHtR and BMI for metabolic syndrome risk.

Statistical analysis was carried out using IBM SPSS Statistics 20.0 software (SPSS/IBM, Chicago, IL, USA). Significance was accepted at $p<0.05$.

Results

Table1 shows clinical, biochemical and anthropometric characteristics of the study participants. Statistically significant differences were found between the MS cases and controls in all anthropometric and biochemical parameters. Women with MS showed significant higher values of BMI, WC, HC, WHR, WHtR, BAI, blood pressure levels, LDL-C,

TG, FBI, FBG, HOMA-IR and lower HDL-C than normal controls ($p < 0.001$).

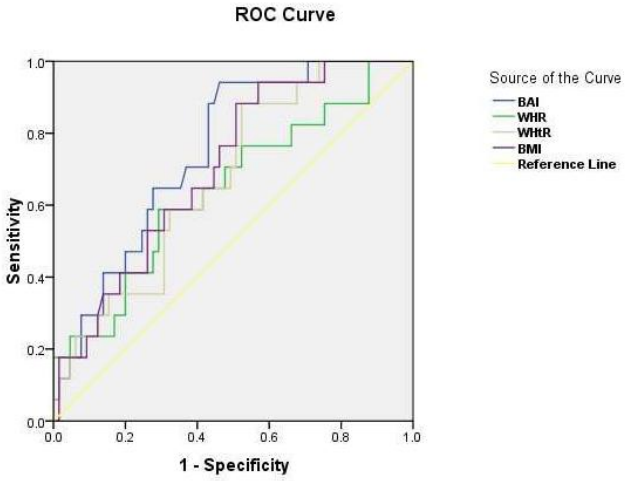

Figure 1: ROC Curve to determine the diagnostic value of body adiposity indices to predict the metabolic Syndrome

Figure 1 shows the ROC curve for BAI,BMI and WHR with respect to the presence of MS. Area Under the Curve(AUC) was 69.8% (95% CI: 62.2% – 81. 3%) for BIA, 67.3% (95% CI:56.8% – 80.9%) for WHR,65.9% (95% CI:56.8% –79.9%)for WHtR and 61.3% (95% CI: 48.2%-78.5%) for BMI. BAI showed higher discriminatory capacity than WHR, WHtR and BMI for MS risk.

Table 2: Partial correlations between adjusted body adiposity indices and metabolic components; to exclude the effect of age

	BAI	WHR	WHtR	BMI
Systolic BP	0.727**	0.560**	0.461**	0.361*
Diastolic BP	0.686**	0.502**	0.434**	0.374**
FBG	0.586***	0.3214**	0.294*	0.283*
FBI	0.575***	0.402**	0.382*	0.361*
HOMA-IR	0.486**	0.482**	0.464**	0.253*
Triglyceride	0.485**	0.404**	0.384*	0.251*
HDL-C	-0.583**	-0.455**	-0.335*	-0.237*
LDL-C	0.486***	0.381**	0.335*	0.235*

Table 2 shows the coefficients of partial correlation between anthropometric measures and metabolic risk factors. BAI had the highest correlation coefficients with metabolic components followed by WHR and WHtR and then BMI.

Discussion

The objective of this cross sectional study was to analyze correlation of body adiposity indices with metabolic risk factors among Egyptian women aged 25-35 years old. Adipose tissue accumulation increases the incidence and risk of adverse metabolic events and diseases.

To our knowledge this study is the first study focused on evaluating the applicability of BAI as a method to determine metabolic risk in a sample of Egyptian women and determine the validity of each of BAI, WHR, WHtR and BMI that might have predictive power for the risk of MS. The main finding of the present study is that BAI a good adiposity predictor for MS and overcome the limitations of BMI and the other indices analyzed. Body fat content, fat distribution or adiposity, therefore, could be considered as important indicators of metabolic risk. Body adiposity index has been developed, to overcome the shortcomings of BMI and it can be used to reflect body fat percentage (BF %) in adults [19, 20]. BAI was suggested to have several advantages over BMI, including that it yields similar associations with BF% for men and women and may be more practical to assess in field studies because it does not require a weight measurement and it can be used to reflect body fat percentage (BF %) in adults. It has been suggested that the BAI can be used to mirror %body fat for adult men and women of differing ethnicities without numerical correction.

BAI was suggested to have several advantages over BMI, including that it yields similar associations with BF% for men and women and may be more practical to assess in field studies because it does not require a weight measurement [21].

Current study tested adiposity indices for identifying MS using ROC curves and detect their sensitivity and specificity in the categorization of MS among Egyptian women.

The present results demonstrated that BAI has the higher discriminatory capacity (higher area under the curve) than the WHR, WHtR and BMI from ROC curves for identifying MS (IDF criteria). Area Under the Curve (AUC) was 69.8% for BIA, 67.3% for WHR,65.9% for WHtR and 61.3% for BMI, indicating that BMI has the weakest predictive power as compared to other adiposity indices. Moreover, partial correlation analysis showed that BAI had the highest correlation with metabolic risk factors compared to other adiposity indices. BAI level in subjects with metabolic syndrome was 35.75 ± 4.22 and 25.65 ± 3.31 in healthy controls.

The syndrome is common and has a rising prevalence worldwide, relating largely to a complex interplay of rapid nutritional alterations, sedentary lifestyle and socioeconomic evolution, increasing affluence, rural -tourban migration, leading to obesity [22].

However other studies reported that BAI could be less useful than BMI when the metabolic health risk is evaluated. Furthermore, this study suggested that WC and WHR may be even better candidates than BMI or BAI as simple (only tape measurements are required) and practical indicators of cardiovascular health risk [23].

In the IDF definition for MS, central obesity (increased WC) is a pre-requisite criterion in addition to two or more of the other major risk factors. The IDF

definition was adopted in this study for identifying subjects with MS and studying the relation between different risk factors. Different measures of central obesity have been developed over time including WHR, BMI, WC, and WHtR [22-24]. The first definition of MS by World Health Organization (WHO) used BMI and WHR. The ATPIII used BMI and WC to indicate central obesity, whereas the IDF only used WC in their MS criteria.

Our study showed significant partial correlations between adiposity indices and metabolic syndrome risk factors after controlling for age with highest correlations BAI followed by WHR then WHtR and BMI. In agreement to the current study, several studies have shown that the BAI supposes a new approach in order to determine the adiposity and MS [19-24].

WHtR ratio may reflect visceral fat more accurately than WHR, since the latter indicator does not reflect visceral fat properly as it may stay the same because WC and hip circumference can increase or decrease proportionately. However, previous study from Iran demonstrated that increased WHR was a better predictor for CVD risk factors than BMI, WC and WHtR, in all age groups [25]. BAI has been suggested to have several advantages over BMI. BAI gives similar associations with BF% for men and women and may be more practical to assess in field studies because it does not require a weight measurement. Many techniques have been developed for assessing and/or determining body fat or adiposity. These include the BMI, WC, WHtR, skinfold thickness, dual energy X-ray absorption (DXA) and hydrostatic densitometry [22-26].

In our study, BMI, BAI were and WHR were significantly correlated with metabolic parameters; correlations of BAI were stronger than WHR and BMI. ROC analysis revealed also superior discrimination of BAI compared to BMI and WHR.

A recent study done in north India concluded that the correlation of BMI to percentage of body fat was better than that of BAI to percentage of body fat, the sensitivity and specificity of BAI were similar to, if not better than, BMI [27, 28] .

The BAI can be measured without weighing, which renders it BAI was developed and validated in studies of Mexican American and African-American adults. Several studies of BAI values for predicting fat content or metabolic disorders in European-American, Mexican-American, Caucasian and Asian subjects have reported controversial results [29-31]. In Caucasians, BAI is a better estimate of adiposity than BMI in non-obese subjects, but less effectively than BMI in obese men and women. Another study reported that BMI more strongly correlated with BF% than BAI, and more highly associated with diabetes risk in Caucasian [32]. BMI was more accurate surrogate for adiposity in American [33, 34], Mexican Americans [35] Caucasian [36, 37] and Asian subjects [38]. Lifestyles have been changed over the preceding decades in developing countries, especially in Egypt. Increase in sedentary lifestyles has been observed that likely contribute to an increased incidence of MS [39]. Moreover, MS and its components (high waist circumference, high triglyceride levels, and low high density lipoprotein cholesterol levels) were significantly associated with menstrual irregularity in women of reproductive age [40].

In conclusion, BAI is practical predictor for MS and has a significant diagnostic accuracy for diagnosing MS among Egyptian women and can be used as a useful predictor in addition to other adiposity indices (WHR, WHtR and BMI) for identifying MS in the field studies.

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Basic Science

Relation between microRNAs and Apoptosis in Hepatocellular Carcinoma

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Abstract

AIM: To determine the relation between serum microRNAs and apoptotic markers as regards development of HCC to understand the underlying mechanism of HCV related hepatocarcinogenesis.

PATIENTS AND METHODS: A total of 65 serum samples (25 samples from controls, 20 samples from hepatitis and 20 samples from HCC patients) were collected for miRNAs (mir 21, mir 199-a, and mir 155) detection. Human Programmed cell death protein-4 (PDCD-4) and Human Cytochrome-C (CYT-C) were determined.

RESULTS: miRNAs 21 and 155 were over expressed in sera of patients with HCC compared to patients with chronic hepatitis ($p < 0.0001$). While serum means values of miR 199a was significantly decreased among HCC group patients when compared to patients with chronic hepatitis ($p < 0.0001$). The serum levels of PCDC4 and CYTC were increased in patients with HCC when compared to chronic hepatitis patients. They were also increased in patients with chronic hepatitis when compared to controls ($p < 0.05$, significant). There was direct correlations between apoptotic markers and oncomirs miRNAs 21 and 155 while apoptotic markers were inversely correlated with miRNA 199-a.

CONCLUSION: Both microRNAs and apoptotic markers have roles in HCC pathogenesis. It seems that oncogenic microRNAs induce liver carcinogenesis in HCV patients irrespective of suppression of apoptosis.

Introduction

Liver cancer is one of the most common malignancies all over the world and among the important causes of malignancy-related death [1]. Similar to other malignancies, the pathogenesis of liver cancer is a complex with contribution of genetic and epigenetic changes [2].

MicroRNAs (miRNAs) are a class of phylogenetically conserved short RNAs that suppress protein expression through base-pairing with the 3'-untranslated region (3'-UTR) of target mRNA [3]. Many studies suggest that miRNAs act significant roles in diverse biological processes and the dysfunction of miRNAs is included in the cancer development [4].

Cell cycle dysregulation is an important step in the induction and development of human malignancies, including liver cancer. Accumulating evidence has shown that deregulated miRNAs may affect HCC cell proliferation through direct interaction with critical regulators of cell cycle machinery [5].

Apoptosis is a natural barrier to tumorigenesis and malinancy progression. Cancer cells struggle to

avoid apoptosis to escape from the supervision of the body and to survive in the difficult tumor environment [6].

There are many miRNAs that target anti-apoptotic members of the Bcl-2 family. Most are significantly downregulated in HCC. For instance, miR-16 and miR-29 are down regulated in HepG2 cells, and one of their target genes is confirmed to be Bcl-2 [7]. There are some other miRNAs whose target gene is Mcl-1. Apart from silencing of Bcl-2, miR-29 can also directly target Mcl-1 in mitochondrion-mediated apoptotic pathway [8]. In addition, miR-101, miR-193b, miR-125b, and let-7c, which are downregulated in HCC cells, might exert anti-apoptotic action via targeting Mcl-1 [9].

Xiong and his colleagues have reported a significant down-regulation of miR-29 family members, including miR-29a, miR-29b, and miR-29c (miR-29a/b/c), in tissues of liver cancer [10]. This is in accordance with previous observations in other types of human neoplasm [11]. It has been shown that ectopic expression of miR-29b inhibits cell growth and promotes tumor necrosis factor–related apoptosis inducing ligand–triggered apoptosis [12].

In this study, we found differentially deregulated microRNAs (mir21; mir199-a; mir155) among patients with HCV related hepatocellular carcinoma. Moreover, apoptotic markers: Programmed Cell Death Protein 4 (PDCD4) and Cytochrome C (CYTC) were up regulated in the same patients. Here we try to clarify the relation between serum microRNAs and apoptotic markers as regards development of HCC to understand the underlying mechanism of HCV related hepatocarcinogenesis.

Patients and Methods

Between June 2011 and June 2013, a total of 65serum samples (25 samples from controls, 20 samples from hepatitis and 20 samples from HCC patients) were collected from patients who underwent liver resection or living donor liver transplantation (LDLT) at the First Affiliated El Sahahel Teaching Hospital and Dr. Refaat Kamel Hospital (Cairo, Egypt). Serum samples were also collected from 25 healthy volunteers who served as control group. All of the HCC patients were diagnosed by liver biopsy or by the findings of at least two radiological tests of HCC, including abdominal ultrasound, magnetic resonance imaging (MRI), hepatic angiography and contrast-enhanced dynamic computed tomography or by increased AFP (AFP ≥200 μg/mL). Patients with secondary or recurrent tumors, a history of other malignant tumors; patients with hepatitis B virus infection or being included in other studies were excluded from this study. For the 20 chronic hepatitis cases, the diagnosis was based on the serum tests. Serum hepatitis B surface antigens (HBsAg) and anti-HCV antibody were assayed by microparticle enzyme immunoassay using commercial kits to determine hepatitis B or hepatitis C infection. A total of 25 cancer-free controls were attached at the physical examination center. Controls that had clinical liver diseases were excluded. The current study was approved by Ethical Committee of National Research Center. After signing an informed consent, all subjects were asked to fill a questionnaire to investigate the demographic characteristics, disease history, and the history of cancer and alcohol or tobacco use. Consent to publish data of the current research was obtained from every participant. The clinical characteristics including tumor differentiation, tumor size, metastasis, Child-Pugh class, were collected from medical records.

miRNA extraction and quantification

Isolation of miRNAs from blood of patients and controls followed the protocol for miRNeasy RNA isolation kit (Qiagen, Germany). Separation of serum took place immediately within 2 h from blood sample collection. The extracted total RNA including miRNA from tissue and serum are subjected to reverse transcription.

RT-PCR

TaqMan miRNA assays (Life Technologies, CA) were used to quantify the expression levels of mature miR-122 Total RNA extracted by miRvana (life technologies) was reverse transcribed in reaction mixture containing miR-specific stem-loop RT primers. Quantitative real time polymerase chain reaction (qPCR) was performed with 3 microliter of each cDNA on aStep One TM Plus Real-Time PCR System (ABI) in duplicates reactions containing the prepared cDNA and TaqMan specific primers in Universal Master Mix without Amp Erase UNG (Applied Biosystems) and threshold cycles (CT) were calculated using Sequence Detection Software (SDS v2.2.1, Applied Biosystem). All mRNA quantification data were normalized to 18S RNA. All miRNA data are expressed relative to a RNU48 small nuclear (sn)RNA TaqMan PCR performed on the same samples. Fold expression was calculated from the mean CT values using the 22DDCt method. Relative quantity (RQ) of miRNAs 21 and 199-a was calculated by the formula ($RQ=2-\Delta\Delta Ct$), where Ct is defined as the fractional cycle number at which the fluorescence generated by cleavage of the probe passes a fixed threshold above baseline.

Detection of Human Programmed cell death protein-4 (PDCD-4) and Human Cytochrome-C (CYT-C) in blood samples (serum)

Detection of apoptotic markers in serum was carried out using this assay employed the quantitative sandwich enzyme immunoassay technique. The kits (Glory Science Co., Ltd, USA) use double – antibody sandwich enzyme- linked immunosorbent assay (ELISA) to assay the level of Human Programmed cell death protein 4 (PDCD4) and Human Cytochrome C (CYTC) in blood samples (serum). The procedure for each parameter is as follows:

- Add PDCD4 to monoclonal antibody Enzyme well which is pre-coated with Human Programmed cell death protein monoclonal antibody, incubation; , or add CYTC to monoclonal antibody Enzyme well which is pre-coated with Human cytochrome C monoclonal antibody; then
- Add PDCD4 or Cytochrome c (CYTC) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue, and the effect of acid, the color finally becomes yellow. The chroma of color and the concentrations of the human substance Programmed cell death 4 (PDCD4) or Cytochrome C (CYTC) of sample were positively correlated.
- We calculate the O.D value of all the wells with the standard and the wells with samples, make the standard curve diagram with concentration of the standard from the low to the high and the left to the right as abscissa {X} and O.D values of the wells at 450nm as ordinate {Y} axis.
- Then find out the corresponding concentration range of each sample on this standard curve diagram according to their O.D values.

Statistical analysis

Data are expressed as mean ± SD unless otherwise indicated. Categorical data are described as frequency of the subjects with a specific characteristic. Chi-square test or Fisher's exact test was used for comparing categorical data and Student's t-test, Mann-Whitney-U-test, one-way ANOVA or Kruskal-Wallis test, when appropriate, was used for comparing continuous variables. Two-tailed p-values less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA).

Results

General characteristics for the studied patients

Two groups of chronic HCV patients were enrolled in the current study; HCC group consists of 20 patients, 19 males and one female with mean age of 56.25 ± 8.13 years. Chronic hepatitis group consists of 20 patients, 15 males and 5 females with mean age of 46.22 ± 8.45years. Frequency of past history of Schistosomiasis was 55% among HCC group while it was 60% among patients of chronic hepatitis group as shown in Table 1. There were no any significant differences between two studied groups of patients as regards liver function tests. As a traditional marker for HCC, AFP was significantly increased among HCC group of patients. Macroscopic examination of hepatocellular malignant lesions revealed that distribution of these lesions were more prevalent in both right and left lobes, then the right lobe and were least found in left lobe with mean size of 3.65 ± 1.75 cm. the frequency of single lesion was more than that of multiple lesions.

Table 1: Demographic; clinical; biochemical and pathological data of the two studied groups of patients

Variables		HCC group N= 20	Chronic hepatitis group N= 20
Age in year's	mean± SD.	56.25 ± 8.13	46.22 ± 8.45
Sex			
Male No (%)		19 (95)	15(75)
Female: No (%)		1 (5)	5 (25)
PH of anti-Sch. ttt.			
Negative No (%)		9 (45)	8 (40)
Positive No (%)		11 (55)	12 (60)
AST IU/L	median (range).	98 (33-300)	53 (33-83)
ALT IU/L	median(range)	35.6 (16-221)	57 (28-192)
Albumin gm/dl	median(range)	2.5 (1.5-3.2)	2.8 (1.8-3.5)
Total bilirubin mg/dl	median(range)	1.6 (1-2.2)	1.4 (1-2.2)
AFP ng/ml	median (range).	21 (8-7460)	5 (1.30-75)
CHOL mg/dl	mean ± SD.	108.00 ± 16.35	121.66 ± 33.29
TRG mg/dl	mean ± SD.	91.20 ± 44.69	74.00 ± 13.11
HDL mg/dl	mean ± SD.	41.00 ± 18.29	28.00 ± 15.11
LDL mg/dl	mean ± SD.	42.30 ± 18.46	114.00 ± 16.35
HCC lesions Number:			
1	No (%)	9 (45)	
Multiple	No (%)	11 (55)	
Site:			
Rt. lobe No (%)		7 (35)	
Lt. lobe No (%)		1 (5)	
Both No (%)		12 (60)	
Size cm		3.65 ± 1.75	
Grade :			
I No (%)		4 (20)	
Π No (%)		16 (80)	
Type:			
Trabecular No (%)		8 (40)	
Mixed No (%)		12 (60)	
Liver background :			
1-Mixed cirrhosis with mild activity	No (%)	2 (10)	5 (25)
2-Mixed cirrhosis with moderate activity	No (%)	18 (90)	15 (75)

PH of anti-Sch. ttt: past history of antischistosomal treatment. AST: aspartate transaminase; ALT: alanine transaminase; AFP: α-fetoprotein; CHOL: cholesterol; TRG: triglycerides; HDL: high density lipoprotein; LDL: low density lipoprotein.

microRNAs expression

Calculated RQ expression of miRNAs 21;199a and 155 revealed that miRNAs 21 and 155 were over expressed in sera of patients with HCC

compared to patients with chronic hepatitis (p < 0.0001) (Table 2). While serums mean values of miR 199a was significantly decreased among HCC group patients when compared to patients with chronic hepatitis (p < 0.0001) (Table 2). Correlation of serum miR 21 to age; tumor size and biochemical and molecular investigations revealed that miR 21 was directly correlated to α-fetoprotein (P = 0.006, significant); HDL (p < 0.0001, highly significant); miR -199 was inversely correlated to α-fetoprotein (P = 0.009, significant); miRNA 155 as directly correlated to α-fetoprotein (p = 0.001, significant) and serum total bilirubin (p = 0.037, significant). Otherwise, there was no significant correlation of serum miRNAs to other studied variables. There were no significant impacts of tumor characters as regards number; site; size and microscopic features on serum or tissue expression of both studied miRNAs.

Table 2: Comparison between HCC group and chronic hepatitis group as regards mean values of serum mirNAs and apoptotic markers

mirRNAs (Mean ± SD)	HCC group N = 20	Chronic hepatitis group N = 20	Controls N = 25	P value
Serum miR 21	7.76 ± 2.66	2.96 ± 1.92	1.2 ± 0.8	0.0001**
Serum miR 199-a	0.79 ± 0.62	2.15 ± 1.048	2.5 ± 1.01	0.0001**
Serum miR 155	10.25 ± 3.31	5.18 ± 2.45	1.1 ± 0.7	0.0001**
Serum PDCD4	12.78 ± 5.36	6.87 ± 1.76	4.37 ± 1.13	0.0001**
Serum CYTC	12.29 ± 4.87	7.63 ± 1.92	4.09 ± 0.92	0.01*

*: P value is significant; **: P value is highly significant.

Serum values of apoptotic markers

We found that serum levels of PCDC4 and CYTC were increased in patients with HCC when compared to chronic hepatitis patients. They were also increased in patients with chronic hepatitis when compared to controls (p < 0.05, significant) as shown in Table 2.

Correlations of apoptotic markers with clinical and biochemical investigations revealed that serum values of PDCD4 was directly correlated with age and α fetoprotein (p = 0.004; 0.029 respectively, significant). On the other hand, serum values of CYTC was inversely correlated with SGPT (p = 0.007, significant) and directly correlated with total serum bilirubin (p = 0.013, significant). Analyzing the impact of HCC characters on serum values of PDCD4 and CYTC proved that the mean values of both PDCD4 and CYTC were significantly higher in cases with single lesion of HCC (p < 0.05, significant). Right lobe location of HCC lesions has highest mean values of PDCD4 (p < 0.05, significant). As regards grade of differentiation, grade Π have higher mean values of CYTC (p < 0.05, significant).

Correlations between apoptotic markers and miRNAs revealed that there was direct correlations between apoptotic markers and oncomirs miRNAs 21 and 155 while apoptotic markers were inversely correlated with miRNA 199-a as shown in Table 3.

Table 3: Correlations between serum mirNAs and apoptotic markers

Variables	Serum PDCD4	Serum CYTC
Serum miR 21 Mean ±SD	R = 0.538** P = 0.0001	R = 0 .570** P = 0.0001
Serum miR 199-a Mean ±SD	R = - 0.330* P = 0.041	R = - 0.511** P = 0.001
Serum miR 155 Mean ±SD	R =0.575** P = 0.0001	R = 0.334* P = 0.038

*:P value is significant; **: P value is highly significant.

Diagnostic accuracy of both microRNAs and apoptotic markers for HCC

As regards the diagnostic accuracy of the studied mirNAs and apoptotic markers for HCC, it was found that highest sensitivity was achieved by mir 21 and α fetoprotein while least sensitivity was achieved by CYTC. Serum α-fetoprotein has the least specificity while mir 21 and CYTC have the highest specificity. Combining mirNAs with each other resulted in 100% sensitivity and specificity except combined miR199-a and miR 155 have 88.90% specificity. Combined PDCD4 and CYTC can diagnose HCC with 94.70% sensitivity and 88.90% specificity as shown in Table 4.

Table 4: Diagnostic accuracy for both serum mirNAs and apoptotic markers as early detectors for HCC

Group	Parameter	Area under the curve	Cutoff value	Sensitivity %	Specificity %
HCC	Serum miR 21	0.981	3.800	100.0 %	88.90%
	Serum miR 199-a	0.843	1.150	83.30%	77.80%
	Serum miR 155	0.907	6.300	94.40%	77.80%
	Serum PDCD4	0.895	8.337	89.50%	77.80%
	Serum CYTC	0.798	9.610	63.20%	88.90%
	Serum α-fetoprotein	0.832	8.050	100.0 %	69.2 %
	combined miR 21 and miR 199-a	1.000		100.00%	100.00%
	combined miR 21 and miR 155	1.000		100.00%	100.00%
	combined miR199-a and miR 155	0.987		100.00%	88.90%
	Combined PDCD4 and CYTC	0.918		94.70%	88.90%

Discussion

One hundred seventies (170) million people are infected with Hepatitis C virus (HCV) all over the world [13]. Progression of hepatic inflammation to liver fibrosis, advanced fibrosis and liver cirrhosis present the platform for HCC development [14]. It was supposed that contribution of the apoptotic process during liver fibro genesis may be important for chronicity persistence, failure of response to antiviral drugs, fibrosis progression, and liver carcinogenesis [15].

It was found that miRNAs represents the genetic signature for many diseases including liver

cancer [16]. miRNAs usually target many genes, half of these genes are located in cancer-associated regions, including common chromosomal breakpoints, regions of loss of heterozygosity (LOH), amplified regions, fragile sites and hotspots for papilloma virus integration sites [17].

Apoptosis counterbalance the cell proliferation; so that, it is important for tissue homeostasis. Some of miRNAs influence cancer development might be by regulating apoptosis. This was approved by many studies [18].

Hou et al. used NGS, which is a high-throughput technology that supplies universal information on all miRNAs in only one sample [19]. They investigated the miRNomes in human normal liver, inflamed liver due to viral hepatitis, and liver cancer. They found nine miRNAs accounted for ~88.2% of the miRNome in human liver. The three most represented miRNAs were mRr-122, miR-192, and miR 199 a/b-3p. In HCC miR-199a/b-3p is lowered, this is accompanied with bad prognosis. Moreover, both in vitro and in vivo, the PAK4/Raf/MEK/ERK pathway is inhibited by miR-199a/b-3p targeting tumor-promoting PAK4 to suppress HCC growth.

We suggest that rate of hepatocyte death increases during liver carcinogenesis and this may explain high serum levels of PCDC4 and CYTC among patients with chronic hepatitis and HCC. All HCC cases in the current study have liver cirrhosis as a background. This background with continuous progression of inflammation may have an impact on increased serum values of apoptotic markers among cirrhotic and malignant patients.

Programmed cell death 4 (PDCD4) proteins is a translational repressor that blocks helicase activity leading to negative effects on/control of the inception of mRNA translation [20]. Some studies have found a task for PDCD4 as a tumor oppressor that is missed in definite aggressive malignancies [21]. Surprisingly, recent evidence also proved that the mission of PDCD4 can be changed by the cofactor protein arginine methyltransferase 5 (PRMT5) and that arginine methylation of PDCD4 leads to progression of malignant transformation [22]. Therefore, there is strong evidence that PDCD4 has a serious role in the regulation of carcinogenesis and that its deregulation has significant outcome in cell growth and carcinogenesis.

In our clinical practice, we find that late diagnosed HCC is an aggressive disease with high mortality rate; decreased survival rate and poor prognosis. So that search for novel molecular biomarkers for early detection of HCC will equal early therapeutic intervention; increased survival rate; better prognosis with improved quality of life for these patients.

In the current study, we research for the relation between serum values of microRNAs and serum values of apoptotic markers in a trial to provide an in-depth view of genetic alteration patterns occurring in HCC and enable the discovery the mechanism by which microRNAs act in pathogenesis of HCC that might potentially be new diagnostic and/ or targets for HCC treatment

We found that apoptotic markers were directly correlated with oncomirs 21 and 155 while they were inversely correlated with tumor suppressor mir 199-a. Our findings were against findings of previous studies, as Chu and his colleagues who reported that oncomirs inhibit apoptosis and down regulated miRNAs induce apoptosis during pathogenesis of HCC [23].

Zhu and his colleagues demonstrated that miR-21 was increased in liver cancer tissues and cell lines and was a strong stimulator of the migrative/invasive abilities of malignant liver cells. They also proved that a miR-21 inhibitor restricted HepG2 cell immigration and metastasis by direct/indirect control of PDCD4 and downstream signaling pathway molecules (p-c-Jun/AP-1, MMP-2 and MMP-9), and that the transcription factor AP-1 directly activates miR-21 transcription. They, therefor, suggest that a positive feedback loop of miR-21-PDCD4-AP-1 maintains the miR-21-mediated biological effects of the liver cancer phenotype. They concluded that molecular introduction design interfering with the miR-21-PDCD4-AP-1 feedback loop might supply strong base for inhibiting invasion/metastasis in liver cancer in the near future [24].

Cheng and his colleagues used antisense RNA library to specifically knock down 90 human miRNAs in two different cell lines – HeLa (cervical carcinoma) and A549(lung carcinoma) – and tested for alterations in cell proliferation or apoptosis. In HeLa cells, inhibition of 19 miRNAs led to reduced cell growth, while inhibition of two miRNAs, miR-21 and miR-24, resulted in greater cell growth. Interestingly, inhibition of miR-24 in A549cell s led to the complete opposite phenotype: significant decreased of cell proliferation [25].

This result supposed that depending on the cellular environment or context, the same miRNA may have totally different effects. The ant proliferative activity of miR-21 in HeLa cells is also intriguing, because miR-21 has been proved to act as an ant apoptotic factor in human glioblastoma cells; therefore, one would have expected inhibition of miR-21 to result in a reduction of cell growth. Finally, inhibition of miR-21 in A549cells did not lead to either specific up- or downregulation of cell proliferation. Therefore, likely to miR-24, miR-21 might have different tasks in different organs. Cheng and his colleagues also reported that inhibition of seven miRNAs (miR-1d, 7, 148, 204, 210, 216 and 296) result in increased caspase-3 activity and one miRNA

(miR-214) results in reduced activity. How the various miRNAs affect apoptosis remains to be investigated. Nevertheless, these experiments show the potential of such global screens to detect important candidate miRNAs, which can then be investigated further using more significant procedures. As discussed above, miR-21 can also act as an antiapoptotic factor. Glioblastoma, a highly malignant human brain cancer, strongly increased miR-21. Chan and his colleagues found greatly elevated levels of miR-21 in human glioblastoma malignat tissues, in early-passage glioblastoma cultures and in six established glioblastoma cell lines. Knock down of miR-21 in cultured glioblastoma cells led to a marked decrease in cell number. This reduction was not due to large differences in cell growth, but rather due to an increase in apoptosis, as detected by caspase-3 and -7 enzymatic activities and TdT-mediated dUTP nick-end labeling (TUNEL) staining. How miR-21 down regulates apoptosis, and whether it acts directly or indirectly remains to be detected [26].

Palma and his colleagues reported an anti-leukaemic role for miR-155 in human FLT3-wildtype AML, by initiating cell apoptosis and myelomonocytic differentiation, which is in contrast to its previously suggested role as an oncogene. This highlights the complexity of gene regulation by microRNAs that depending on disease context or tissue type may have tumor repressor or oncogenic effects [27]. Here in the current study we can report that deregulation of the studied microRNAs have significant roles in development of HCC. They are powerful early diagnostic noninvasive markers for HCC. HCC usually develops on cirrhosis in cases of HCV infection as we found that liver background among HCC patients was mixed cirrhosis and the activity was mostly moderate which support that the process of inflammation in patients with HCV related HCC is continuous. The underlying mechanism for liver carcinogenesis is completely complex.so that, elevated serum apoptotic markers among HCC patients may be referred to continuous inflammatory process of underlying cirrhosis. According to our findings, it seems that oncogenic microRNAs induce liver carcinogenesis in HCV patients irrespective of suppression of apoptosis and the down regulated microRNA did not induce apoptosis to control tumorigenesis among HCV patients. We can suggest another explanation for the relation between microRNAs and apoptotic markers in the present study which is the process of underlying inflammation during HCV infection is not only continuous but it also exceeds the process of carcinogenesis. However, our findings may pave the way for search for treatment that target apoptosis with treatment target overexpressed or replace down regulated microRNAs to control the underlying mediators of HCC pathogenesis.

In conclusion, both microRNAs and apoptotic markers have roles in HCC pathogenesis. They could be used as early diagnostic markers for HCC. Further efforts must be paid to pave the way for emerging therapies to target apoptotic markers and expressed micoRNAs to control HCC development.

Author contribution

WME, KSA and MA proposed the study. RRK supplies tissues and blood samples. YAE, HHF and AEH help in collecting samples, all cooperate in writing the paper.

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Clinical Science

Fractional Carbon Dioxide Laser in Treatment of Acne Scars

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Key words: Acne scars; aesthetic dermatology; carbon dioxide laser systems; subjective evaluation of adverse effects by the therapy; scale of satisfaction by the treatment.

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Abstract

BACKGROUND: Scars appear as a result of skin damage during the process of the skin healing. There are two types of acne scars, depending on whether there is a loss or accumulation of collagen: atrophic and hypertrophic. In 80-90% it comes to scars with loss of collagen compared to smaller number of hypertrophic scars and keloids.

AIM: The aim of the study was to determine efficiency and safety of fractional carbon dioxide laser in the treatment of acne scars.

MATERIAL AND METHODS: The study was carried out in Acibadem Sistina Clinical Hospital, Skopje at the Department of Dermatovenerology, with a total of 40 patients treated with fractional carbon dioxide laser (Lutronic eCO2). The study included patients with residual acne scars of a different type.

RESULTS: Comedogenic and papular acne in our material were proportionately presented in 50% of cases, while the other half were the more severe clinical forms of acne - pustular inflammatory acne and nodulocystic acne that leave residual lesions in the form of second, third and fourth grade of scars.

CONCLUSION: The experiences of our work confirm the world experiences that the best result with this method is achieved in dotted ice pick or V-shaped acne scars.

Introduction

Acne is a common disease. Acne has a prevalence of 90% in adolescents and persists to adulthood in approximately 12-14% of cases, with serious psychological and social implications.

Scars appear as a result of skin damage during the process of the skin healing. There are two types of acne scars, depending on whether there is a loss or accumulation of collagen: atrophic and hypertrophic. In 80-90% it comes to scars with loss of collagen compared to smaller number of hypertrophic scars and keloids.

Atrophic scars are classified into ice pick, rolling and boxcar type. Dotted, ice pick represents 65-70% of atrophic scars, rolling type 20-30% and boxcar type 15-25%.

Ice pick scars are narrow (2 mm), dotted and deep scars, with a wide opening and a deep infundibulum in the form of the letter V.

Rolling scars are wider (up to 5mm) and they reach to the subcutaneous adipose tissue. They give a distorted appearance to the skin, in the form of the letter M.

Boxcar scars have clearly visible vertical edges; they are wider than ice pick scars and take U shape with a broad and visible base [1].

Qualitative scarring gradation is applied according to Goodman and Baron [2]

The first grade consists of macular hyper- and hypopigmentations that are visible. The second grade includes mild atrophy or hypertrophy, but can be concealed with cosmetics. The third grade consists of moderate atrophic or hypertrophic acne that can not easily be concealed with makeup and can be visible at

a distance greater than 50 cm. The most severe grade is highly atrophic or hypertrophic acne visible at a distance greater than 50 cm that are not flattened with pressure of the skin around the scars.

Table 1: Qualitative scarring grading system (Goodman and Baron) [2, 3]

Qualitative scarring grading system		In 2007 Goodman and Baron suggested global classification of acne scars in four grades
Grades of post acne scars	**Level of disease**	**Clinical features**
1	Macular	These scars can be erythematous, hyper- or hypopigmented flat marks. They do not present a problem of skin contours like other scars but of color.
2	Mild	Mild atrophy or hypertrophic scars may not be visible at a distance of 50 cm or greater, and can be covered with makeup or shadow of shaved beard hair in men.
3	Moderate	Moderate atrophic or hypertrophic scars are visible at social distance of 50 cm or greater, and are not easily covered with mascara or shadow, but still can be flattened by manual pressure of the skin.
4	Severe	Severe atrophic or hypertrophic scars are evident at a distance greater than 50 cm and are not easy covered with makeup, and can not be flattened by manual stretching of the skin.

Acne scars have a major impact on the quality of life, making their treatment to be efficient and to start as early as possible [3].

The treatment of acne scars use a variety of therapeutic modalities, such as chemical peelings [4], methods of tissue augmentation [5], dermabrasion and microdermabrasion, as well as other micro-surgical procedures.

Carbon dioxide laser

The purpose of minimally invasive treatments in aesthetic dermatology is to obtain better effects with as much smaller thermal trauma to the skin as possible, while keeping the epidermis intact. These methods could be laser and non-laser. Lasers used for that purpose could be non-ablative and ablative. Fractional carbon dioxide laser is the most used today of all laser treatments.

Carbon dioxide laser systems that emit light of 10600 nm maintain the leading position in dermatology. They are the gold standard in the treatment of acne scars. The advantages of carbon dioxide lasers over conventional surgery are less tissue damage and less edema, as well as shorter recovery time. However, the noticed thermal trauma to the surrounding tissue constituted a major problem. [6] The first continuous wave lasers were limited in their use due to their non-specific thermal effect. Improving laser technology led to the use of pulse and ultrapulse laser systems that minimize thermal trauma [7].

eCO_2 laser is the latest generation of carbon dioxide fractional lasers, with combined fractional technology and deep ablative effect of CO_2 laser. Micro-ablative columns of laser penetrate deep into the skin with a maximum depth of 2.5 mm. The wavelength of 10600 nm has a high rate of water absorption. Thus greater epidermal damage is avoided, while lateral thermal damage is reduced. In comparison to similar CO_2 laser systems it causes less damage and also affect the remodelling of collagen fibers in the reticular dermis.

CO_2 lasers have a double effect – they encourage renewable processes of the wound and incite increased production of myofibroblasts and matrix proteins such as the hyaluronic acid.

Previous clinical and histological studies had shown efficacy of CO_2 laser skin renewal in atrophic acne cicatrixes, with improvement of 50-80% [8].

Candidates for laser treatment should not receive oral retinoids at least a year, have no active herpes viral infection 6 months prior to the treatment, nor a history of keloids and hypertrophic scars. Patients with a higher skin phenotype are at higher risk of hyperpigmentation than patients with a lower phenotype. All ablative lasers have a risk of complications and adverse effects. The adverse effects of the ablative lasers of first generation are classified as short-lived (bacterial, fungal or herpetic infections) and long-lasting (persistent erythema, hyperpigmentation, scarring) [9, 10].

Complications

As a complication after laser treatment with carbon dioxide laser, the scars are result of excessive treatment on the area (including excessive energy or density or both of them). It is especially important to take this into account when treating sensitive parts such as eyelids, upper neck, and particularly lower part of the neck, neckline and chest [11, 12]. The new concept of fractional photothermolysis is designed to create microscopic thermal wounds that cause homogeneous thermal damage to a certain level of the skin, in contrast to chemical peeling and traditional laser remodelling. Previous studies have proved the efficiency of fractional photothermolysis in the treatment of acne scars [13] with particular attention to the dark skin to avoid post-inflammatory hyperpigmentations [14]. A new mode of treatment is the so-called fractional photothermolysis, with specific laser devices that create thin microscopic wounds surrounded by undamaged tissue in the patients, which is the advantage of these new devices. These laser systems have a more modest result than traditional laser systems but have very few adverse effects and short recovery period [15].

Topographic analyses showed that the depths of acne scars have significantly improvement that ranges between 43-80%, an average of about 66.8% [16]. Different experiences of numerous authors suggest that combining the technology with fractional photothermolysis is a safe and effective treatment in acne scars. In comparison to the conventional

ablation with conventional CO_2 laser, the adverse effects with fractional photothermolysis are much improved. It is believed that rapid re-epithelialiazation of the surrounding undamaged tissue is responsible for comparatively faster recovery [17-19].

Abnormalities in pigmentation that accompany the laser treatment are always worrying. Alster and West found 36% incidence of hyperpigmentation after using the conventional CO_2 resurfacing, compared to the ablative photothermolysis (AFR), probably associated with a shorter period of recovery and post-treatment erythema. And treatment strategy to optimize the treatment parameters is to apply an optimal energy and interval between the sessions, and a longer follow-up period.

The aim of the study was to determine efficiency and safety of fractional carbon dioxide laser in the treatment of acne scars.

Material and Method

The study was carried out in Acibadem Sistina Clinical Hospital, Skopje at the Department of Dermatovenerology, with a total of 40 patients treated with fractional carbon dioxide laser (Lutronic eCO_2). The study included patients with residual acne scars of a different type.

Exclusionary criteria in the study were the use of oral retinoids in period of 6 months prior to the laser treatment; the use of anticoagulation therapy; age under 17; and the presence of systemic diseases in the patient.

A questionnaire was prepared to collect the data needed for the objectives of the study. Every patient gave consent to participate in the study after being informed about the nature of the treatment and the research.

The treatment was carried out by different protocols depending on the type of lesion and its depth. Patients were treated with eCO_2 beams of 120 and 300 nm. It used static method directly on the lesion, while the periphery was treated with dynamic module. It used different pulse energy, power, density and number of passes in a single session, and a different number of sessions depending on the clinical features of the patient.

To determine the efficacy and safety of the treatment three methods were used: a questionnaire for subjective evaluation of adverse effects by the therapy (pain, redness, and pigmentation), scale of satisfaction by the treatment and comparison of digital photographs before and after the treatment.

Results

The average age of patients in our study for men and women collectively was 28.2 years - the average age for men was 28.33, and for women 26.26. The average total duration of acne was 4 years in men and 9 years in women. More severe clinical forms of acne conglobata and nodular cystic acne appeared in men. Comedogenic and papular acne in our material were proportionately presented in 50% of cases, while the other half were the more severe clinical forms of acne - pustular inflammatory acne and nodulocystic acne that leave residual lesions in the form of second, third and fourth grade of scars. Average age of acne appearance was 15.5 years. They started earlier in women than in men. Average duration of scars was 12 years.

Table 2: Efflorescence distribution in the population by sex, age and duration

Sex	Men	Women	Total population
Average age (range), years	19-38	18-42	18-41
Average age of the patients (range)	28.33	26.26	28.2
Average total duration of acne (range)	4 (1-15) years	9 (1-16) years	6 months-16 years
Average age at appearance of scars (range)	17-38	15-42	15-42
Average total duration of scars (range)	2-16	1-15	1-16

The success of treatment is objectively seen in photographs taken before and after the treatment.

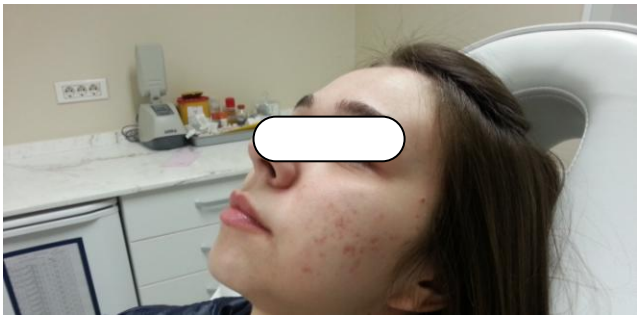

Figure 1: Before laser treatment

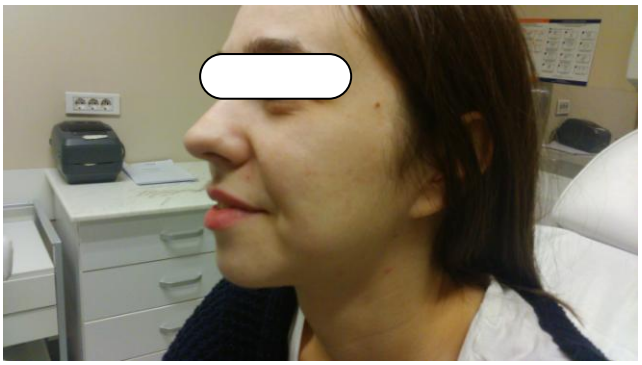

Figure 2: After 3 treatments with fractional carbon dioxide laser

Table 3: Preview of scars' severity before and after the start of treatment and number of laser sessions in average

Type of scars (number of patients treated)	Average score before the treatment (on a scale of scarring grading intensity of 1-5)	Average score after the treatment (on a scale of scarring grading intensity of 1-5)	Average number of treatments
Ice pick (18)	3.46	1.86	3
Rolling (8)	4.1	2.37	4-5
Boxcar (7)	3.5	1.9	3-4
Pigmentations (5)	2.2	1.4	3

Discussion

Fractional photothermolysis was first described by Anderson and Parrish in 1983 in the journal Science [7].

Laser technology has progressed continuously. The application of fractional carbon dioxide laser in the treatment of acne blemishes was approved by the FDA in 2007.

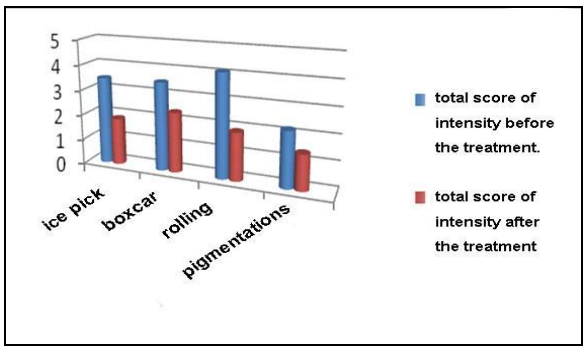

Figure 3: Scarring intensity on a scale of 1-5 before and after the start of the treatment, according to the scar type classified by Goodman/Baron [2]

Numerous researches on the impact of fractional laser on acne scars indicate great success of the treatment [7, 10, 11, 13-15].

Most patients who participated in the researches noticed improvement of the face condition and loss of blemishes [15, 16, 19].

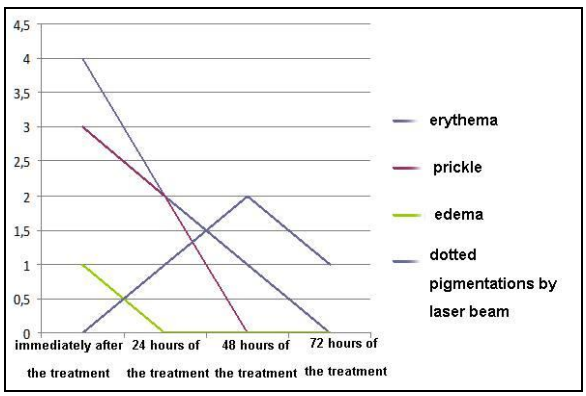

Figure 4: Transient adverse effects on the skin by laser treatment, on a scale of 1-5 /observation based on the average assessment by the doctor, the patient and another doctor at the department

According to relevant studies, ablative CO_2 lasers are ideal for the treatment of grade 3 scars caused by acne [19].

In the treatment of scars, the longer time has passed since the appearance of a certain cicatrix it is more severe and refractory for therapy.

It can be concluded that the selection of patients and response to the therapy in patients participating in this study coincide with world experiences and experiences from great number of research studies.

In conclusion, the results of this study show that fractional laser treatment in these patients is fast and practical. Depending on the scar condition it could be made 6 treatments, and even if the scar is not completely disappeared after the treatment, it significantly improves the look. In 35% of cases there is a complete reduction of scarring, and in 40% a significant reduction of scars with more than 50%. The time period between two treatments is one month. Dotted and atrophic scars showed the best response to treatment with CO_2 laser. Fractional photothermolysis using fractional eCO_2 laser is effective and safe method for treating acne scars. The experiences of our work confirm the world experiences that the best result with this method is achieved in dotted ice pick or V-shaped acne scars.

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Association between Nephrolithiasis, Hypertension and Obesity in Polycystic Kidney Disease

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Key words: nephrolithiasis; hypertension; polycystic kidney disease; body mass index, anatomic and metabolic factors.

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Abstract

AIM: We aim to define the correlations between nephrolithiasis, hypertension, age and obesity in patients with autosomal dominant polycystic kidney disease (ADPKD) in Albania.

MATERIAL AND METHODS: We included 100 patients with autosomal dominant polycystic kidney from 2011 to 2014. The patients underwent X-ray and renal ultrasonography. We performed the metabolic evaluation of blood and urine.

RESULTS: The patients with renal stones had a higher level of mean systolic and diastolic blood pressure compared with patients without stones (155 ± 12 mmHg vs. 145 ± 8 mmHg, and 105 ± 0.9 mmHg vs. 92 ± 1.28 mmHg, respectively). Patients with renal stones were older (47 ± 15 vs. 38 ± 5 years), had a higher prevalence of obesity [body mass index (BMI): 28 ± 2.4 vs. 25.7 ± 0.6], had higher levels of total cholesterol level (220 ± 5 mg/dl vs. 203 ± 4 mg/dl) as well as triglyceride levels (160 ± 9 mg/dl vs. 126 ± 4 mg/dl), compared with no renal stone individuals.

CONCLUSION: ADPKD patients with renal stones in our study had a higher mean level of systolic and diastolic blood pressure, BMI and cholesterol and triglycerides levels compared with individuals without renal stones.

Introduction

Nephrolithiasis is an important manifestation of autosomal dominant polycystic kidney disease (ADPKD), which ranges from 8% to 36% in different studies [1-10], about twice higher than in general population.

Most ADPKD patients affected by nephrolithiasis are above 40 years old. The disease frequency begins to rise between 20-40 years, making very uncommon finding nephrolithiasis under the age of 20 years [11, 12]; even so there are studies that have found cases of nephrolithiasis in ADPKD under the age of 20 years [9, 13].

Recent reports suggest that the etiology of stone disease in ADPKD is multifactorial in nature, including both anatomic and metabolic factors, and a combination of these factors [6, 7, 9, 11, 12, 14]. Hypertension and obesity are also social diseases with important epidemiological similarities to nephroloithiasis. Various studies have demonstrated high calciuria in hypertensives with a linear relationship between 24-h calciuria and arterial blood pressure.

Also, body mass index (BMI) and body weight are independently associated with an increase in stone risk even though, due to a number of bias (limited weight categories, low number of obese persons in the study populations, no control group, no recording of food intake) the studies published failed to be conclusive.

We aim to define the correlations between nephrolithiasis, hypertension, age and obesity in patients with ADPKD in Albania.

Material and Methods

We included 100 patients with autosomal dominant polycystic kidney from 2011 to 2014 in a prospective controlled study. The ethic committee of the Faculty of Medicine had approved the study. The patients underwent X-ray and renal ultrasonography. We performed the metabolic evaluation of blood and urine. The diagnosis for ADPKD is done based on criteria established by Ravine et al. in 1994 [15]: the presence of three or more (unilateral or bilateral) renal cysts for individuals aged between 15 to 39 years, two or more cysts in each kidney for individuals aged 40 to 59 years, and four or more cysts in each kidney for individuals over 60 years old.

All patients underwent renal ultrasound to determine cyst number and predominant cyst size. Patients with nephrolithiasis were defined as those with stones within the collecting system. To diagnose renal stones we used all imaging methods, renal ultrasound (identification of an echogenic focus with posterior acoustic shadowing within the kidney), plain abdominal kidney–ureter–bladder film (KUB) for radiopaque stones, intravenous pyelography (more rarely), which can provide both anatomical and functional information on stones and the urinary tract and non-contrast helical computed tomography (CT) scan in cases when nephrolithiasis was not observed by KUB or renal ultrasound.

Subjects were considered normotensive if they had not taken medication for hypertension, the mean of three systolic reading was less than 140 mmHg, and the mean of three diastolic reading was less than 90 mmHg at baseline [16, 17].

Table 1: Demographic data of patients

	Patients with nephrolithiasis (58 patients)	Patients without nephrolithiasis (42 patients)	*P* value
Age	47 ± 15 years	38 ± 5 years	<0.05
Sex			
Females/Males	39/19	19/23	NS
Area of origin			
Rural area/Citizen area	40/18	28/14	<0.001
Renal function			
GFR≥60 ml/min/GFR<60 ml/min	42/16	30/12	NS
BMI (kg/m^2)	28 ± 2.4	25.7 ± 0.6	<0.05
Smoking (Yes/No)	18/40	19/33	NS
Mean blood pressure values (mmHg)			
Mean systolic pressure	155 ± 12	145 ± 8	<0.05
Mean diastolic pressure	105 ± 0.9	92 ± 1.28	

GFR- glomerular filtration rate, BMI- body mass index, NS- not significant.

All variables are presented as mean ± SD. Differences were considered significant at the $p < 0.05$ levels.

Pearson's correlation was used in order find the associations between diastolic blood pressure and kidney size.

Results

Nephrolithiasis was present in 58 of our patients with ADPKD (58%). Thirty-nine patients with kidney stones were women (Table 1). The stones were composed primarily of urate (47%) and calcium oxalate (39%), and other compounds 14%. Sixty-six per cent of patients with nephrolithiasis (38 patients) had hypertension and 40% of them had increased BMI. The patients with renal stones had a higher level of mean systolic and diastolic blood pressure compared with patients without stones (155 ± 12 mmHg vs. 145 ± 8 mmHg, and 105 ± 0.9 mmHg vs. 92 ± 1.28 mmHg, respectively). Patients with renal stones were older (47 ± 15 vs. 38 ± 5 years), had a higher prevalence of obesity (mean BMI: 28 ± 2.4 vs. 25.7 ± 0.6), had higher levels of total cholesterol level (220 ± 5 mg/dl vs. 203 ± 4 mg/dl) as well as triglyceride levels (160 ± 9 mg/dl vs. 126 ± 4 mg/dl), compared with no renal stone individuals. Ten patients were with diabetes mellitus; from there 6 patients were with stones from uric acid and all of them with increased BMI.

Table 2: The correlation of hypertension with kidney size and renal volume

	Hypertensive patients	Normotensive patients	Pearson's correlation	*P* value
Kidney size	16.31 ± 1.7 cm	12.4 ±1 cm	r=0.60	<0.039
Mean renal volume	580 ± 41 cm3	360 ± 42 cm3	r=0.75	<0.005

The kidney size (longitudinal diameter) was significantly greater in the hypertensive patients compared with those normotensive (16.31 ± 1.7 cm vs. 12.4 ± 1 cm, $p < 0.039$) (Table 2). Systolic and diastolic blood pressure correlated with kidney size ($p < 0.05$; $r = 0.55$; $r = 0.63$). Also, mean renal volume was significantly greater in the hypertensive patients versus the normotensive patients (580 ± 41 cm^3 vs. 360 ± 42 cm^3, $p < 0.005$) (Table 3).

Table 3: The correlation of blood pressure with kidney size

Blood pressure	Mean ± SD	Kidney size	Pearson's correlation	P value
Systolic blood pressure	155 ± 12	16.8 ± 1.2 cm	R = 0.55	0.04
Diastolic blood pressure	105 ± 0.9	11.5 ± 0.9 cm	R = 0.63	0.03

Discussion

Our study confirmed that incidence of stone disease is greater in hypertensives than in normotensives also in ADPKD patients. By the same token, the incidence of hypertension is greater in stone formers than in non stone formers, but it is not clear whether nephrolithiasis is a risk factor for hypertension or vice versa. It has been suggested a

relationship between structural deformation and hypertension in the ADPKD patients [18]. In the present study, hypertension was associated with greater renal structural abnormalities. Specifically, the hypertensive ADPKD patients have greater renal volumes and cystic involvement than well-matched normotensive ADPKD patients. This supports the hypothesis that cyst decompression has been associated with a decrease in blood pressure and an improvement of renal function [19, 20].

Metabolic factors are equally important in the stone forming process, as are anatomical anomalies in ADPKD. Gambaro et al. observed that metabolic disturbances were more frequent in stone-forming patients with renal anatomical anomalies [7].

Uric acid and calcium stones are the most frequent types of stones in ADPKD patients even though hyperuricosuria and hypercalciuria do not occur consistently in ADPKD patients [6, 10]. Umbreit et al. in his study also found that 55% of treated stones in ADPKD were primarily uric acid calculi [21]. Meanwhile, Daudon found that uric acid stones are the predominant type in cystic renal abnormalities [22]. The reason for this predominance of uric stones in this kind of patients is still a debate because of the low prevalence of hyperuricosuria (18-28%) in ADPKD with normal renal function [4, 13], and more over the lack of statistically difference in uricosuria between ADPKD patients with or without lithiasis [9]. The metabolic abnormalities in these patients include hypocitraturia, hyperuricosuria, hyperuricemia and presence of diabetic patients among them with stones from uric acid confirm the relationship between diabetes, hyperuricemia, metabolic syndrome, increased BMI and nephrolithiasis.

On the other hand, in the high frequency of nephrolithiasis in ADPKD patients contribute some non intrinsic factors such socioeconomic status of patients, geographic zones and dietary habits (most of them were from rural areas, consuming less water, more vegetable and animal proteins) [13].

In the final analysis, stone disease, arterial hypertension and excess weight/obesity prove to be closely interconnected and it is possible to intervene with targeted diets aimed at reducing the risk of illness and death from these diseases.

In conclusion, future studies in Albania should include larger population-representative samples. The association between nephrolithiasis and hypertension in patients with ADPKD is important in our patients. Except anatomic and metabolic factors, there are other contributor factors to this association like dietary habits. Both hypertension and stones might be addressed through lifestyle modification to prevent weight gain.

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Prosthetic Subclavian-Aortic Bypass as a Safe Surgical Technique for the Coarctation of the Aorta in Adults

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Key words: coarctation; adults; bypass graft; hypertension; technique.

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Abstract

BACKGROUND: Coarctation represents 5-8% of congenital heart disease. Residual hypertension remains the main problem after late correction. Surgical treatment in the adult remains a challenge for the surgeon. Our prefered method used in this category is the Subclavian-aortic bypass.

MATERIAL AND METHODS: We have reviewed our registry for the period of 12 years (1998-2010) and we found a group of 18 adult patients being operated for coarctation of the aorta. The mean age of this group of patients was 24.7 ± 8.43 years (range 16-42 years). 13 were males and 5 females.

RESULTS: Sugical technique: Most of the patients (13 pts, 72%) which were obviously treated with subclavian-aortic bypass with a Dacron prostheses. Mean preoperative and postoperative pressure gradients measured by echocardiography were 77.7 ± 20.16 mmHg and 22.3 ± 9.14 mmHg respectively. No mortality was observed in this series of patients. Chylothorax was the only complication observed in one patient in the early postoperative period.

CONCLUSION: Coarctation of the aorta in adults is treated with optimal early results at our surgical centre. Subclavian-aortic bypass grafting requires less aortic dissection, can be performed with a partially occluding clamp, and does not compromise the spinal cord vascularization.

Introduction

Aortic coarctation is a congenital luminal narrowing (usually around the origin of the left subclavian artery), which obstructs blood flow. Coarctation represents 5-8% of congenital heart disease [1]. Since the first successful repair of aortic coarctation [2], treatment is now well established, but residual hypertension remains the main problem after late correction [3].

However, despite a 60-year experience, surgical repair of coarctation remains a technical challenge for the surgeon in the subset of older patients with atypical anatomic forms of coarctation: long coarctation, aortic wall calcifications, extensive or minimal collateral circulation, and multiple previous operations. These patients are exposed to major operative risks with use of the various surgical anatomic techniques involving direct exposure of the diseased aorta: end-to-end anastomosis, subclavian flap aortoplasty, subclavian displacement, prosthetic patch aortoplasty, or prosthetic patch interposition grafting. Specific risks are linked either to the dissection of the aortic and periaortic structures or to spinal cord ischemia associated with total aortic cross-clamping. To minimize these drawbacks in these patients, our prefered method used in this category is a bypass graft (subclavian artery-descending aorta).

The purpose of this study was to review our 12-year experience with this proce-dure and report the early results.

Material and Methods

We have reviewed our registry for the period of 12 years (1998-2010) and we found a group of 18 adult patients being operated for coarctation of the aorta. The mean age of this group of patients was 24.7 ± 8.43 years (range 16-42 years), 13 were males and 5 females. In three patients we had an association with bicuspid aortic valve and in one of them a dilated ascending aorta which was replaced in the same procedure via sternotomy. All patients presented with arterial hypertension and used two or more antihypertensive drugs.

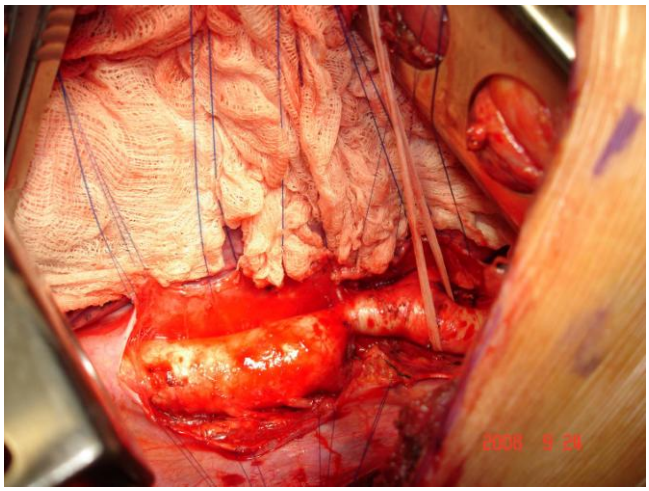

Figure 1: Surgical view of coarctation of the aorta

Results

Operative technique

Most of the patients (13 pts, 72%) were obviously treated with subclavian-aortic bypass with a Dacron prostheses. A left posterolateral thoracotomy was used in these patients. The dissection was limited to the area of the anastomoses. Proximal implantation was done on the left subclavian artery. Distal implantation was performed on the descending thoracic aorta in all instances. Both proximal and distal end-to-side anastomoses were performed under partial aortic cross-clamping using continuous 5-0 polypropylene suture.

In 3 patients resection and primary anastomosis was used. The surgical option for one patient was synthetic patch aortoplasty and for another patient resection and graft interposition.

Table 1: Patients according to the applied surgical technique

Type of operation	Number of patients
Subclavian-aortic bypass with dacron prostheses	13 pts
Resection and end to end anastomosis	3 pts
Synthetic patch aortoplasty	1 pts
Resection and graft interposition.	1 pts

No mortality was observed, and the postoperative period was generally uneventful this series of patients.

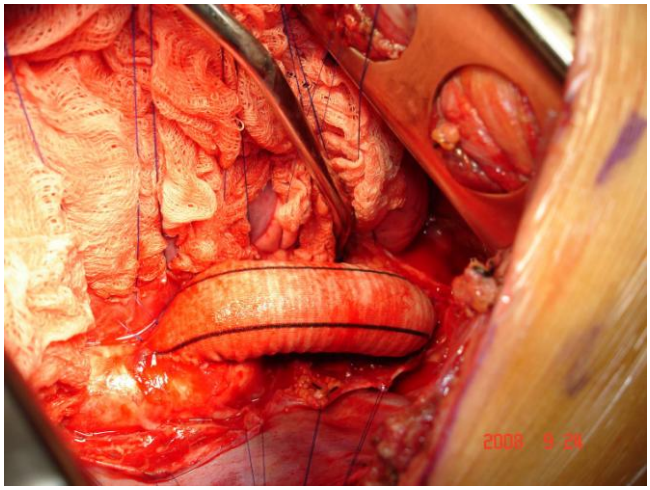

Figure 2: Prosthetic subclavian aortic bypass grafting

Mean preoperative and postoperative pressure gradients measured by echocardiography were 77.7 ± 20.16 mmHg and 22.3 ± 9.14 mmHg respectively.

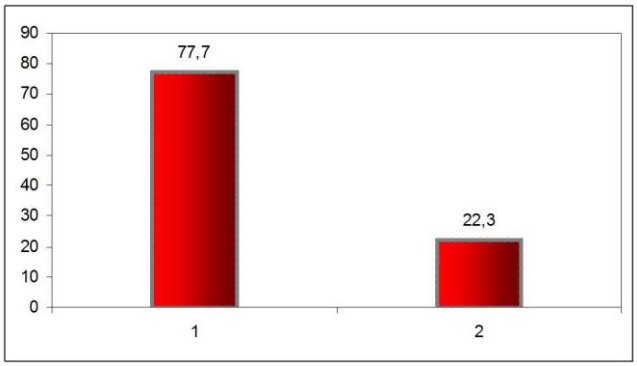

Figure 3: Reduction of gradients measured by echocardiography postoperatively

Chylothorax was the only complication observed in one patient in the early postoperative period. The patient was treated successfully in a conservative regimen. Early after the correction 12 patients (66%) were normotensive and the rest of them (6 patients, 34%) used only one drug to control the hypertension.

Discussion

Unrepaired coarctation of the aorta results in high morbidity and mortality from hypertension and associated problems, including myocardial infarction, heart failure, intracranial hemorrhage, aortic rupture,

and infective endocarditis. Without correction, most patients die before the age of 50 years [3]. Usually adult patients present with hypertension which is the main complication associated with the pathology at this age. All of our patients were diagnosed based on a work-up for hypertension. Several authors confirm that surgical repair of aortic coarctation in patients older than 20 years of age reduces systolic hypertension [4, 5].

On the basis of the worldwide experience with coarctation of aorta in adults, repair is recommended even at advanced ages. In a series, reported by Wells et al patients up to the age of 60 years appear to have benefited from the operation as indicated by improved control of systolic hypertension [5]. In our group of patients early after the repair 66% of the patients were normotensive at rest without medication. Because the risk of operation is extremely low, operation is recommended even for patients with mild preoperative hypertension [5].

Different techniques are available for the correction of this anomaly in adults. Resection and end-to-end anastomosis, which is the best choice in the pediatric population cannot always by applied in adults, and furthermore is linked with and increased risk for complications. Compared with the aorta of infants and children, the aorta of the adult patient with coarctation is relatively immobile, and there are frequently large collaterals immediately adjacent to the narrow segment. This makes bypass grafting from the left subclavian or distal aortic arch to the descending aorta, which can be performed with a partially occluding clamp, an attractive option. This is the likely explanation for its frequent use at our institution. These techniques are simple, safe, and feasible [6].

On the other hand, Campbell et al have concluded that patch aortoplasty repair of aortic coarctation should be abandoned in adults, because of the high incidence of aneurysm formation after the procedure [3].

Coarctation of the aorta in adults is treated with optimal early results at our surgical centre. Subclavian-aortic bypass grafting requires less aortic dissection, can be performed with a partially occluding clamp, and does not compromise the spinal cord vascularization. We conclude that this is a safe choice for the treatment of aortic coarctation in the adult population where other techniques are more hazardous.

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Clinical Science

Analysis of Lymphocyte Immunological Reactivity in Patients with Pleural Effusions of Different Aetiology

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Key words: pleural effusions; lymphocyte; CD markers; malignant pleural fluid; tuberculous pleural effusions.

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Abstract

BACKGROUND: The proportion of T and B lymphocytes in pleural fluids and blood may point to the presence of local immunological phenomena in pleural disorders.

AIM: Aim of study was to evaluate the lymphocyte phenotype and the ratio between helper (CD4+) and cytotoxic/suppressor (CD8+) lymphocytes in malignant and non-malignant effusions.

MATERIAL AND METHODS: We studied 48 patients with pleural effusions. First group had 18 patients with tuberculosis pleural effusions; second group had 20 patients with malignant pleural fluids, third group had 10 patients with transudates and 30 healthy controls. We investigated the distribution of T and B lymphocytes, T cells with helper/inducer CD4 or suppresser/cytotoxic CD8 phenotypes and the CD16 subset.

RESULTS: Results showed decreases levels of CD3, CD4, and CD16 T cells in blood of patients versus healthy controls. There were increases in the percentage of the CD3 and CD4 T cells in the pleural fluid compared with values in the blood with statistical significance in tuberculous pleurisy. The values of CD8 were similar in the pleural fluid and in blood. Levels of CD16 were non-significantly higher in pleural fluid in all groups.

CONCLUSION: This study confirms the hypothesis that pleural cavity is compartment with immunological reactivity and results could be used in differential diagnosis together with other examinations.

Introduction

Lymphocytes are the primary effectors of cellular and humoral immunocompetence in humans. Lymphocytic pleural effusions are characterized by divergent cellular responses depending on the etiology of disease [1]. The accumulation of fluid in the pleural space indicates the presence of systemic or local disease. Pleural exudates involve the migration of immune cells to the pleural cavity [2]. Lymphocytes dominance occurs in the most chronic pleural effusions [3, 4]. The proportion of T and B lymphocytes in pleural fluids relative to that in peripheral blood may point to the presence of local immunological phenomena in various pulmonary and pleural disorders. Tuberculosis and malignant disease are among most frequent causes of pleural effusions. In both causes, the pleural fluid is generally lymphocytic, with predominance of T lymphocytes, particularly CD4+ positive T cells [2, 5, 6]. Malignant effusions are a relatively easily accessible source of tumor-associated T cells and this represent a suitable model for the study of interactions between tumor cells and the host immune system [7].

Considering the compartmentalization of the pleural space, the association between the local and systemic cellular responses should be analyzed.

Material and Methods

We have investigated the distribution of T and B lymphocytes, T cell with helper/inducer CD4+ or suppresser/cytotoxic CD8+ phenotypes and the subset of cells with natural killer NK activity. We have used the analysis of T cell subsets by monoclonal antibodies defined markers. We studied 48 patients from Clinic of Pulmonology and Allergology with pleural effusions, divided in three groups. First group had 18 patients with tuberculous pleural effusions, Second group had 20 patients with malignant pleural effusions (mesothelioma, lung carcinoma or metastatic pleural effusions) and third group had 10 patients with transudates secondary to cardiac failure. We have also examined a group of 30 healthy controls.

In our study we evaluated:

1) the frequency of lymphocyte predominance in different malignant and non-malignant pleural effusions;
2) lymphocyte phenotype and the ratio between helper (CD4+) and cytotoxic/suppressor (CD8+) lymphocytes in malignant and non-malignant effusions.

Results were statistically elaborated according to the Student t-test and Analysis of Variance (ANOVA).

Results

According to our results there were a significant decrease of the levels of CD3, CD4, CD16 and CD22 positive cells in peripheral blood of patients with tuberculous pleural effusions versus healthy controls (Table 1). It is due to suppresser activity of lymphocytes in peripheral blood, but also to the immunological reactivity of the pleural effusions.

Table 1: Values of CD markers in blood of patients with tuberculous effusion versus healthy controls

	CD3%	CD4%	CD8%	CD16%	CD22%	CD4/CD8
Controls (N=30)	65	38	23	15	15	1.65
Tuberculous (N=18)	59.33	31.66	22	12.67	11.78	1.44
t-test	2.31	2.91	0.56	2.09	2.06	
Significance	S	S	NS	S	S	

Evaluation of the values of CD markers in peripheral blood with malignant effusions versus healthy controls, demonstrate significant decrease in patients with malignant effusions only for CD22 cells. Changes of the values for CD3, CD4, CD8 and CD16 T cells were not significant (Fig. 1)

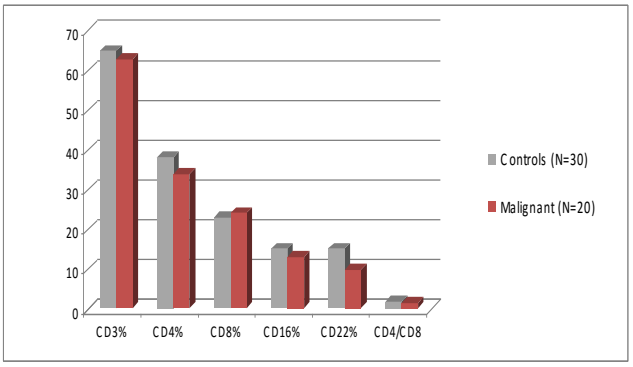

Figure 1: Values of CD markers in the peripheral blood of patients with malignant pleural effusions versus values of CD markers in peripheral blood in healthy controls

Values of CD markers in peripheral blood with transudates versus healthy controls demonstrate significant decrease in patients with transudates only for CD22 cells. Changes of the values for CD3, CD4, CD8 and CD16 T cells were not significant (Table 2).

Table 2: Values of CD markers in blood of patients with transudates versus healthy controls

	CD3%	CD4%	CD8%	CD16%	CD22%	CD4/CD8%
Controls (N=30)	65	38	23	15	15	1.65
Transudates (N=10)	59.6	33.2	22.6	13.4	9.2	1.6
T-test	1.63	0.62	0.17	1.36	3.21	
Significance	NS	NS	NS	NS	S	

In our study, the analysis of the values of CD markers in patients with tuberculous effusions, demonstrate significant increase of the percentage of CD3 and CD4 in the pleural fluid versus blood. We also noticed significant decrease of CD22 cells in pleural tuberculous effusion ($p<0.05$) (Fig. 2).

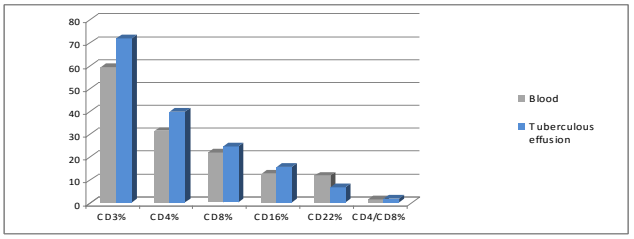

Figure 2: Values of CD markers in blood and pleural effusions in patients with tuberculous pleurisy

In patients with malignant effusions, we noticed increase of the values of CD3, CD4, CD8 and CD16 cells in the pleural malignant fluid versus blood, but these changes were not significant, ($p<0.05$) (Table 3).

Table 3: Comparison of values of CD markers in blood and pleural effusions in patients with malignant effusion

	CD3%	CD4%	CD8%	CD16%	CD22%	CD4/CD8%
Blood	63	33.8	24	13	9.7	1.41
Malignant effusion	67.68	38.63	25.37	15.67	7.67	1.52
T- test	1.18	1.62	0.66	1.42	1.26	
Significance	NS	NS	NS	NS	NS	

According to our results, in patient with transudates, the values of CD3, CD4, CD8 and CD16 cells were not significantly changed, ($p<0.05$) (Fig. 3).

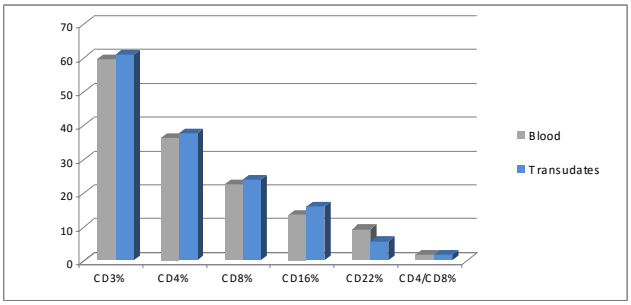

Figure 3: Values of CD markers in blood and pleural effusions in patients with transudates

Discussion

This study evaluated the concept of compartments and it also investigated the significance of immunological reactivity of pleura. In our study, according to our results, there was a significant decrease of the levels of CD3, CD4, CD16 and CD22 positive lymphocyte cells in peripheral blood of patients with tuberculous pleural effusion versus healthy controls. Shiratusci in his study demonstrate the evaluation of the values of the lymphocyte subsets and there are noticed the decrease of values of CD3 and CD4 cells in patients with tuberculosis versus healthy subjects [8]. Compared with controls, the cases showed total lymphocytopenia in peripheral blood and also major depletion of the peripheral T lymphocytes [9].

According to our results, in patients with malignant pleural effusion we noticed decreased values for CD3, CD4 and CD16 positive T cells versus healthy subjects and increases for CD8 positive T cells, but there were not significant. We noticed significant decrease only for CD22 lymphocytes. In literature it was observed lower percentage of CD4+ lymphocytes subsets and higher percentage of CD8+ lymphocytes subsets in malignant when compared to non-malignant fluids [10]. Values of CD markers in peripheral blood with transudates versus healthy controls demonstrate significant decrease in patients with transudates only for CD22 cells. Changes of the percentage for CD3, CD4, CD8 and CD16 positive T cells were not significant due to low inflammatory reactivity in the pleural space of patients with cardiac failure.

In our study, the analysis of the values of CD markers in patients with tuberculous effusions, demonstrate significant increase of the values of CD3 and CD4 positive cells in the pleural fluid versus blood. We also noticed significant decrease of the percentage of CD22 cells in pleural tuberculous effusion versus blood. In literature, it is demonstrated that pleural involvement is associated with migration of immune cells to the pleural cavity [2] and it is noticed the predominance of T helper cells into pleural space [11-13]. The lymphocytic subpopulation study confirms the concept of compartmentalization in tuberculous pleuritis, as shown by the greater number of activated T lymphocytes present in pleural fluid in comparison with peripheral blood in patients with tuberculous pleural effusions [14]. and predominance of helper cells (CD4+) in pleural fluid [12, 14, 15]. According our study the CD4/CD8 ratio was greater in pleural fluid then in peripheral blood as it is known in literature [14].

In patients with malignant effusions, we noticed increase of the values of CD3, CD4, CD8 and CD16 positive T cells in the pleural malignant fluid versus blood, but these changes were not significant due to different etiologies of malignancy. Some authors demonstrate increased CD4+ T lymphocyte subset in malignant pleural effusion [16, 17]. In the literature there are similar findings with explanation that it is due to suppresser activity of lymphocytes in peripheral blood, but also to the immunological reactivity of the pleural effusions [18]. In malignant effusions, the inflammatory processes and the immune responses induce the recruitment of cells into the pleural space [19]. The levels of CD16 (NK cells) were non-significantly higher in pleural fluid in all three groups and it show that they have not a relevant role in immunological reactivity of the pleura and diagnosis of pleurisy. Some authors found that despite a higher percentage of circulating NK cells in patients with pleural malignancies than in healthy subjects, there was a defect in recruiting NK cells in malignant pleural effusions [11, 20].

The patients with transudates secondary to cardiac failure have very small difference of the values versus other groups that is according to low immunological and inflammatory reactivity in the pleural space of these patients.

In conclusion, the values of CD3 and CD4 positive T cells were significantly higher in the pleural fluid of patients with tuberculous pleural effusions. The results suggest that the responding T lymphocytes have been portioned from the peripheral blood to the site of inflammation. The lymphocytes chemoattractans are present in the pleural fluid and these factors enhance the accumulation of T cells to the pleural cavity (especially in patients with tuberculous pleuricity). The patients with transudates secondary to cardiac failure have very small difference between the percentages in all three groups. We can say that this study confirms the hypotheses that the pleural cavity is compartment with its own immunological reactivity. The results of this study could be used in differential diagnosis only together with other clinical and biochemical examinations.

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Clinical Science

Maternal and Neonatal Effects of Vasopressors Used for Treating Hypotension after Spinal Anesthesia for Caesarean Section: A Randomized Controlled Study

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Key words: ephedrine; caesarean section; hypotension; maternal; neonatal outcome; phenylephrine.

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Abstract

AIM: The aim of the study was to examine whether ephedrine and phenylephrine were different in their efficacy for managing maternal hypotension and their effect of adverse maternal and neonatal outcome.

METHODS: A double-blind randomized controlled study in healthy pregnant women ASA physical status 2, which underwent elective caesarian delivery under spinal anesthesia. Patients were randomized to receive an intravenous bolus of either phenylephrine (Ph group) or ephedrine (E group) immediately after the episode of hypotension after spinal anesthesia. Maternal and neonatal outcomes were recorded.

RESULTS: Two hundred and two (202) pregnant women at term were entered in this study. There were no differences between group E and group Ph regarding the incidence of hypotension after vasopressor therapy, and the incidence of nausea and vomiting. There was no significant difference between groups in the first-minute and the 5th minute Apgar score, none of the neonates had the true fetal acidosis.

CONCLUSIONS: Ephedrine and phenylephrine have the same efficacy in treating hypotension after spinal anesthesia for caesarean section. The use of Phenylephrine was associated with better fetal acid-base status, and there were no differences on Apgar score values and on the incidence of maternal bradycardia and hypotension.

Introduction

Hypotension associated with spinal anesthesia is a common complication during caesarean section and can result in adverse effects for both mother and infant [1, 2]. When maternal hypotension associated with spinal anesthesia for cesarean section is severe and sustained, it can lead to maternal complications (nausea, dizziness, faintness) as well as impairment of the uterine and intervillous blood flow, with consecutive fetal hypoxia, acidosis, and neonatal depression [3]. The sympathectomy resulting from the neuraxial blockade is exaggerated by the physiological changes of pregnancy and puerperium, leading to hypotension in as much as 55%-90% of the mothers receiving spinal anesthesia for Cesarean section [4]. Phenylephrine and ephedrine are the vasoconstrictor agents which are currently being recommended and used for controlling hypotension, but still nowadays the choice of vasopressor has been debated [5].

Phenylephrine is the α-agonist recommended to treat hypotension that affects approximately half of caesarean sections under spinal anaesthesia [6, 7]. Also, ephedrine was the vasoconstrictor agent of choice in obstetric anaesthesia for many years due to

its favourable pharmacodynamic profile; many animal models have demonstrated a marked increase in uteroplacentary blood flow [5, 8]. Results of several trials suggest that phenylephrine may have similar efficacy to ephedrine for preventing and treating hypotension during spinal anesthesia [9-12]. However, the relative effects of these vasopressors on neonatal outcome [8] and maternal outcome are unclear, and there is need for a large double-blind randomized controlled trial with emphasis on important maternal and neonatal outcomes [13].

The aim of the study was to examine whether ephedrine and phenylephrine were different in their efficacy for managing maternal hypotension after spinal anesthesia for Caesarean section and their effect of adverse maternal and neonatal outcome.

Materials and Methods

This study was approved by the Ethics Committee of the University of Medicine, Tirana, Albania. It has been performed in accordance with the ethical standards displayed in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all patients. Data were made anonymous for analysis.

A double-blind randomized controlled study in healthy pregnant women ASA (American Society of Anesthesiologists) physical status 2, that underwent elective caesarian section under spinal anesthesia, during January 2013 - May 2015 period in University Hospital of Obstetrics and Gynecology "Koço Gliozheni" in Tirana.

The exclusion criteria were: emergency cesarean section, active labor, high risk pregnancies (multiple gestations, intrauterine growth retardation, preeclampsia maternal cardiovascular or pulmonary diseases) and other active medical disorders requiring regular medication), and any contraindication of spinal anesthesia (patient refusal, coagulopathy, hemorrhage or hypovolemic shock).

All patients fasted for at least 8 hours before induction of spinal anesthesia. Upon arrival to the operating room, all patients were monitored for basal vital signs (heart rate: HR, systolic and diastolic blood pressures: BPs, and pulse oximetry: SaO_2). Baseline systolic arterial blood pressure was measured by averaging 3 readings taken 1 minute apart using an automated device for non-invasive blood pressure assessment. A 16-G IV catheter was placed in a peripheral vein in the patient's upper limb, and before performing spinal anesthesia, all patients received a preload of 500 ml and a coload of 1000 ml lactated Ringer's solution.

After completion of fluid infusions all patients received spinal anesthesia by an anesthesiologist, in sitting position at L3-L4 inter vertebral space, using 26-gauge, pencil point needle. Hyperbaric bupivacaine 12.5 mg mixed with preservative-free fentanyl 10 µg and morphine 200 µg was injected over 30 seconds. Immediately after spinal anesthesia, all patients were positioned in the supine position with left uterine displacement. Concomitantly to the intrathecal injection the patient received 10 mL/kg of lactated Ringer's solution.

BP was controlled every minute until delivery and then every five minutes throughout anesthesia. HR and SaO_2 were monitored throughout anesthesia.

Hypotension was considered a decrease in systolic blood pressure > 20% of baseline (prior to drugs being placed in the neuraxis) [11]. Patients were randomized to receive an intravenous bolus of either phenylephrine or ephedrine immediately after the episode of hypotension after spinal anesthesia. Randomization was performed using a computer-generated random number table. The patient and the attending anesthesiologist were blinded to the group allocation. Group allocations were placed in opaque, sealed envelopes on initial randomization. There were two groups of 101 patients: group Ph (Phenylephrine), and group E (Ephedrine). According to the randomization, hypotension was treated: with 100 µg Phenylephrine bolus IV, in Ph group or 5 mg bolus Ephedrine, in E group. If, at any time, maternal systolic blood pressure was < 80% baseline, the rescue doses of 100 µg Phenylephrine bolus IV, in Ph group or 5 mg bolus Ephedrine, in E group, were used. The doses of phenylephrine and ephedrine were chosen empirically, based on our clinical experience of the drugs. We recorded the number of total doses of vasopressors given up to the time of uterine incision. Heart rate and rhythm were monitored with ECG and any change from normal (tachycardia, bradycardia) were recorded and treated as needed. Bradycardia: HR < 60 bpm for 2 consecutive readings 1-minute apart [10]. If a patient developed bradycardia, 0.6 mg atropine was administered.

After delivery and clamping of umbilical cord, 1 mL blood was drawn from the umbilical artery for neonatal blood gas analysis.

The primary outcome was the incidence of maternal hypotension and secondary outcomes collected were: intra-operative maternal compli-cations (incidence of hypertension: SBP > 120% baseline, the incidence of bradycardia, the incidence of nausea: reported spontaneously by patients and vomiting: observed by investigators) and neonatal outcome parameters (the first- and fifth-minute Apgar scores: assessed by the attending paediatrician, and the umbilical artery blood gas analysis, obtained from a double-clamped segment of umbilical cord upon delivery). True fetal acidosis was defined as an umbilical arterial pH value of < 7.20 [14].

Statistical analysis

Continuous variables were presented as the mean ± SD (standard deviation). Categorical variables were expressed as actual numbers (n) and percentages (%). Chi-square analysis was applied to compare frequencies between -groups, and Student's t-tests, one-way ANOVA or non-parametric tests when necessary were employed for quantitative variable analysis. Analysis was performed using the statistical software Statistics Package for Social Scientists (SPSS) version 15.0. Statistical significance was considered to be at the level of P ≤ 0.05. All tests were two-tailed.

Results

Two hundred and two (202) pregnant women at term were entered in this study. Details of maternal demographic characteristics are summarized in Table 1.

Table 1: Maternal Characteristics

	Group E	Group Ph	P value
Age (year)	31.5 ± 5.34	30.26 ± 4.11	0.06
Weight (kg)	81.29 ± 12.66	79.74 ± 11.56	0.18
Height (cm)	165.49 ± 3.02	166.02 ± 5.47	0.39
Gestational age (weeks)	39.14 ± 0.85	39.07 ± 0.76	0.73
Induction-to-delivery time (min)	9.54 ± 2.35	9.47 ± 1.97	0.81

Data are given as mean ± SD.

The two groups were similar with respect to maternal age, weight, height, parity, and gestational age. Indications for cesarean section were repeated cesarean in 112 (55.44%) patients, other obstetrical indications for cesarean (cephalopelvic disproportion, breech or other abnormal presentations) in 77 (38.11%), and in 13 patients (6.43%) were patient's preference. The time from induction of spinal anesthesia to delivery was similar in both groups (range from 5 to 15 minutes). Maternal hemodynamic variables are detailed in Table 2.

Table 2: Comparison of systolic blood pressure (BP) and heart rate (HR) between two groups

	Group E	Group Ph	P value
Basal Systolic BP (mmHg)	114.75 ± 8.46	116.72 ± 7.72	0.08
Systolic BP (mmHg) after anesthesia	82.0 ± 8.95	90.05 ± 6.40	< 0.0001
Systolic BP (mmHg) after vasopressor therapy	111.79 ± 15.96	118.13 ± 10.31	0.0009
HR at baseline (bpm)	101.78 ± 15.77	97.9 ± 17.16	0.09
HR after anesthesia (bpm)	68.81 ± 10.29	66.68 ± 8.23	0.10

Data are given as mean ± SD

Maternal complications

Hypotension: 52 patients (51.48%) in group E and 56 patients (55.44%) in group Ph had persistent severe hypotension and needed additional vasopressor therapy. There was no significant difference between the groups regarding the number of doses of vasopressor required. (P = 0.82) (figure 1).

In group E, 25 patients (24.75%) experienced bradycardia and needed one dose Atropine. 34 patients (23.76%), in group Ph had bradycardia. There was no significant difference between groups regarding the incidence of bradycardia (P = 0.65).

Nineteen (19) patients (16.83%) in group E and 12 patients (11.88%) in group Ph had nausea. Vomiting occurred in 4 patients (3.96%) in group E and in 10 patients (9.9%) in group Ph. There was no significant difference when comparing the incidence of nausea and vomiting between groups, respectively P = 0.87, and P = 0.47.

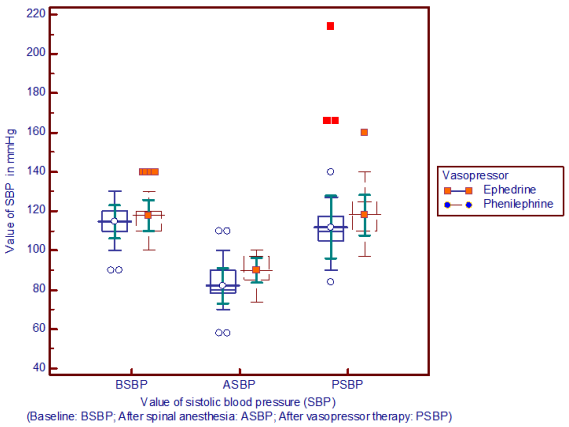

Figure 1: Comparison of systolic blood pressure value between groups

Neonatal complications

First-minute Apgar scores were as follows: 6 neonates of group E (5.94%) and 7 neonates of group Ph (6.93%) had Apgar score 7; 17 neonates of group E (16.83%) and 12 neonates (11.88%) of group Ph had Apgar score 8; 78 neonates of group E (77.22%) and 82 neonates (81.18%) of group Ph, had Apgar score 9. There was no significant difference between groups regarding the first-minute Apgar scores.

Fifth-minutes Apgar scores were: two neonates in group E (1.98%) and none neonate (0.0%) in group Ph had mean Apgar score 8; 44 neonates (43.56%) in group E and 73 neonates (72.27%) in group Ph had mean Apgar score 9; 55 neonates in group E (54.45%) and 28 neonates (27.72%) in group Ph had a mean Apgar score of 10. Also there was no significant difference between groups regarding the fifth-minute Apgar scores.

Table 3: Neonatal Data

	Total	Group E	Group Ph	P value
Umbilical arterial PH	7.32 ± 0.04	7.31 ± 0.04	7.33 ± 0.04	0.0005
HCO_3 (mmHg)	22.55 ± 2.10	22.57 ± 1.87	22.52 ± 2.32	0.86
Base excess (mmol.l^{-1})	- 3.39 ± 1.90	- 3.48 ± 2.02	- 3.30 ± 1.78	0.50

Data are given as mean ± SD

At 1 and 5 min, no neonate in the Ephedrine or Phenylephrine groups had an Apgar score value of < 7.

Umbilical arterial blood gas analyses are summarized in Table 3. Based on the one-way ANOVA test, there was a significant difference in PH between groups. Umbilical arterial PH was significantly lower in ephedrine group (P = 0.0005), but none of the neonates had the true fetal acidosis (Figure 2). There were no differences between groups regarding HCO_3 concentrations and base excess values (Be) in the umbilical arterial blood gas analyses.

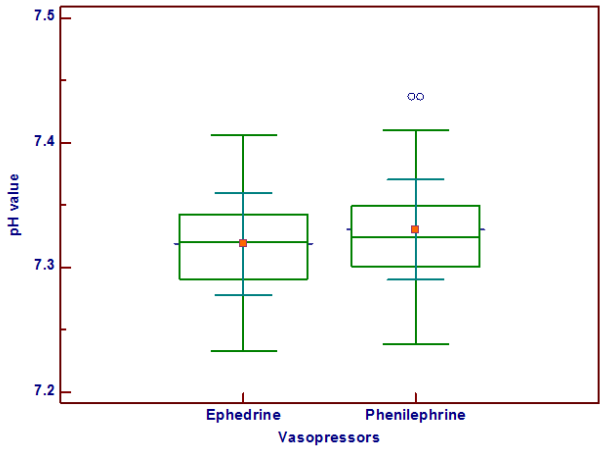

Figure 2: Comparison of umbilical arterial blood pH value between the study groups

Discussion

In this study, we showed no significant differences between ephedrine and phenylephrine in their efficacy for treating spinal-induced intra-operative hypotension during cesarean sections. Also, there were no differences on maternal and neonatal outcomes, although parturients treated with phenylephrine had neonates with higher umbilical pH value than those treated with ephedrine. These results were consistent with the systematic review performed by Lee at al [8] and the meta-analysis performed by Lin et al [15].

As in other studies, [8, 10, 12, 16, 17] we showed there was no difference between groups for the treatment of maternal hypotension with Ephedrine and Phenylephrine. Lin et al [15] in a recent meta-analysis showed that when used to treat hypotension, patients given ephedrine and phenylephrine had comparable incidence of intra-operative hypotension. Our findings support the fact that ephedrine and phenylephrine are equally effective in controlling maternal hypotension [18].

In the contrary with other previous studies, [8, 10, 11, 19] that showed that patients in the Phenilephrine group were more likely than the Ephedrine group to develop bradycardia, in the present study there was no difference on the incidence of bradycardia between two groups.

Cooper showed that ephedrine use, compared with phenylephrine, was associated with a higher incidence of maternal nausea and vomiting [20]. We didn't find any difference in the incidence of maternal nausea and vomiting between two groups.

The findings in the present study are in accordance with other studies [21, 22]: women given phenilephrine had neonates with higher umbilical arterial pH values than those given ephedrine, although there is no risk for true fetal acidosis in none of groups.

The reason for the difference in umbilical arterial pH values is that ephedrine crosses the placenta; [17, 22, 23] therefore, it is possible that ephedrine may have a direct effect on the fetus that contributes to acidosis [8]. In spite of this, fetal clinical adverse effects caused by reduced fetal pH have not been demonstrated [24].

In the present study none of the neonates had Apgar scores < 7, so there was no difference in the risk of low Apgar scores at 1 min or at 5 min (< 7) between the Ephedrine and Phenylephrine groups [8, 25].

Limitations on the study: One possible limitation is that the study was conducted in a single center. Also, it was focused only in elective cesarean section and in healthy non-laboring women, not considering other conditions affecting maternal or neonatal outcome.

In conclusion, which of the two vasopressors: ephedrine or phenylephrine, is superior in treating spinal-induced intra-operative hypotension during cesarean sections, has been argued for years. In summary, this study supports the idea that ephedrine and phenylephrine have the same efficacy in treating hypotension after spinal anesthesia for caesarean section.

The use of Phenylephrine was associated with better fetal acid-base status compared to the use of ephedrine, but there were no differences on Apgar score values and on the incidence of the maternal bradycardia and hypotension after the use ofphenilephrine or ephedrine for treating spinal-induced intra-operative hypotension during cesarean sections.

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Clinical Science

Cardiac Biomarkers and Left Ventricular Hypertrophy in Asymptomatic Hemodialysis Patients

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Key words: hemodialysis; cardiac troponin T; natriuretic peptide; C-reactive protein; left ventricular hypertrophy.

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Abstract

BACKGROUND: Cardiac biomarkers are often elevated in dialysis patients showing the presence of left ventricular dysfunction. The aim of the study is to establish the plasma levels of high-sensitivity cardiac troponin T (hs TnT), precursor of B-natriuretic peptide (NT-proBNP) and high sensitivity C-reactive protein (hs CRP) and their relation to the presence of left ventricular hypertrophy (LVH) in patients undergoing hemodialysis without signs of acute coronary syndrome or heart failure.

MATERIAL AND METHODS: We studied 48 patients - 26 men and 22 women. Pre and postdialysis levels of hs cTnT, NT-proBNP and hs CRP were measured at week interim procedure. Patients were divided in two groups according to the presence of echocardiographic evidence of LVH - gr A - 40 patients (with LVH), and gr B - 8 patients (without LVH).

RESULTS: In the whole group of patients was found elevated predialysis levels of all three biomarkers with significant increase ($p < 0.05$) after dialysis with low-flux dialyzers. Predialysis values of NT-proBNP show moderate positive correlation with hs cTnT ($r = 0.47$) and weaker with hs CRP ($r = 0.163$). Such dependence is observed in postdialysis values of these biomarkers. There is a strong positive correlation between the pre and postdialysis levels: for hs cTnT ($r = 0.966$), for NT-proBNP ($r = 0.918$) and for hs CRP ($r = 0.859$). It was found a significant difference in the mean values of hs cTnT in gr. A and gr. B (0.07 ± 0.01 versus 0.03 ± 0.01 ng /mL, $p < 0.05$) and NT-proBNP (15,605.8 ± 2,072.5 versus 2,745.5 ± 533.55 pg /mL, $p < 0.05$). Not find a significant difference in hs CRP in both groups.

CONCLUSIONS: The results indicate the relationship of the studied cardiac biomarkers with LVH in asymptomatic patients undergoing hemodialysis treatment.

Introduction

Cardiorenal syndrome type 4 often manifests itself with patients undergoing hemodialysis treatment in stage 5 HD on chronic kidney disease (CKD). It is characterized by primary CKD, associated with left ventricular (LV) damage, including left ventricular hypertrophy (LVH), systolic and diastolic dysfunction and congestive heart failure [1]. Echocardiographically-proven LV hypertrophy is detected in 60-75% of patients starting renal replacement therapy and in 60-90% of those on regular dialysis treatment [2, 3] LVH is considered an independent risk factor for cardiovascular mortality in this population [4-6]. Pathophysiologic factors involved in LVH of CKD and ESRD patients have generally been divided into 3 categories [7-9]: (1) related to afterload, (2) related to preload, and (3) not related to afterload or preload. The ones in the first category are represented by an increase in systemic arterial resistance, elevated arterial blood pressure, and reduced large-vessel compliance [7-9] related in part to aortic 'calcification', which is typical in CKD patients; all these factors result in myocardial cell thickening and concentric LV remodeling often together with activation of the intracardiac renin-angiotensin system [9, 10]. Additional roles have inadequate activation of the renin-angiotensin-aldosterone system, hypervolemia, anemia, oxidative stress and inflammation [4, 11, 12]. Oxidative stress

and xanthine oxidase activation as well as the phosphodiesterase-5 pathway may also be involved in the development of LVH [13]. Among the preload-related factors, the role of intravascular volume expansion (salt and fluid loading) has to be underlined, as well as secondary anemia and the presence of arterovenous fistulas [14, 15], resulting in myocardial cell lengthening and eccentric or asymmetric LV remodeling. Both afterload- and preload-related factors operate with additive and synergistic effects. As a result, myocardial hypertrophy induces the activation of cellular apoptotic signals and activates metabolic pathways able to increase extracellular matrix production up to fibrosis [16, 17]. Fibrosis leads to progressive impairment in contractility with stiffening of the myocardial wall, systolic and diastolic dysfunction, dilated cardiomyopathy and congestive heart failure [18]. Renin-angiotensin-aldosterone system activation induces hyperaldosteronemia promoting cardiac fibrosis through the generation of signals leading to profibrotic transforming growth factor production [19]. The LVH can also be promoted by iron and/or erythropoietin [20] or vitamin D deficiency [21]. The presence of arterovenous fistulas can contribute to the development of LVH because of excess blood flow that increases the myocardial workload [22]. Persistent inflammatory condition is recognized as an important risk factor for the development of cardiovascular complications in this group of patients. Their levels of C- reactive protein (CRP) correlate positively with LVH [23]. Cardiac biomarkers reflect LV structure and function and are essential for early diagnosis of heart failure. In recent years, natriuretic peptides, in particular the B-type natriuretic peptide (BNP), are used in screening for the risk of cardiovascular events in the general population [24-27]. BNP is synthesized in ventricular myocytes, undergoing intracellular metabolism to prohormone (proBNP), which is then fragmented into activated (C-terminal) and inactive (N-terminal) proBNP. In practice, NT-proBNP is tested more often because of its longer half-life in the circulation (60-120 min) than that of BNP, which is 23 minutes long [24]. BNP and NT proBNP are biological markers of left ventricular dysfunction [28]. Another biomarker is cardiac troponin T (cTnT), which is released into the circulation and increases sharply if irreversible damage to the heart muscle exists [29]. There are other reasons for the presence of cTnT. These are myocardial damage due to high pressure in the left ventricular wall in hypertrophy, acute or chronic volume overload, microvascular lesions, "quiet" subclinical myocardial ischemia fibrosis and necrosis. In hemodialysis patients, these conditions are very common [30].

Objectives of the study are: -to determine plasma levels of high-sensitivity cardiac troponin T (hs sTnT), NT-proBNP and high sensitivity C-reactive protein (hs CRP) and their changes during hemodialysis; and -to link LVH and cardiac biomarkers in patients undergoing hemodialysis without signs of acute coronary syndrome or heart failure.

Material and Methods

Forty eight patients were studied (26 men and 22 women) undergoing a standard 4-hour bicarbonate dialysis three times a week, over three months. All patients used low-flux dialyzers with polysulfone membranes. The adequacy of dialysis was evaluated by URR%. Clinically they are hemodynamically stable (in sinus rhythm, with values of BP below 150/90, without manifestation of hypertensive crisis, without clinical manifestations of hypotensive reactions), with no evidence of acute coronary syndrome or heart failure in the previous two months. Patients with a diagnosis of cancer, autoimmune diseases and signs of active inflammation were excluded.

Determined pre- and post-dialysis are levels of hs sTnT, NT-proBNP and hs CRP immediately before the interim procedure for the week. hs sTnT and NT-proBNP were analyzed with Elecsys 2010 immunoassay reagents of Roshe Diagnostics. NT-proBNP was reported in pg/mL. The values of NT-proBNP > 125 pg/mL are considered a threshold taken from the manufacturer's package insert above which it can be assumed cardiac dysfunction related to an increased risk of cardiac events (myocardial infarction, heart failure, death). The above hs sTnT reference value is specified by the manufacturer through testing of 533 healthy subjects (99th percentile of healthy) and 0.014 ng/mL. hsCRP was investigated by biochemical analyzer Beckman Coulter AU 480 by immunoturbidimetric method with reagents Roshe Diagnostics and has an upper reference value of 1.0 mg/L. Patients were divided into two groups according to the presence of the echocardiography evidence of LVH - group A (with LVH) and group B (without LVH). Echocardiography was performed on non-dialysis day, 24 hours after the last hemodialysis, with PHILIPS XE 11.

Left ventricular structure was evaluated by 2M echograph mode. LV mass was corrected for body surface area and presented as LV mass index (LVMI). The criteria for LVH are LVMI > 115 g/m^2 in men and LVMI > 95 g/m^2 in women. Compared were the thickness of the free wall of the LV (LVPW d), the thickness of the interventricular septum (IVS d) in diastole, the ejection fraction (EF%) and fraction of shortening (FS%) in both groups of patients. All patients were in sinus rhythm with no abnormalities in the kinetics of the LV wall and without severe valvular heart damage.

To process the statistical data was used R-Project software version 3.1.1. Descriptive statistics was presented and statistical results are reported in mean ± standard error of the average. All parameters were tested for normal distribution using the test of Shapiro-Wilk. The relationship between performances was recorded using linear regression. Statistically significant difference was accepted at $p < 0.05$.

Results

The main demographic and clinical characteristics of the study group of 48 patients were: 26 men and 22 women; mean age 53.04 ± 2.24 years; the average duration of hemodialysis treatment 54 ± 7.74 months; the average URR% - 64.8%. In 17 patients the primary renal disease leading to CKD is chronic tubulointerstitial nephritis, in 14 - chronic glomerulonephritis, in 9 - diabetic nephropathy, in 5 - polycystic kidney disease and three - other disabilities (congenital anomalies, obstructive nephropathy).

Table 1: Values of cardiac biomarkers before and after the dialysis procedure

Biomarker	Predialytic values of biomarkers mean ± sem	Postdialytic values of biomarkers mean ± sem	p	r
hs cTnT (ng/mL)	0.06 ± 0.01	0.07 ± 0.01	p < 0.001	0.966
NT-proBNP (pg/mL)	13,462 ± 1,862	15,279 ± 1,895	p < 0.001	0.918
hs CRP (mg/L)	16.30 ± 4.19	15.44 ± 3.73	p < 0.05	0.859

In all of the patients were found elevated mean values of cardiac biomarkers and hs CRP, both before and after the dialysis procedure. Post-dialysis levels of hs cTnT and NT-proBNP were significantly higher ($p < 0.001$) while hs CRP significantly lower ($p < 0.05$). A statistically significant difference was registered as well as a very high correlation between pre- and post-dialysis levels of markers tested: in hs cTnT ($r = 0.966$), in NT-proBNP ($r = 0.918$) and hs CRP ($r = 0.859$) – the statistical analysis in both groups of patients is based on predialytic values. (Table 1) Predialytic indicators studied in men and women showed significant difference in hs cTnT (0.07 ± 0.02 compared to 0.06 ± 0.01; $p < 0.05$). The remaining two biomarkers are higher in women, but the difference was not statistically significant (Table 2).

Table 2: Predialytic values of cardiac biomarkers in men and women

Value	Men	Women	p
hs TnT	0.07 ± 0.02	0.06 ± 0.01	p < 0.05
NT-proBNP	12,845 ± 2,624	14,192 ± 2,683	p - NS
hs CRP	15.21 ± 6.35	17.65 ± 5.29	p - NS

Predialytic values of NT-proBNP in all investigated patients showed moderate correlation with hs cTnT ($r = 0.47$) and a weaker one with hs CRP ($r = 0.163$). Such dependence is observed in the post-dialysis values of these parameters, respectively compared hs cTnT ($r = 0.482$) and hs CRP ($r = 0.19$) (Table 3).

Table 3: Correlations between the values of NT-proBNP with hs cTnT and hs CRP - before and after hemodialysis

Predialytic values of biomarkers	Correlation coefficient (r)	Postdialytic values of biomarkers	Correlation coefficient (r)
hs cTn T	0.47	hs cTn T	0.482
hs CRP	0.163	hs CRP	0.19

Forty patients from group A (with LVH) take part in the echocardiographic study, while in group B (without LVH) - 8 patients participate: LVH was present in 83% of the study group. Table 4 shows the ultrasound data of the two groups. Patients with LVH had significantly higher values of LVMI, LVPW and IVS, while indicators of systolic function EF% and FS% showed no differences in both groups.

Table 4: Echocardiographic parameters in patients with and without LVH

Echocardiographic parameter	Value mean±sem		p
	GroupA with LVH	Group B without LVH	
LVMI g/m^2	151.97 ± 4.23	94.24 ± 5.27	p<0.005
LVPW d /sm	1.19 ± 0.02	0.94 ± 0.06	p<0.005
IVS d /sm	1.34 ± 0.03	1.08 ± 0.05	p<0.005
EF%	62.8 ± 1.58	68.5 ± 3.29	p - NS
FS%	35.2 ± 1.17	38.4 ± 2.4	p - NS

With all patients studied, levels of NT-proBNP were significantly higher than the threshold values. In group A the average is 15,605.8 ± 2,072.5 pg/mL with 77.5% having values above the limit of chronic heart failure functional class I NYHA (3,410 pg/mL). In group B the recorded average value of this biomarker is 2,745.5 ± 533.6 pg/mL, with only 25% of the patients with the values were above the threshold for chronic heart failure functional class I NYHA. A significant difference was found in the mean values of hs cTnT between patients in group A and B (0.07 ± 0.01 ng/mL compared to 0.03 ± 0.01 ng/mL, $p < 0.05$) and NT- proBNP (15,605.8 ± 2,072.5 compared to 2,745.5 ± 533.6 pg/mL, $p < 0.05$) before hemodialysis. Although the values of hs CRP were higher in patients with LVH, there wasn't a significant difference in this indicator in both groups. A similar dependence was observed in post-dialysis values of biomarkers (Table 5).

Table 5: Pre- and post-dialysis values of cardiac biomarkers in the two groups of patients

	GroupA with LVH	Group B without LVH	p
Predialytic values of biomarkers			
hs cTnT	0.07 ± 0.01	0.03 ± 0.01	p < 0.05
NT-proBNP	15,605.8 ± 2,072.5	2,745.5 ± 533.6	p < 0.005
hs CRP	17.87 ± 4.85	7.35 ± 4.05	p=0.054
Postdialytic values of biomarkers			
hs cTnT	0.08 ± 0.01	0.04 ± 0.01	p < 0.05
NT-proBNP	16,807.7 ± 2,099	7,635 ± 3,455.9	p < 0.05
hs CRP	16.66 ± 4.39	9.5 ± 4.59	p - NS

Statistical analysis established that there was

a positive moderate correlation between hs cTnT and NT proBNP (r = 0.43). of hs cTnT with hsCRP (r = 0.61). and a weaker. but also positive correlation between NT proBNP and hsCRP (r = 0.10) in patients with LVH. In this group there is a positive correlation of each of the biomarkers with LVMI. LVPW IVS and it is most pronounced compared to the thickness of the interventricular septum (Table 6). Three cardiac biomarkers showed negative correlation with EF% and FS%.

Table 6: Correlation analysis between cardiac biomarkers and echocardiographic parameters in patients with LVH

	hs cTnT	NT proBNP	hs CRP	LVMI	LVPW	IVS	EF%	FS%
hs cTnT	-	r=0.43	r=0.61	r=0.23	r=0.28	r=0.46	r= -0.04	r=0.001
NT-proBNP	r=0.43	-	r=0.10	r=0.31	r=0.24	r=0.32	r=-0.21	r=-0.21
hs CRP	r=0.61	r=0.10	-	r=0.18	r=0.39	r=0.50	r= -0.25	r= -0.08

Discussion

Our study found a very high prevalence of LVH in hemodialysis patients, i.e. 83%. Similar results presented by other studies indicate that 75-78% of these patients had LVH in stage 5 CKD on HD [31, 32]. We found a positive correlation of hs CRP with ultrasound parameters LVMI. LVPW and IVS (r = 0.18; r = 0.39; r = 0.50) in group of patients with LVH. This, on its own part, defines the essential role of inflammation in the development of LVH in patients on hemodialysis. Along with hypertension and volume overload, systemic inflammatory state may contribute to the development of LVH by changing the structure and function of vascular smooth muscle cells. leading to increased vascular stiffness [33]. On the other hand, it is considered that chronic subclinical inflammation alters LV structure and geometry by disrupting the balance between cell growth and apoptosis in cardiac tissue [34].

When using a low-flux dialyzers. there was a significant increase in the levels of NT-proBNP in all investigated patients. Being released into the circulation in abnormally high volume, it stretches cardiomiofibres. The established increased level after an adequate hemodialysis procedure and normal hemodynamics, suggests the role of other factors in increased plasma levels. Wahl et al [35] reported that pre- and post-dialysis levels of NT-proBNP are dependent on the type of membrane used during HD. High-flux membranes have a higher ultrafiltration rate than low-flux membranes. They tend to have larger pores, which means that the clearance of NT-proBNP with a molecular weight of 8.5 kDa is higher than when using low-flux membranes. where the post-dialysis concentration increased. In our study we found that NT proBNP was, to a high degree, correlated with some echocardiographic parameters – LVMI, LVPW and IVS in group of patients with LVH. This biomarker of cardiomyocyte microinjury and hemodynamic stress may stimulate fibrosis-related mechanisms and facilitate the diagnosis of subclinical LV remodelling and LVH in this population.

hs cTnT values above the 99th percentile in healthy frequently found in patients with LVH and heart failure in the absence of acute coronary syndrome and in patients with CKD and without clinical heart disease [36-38]. The mechanism of this subclinical myocardial injury with elevated sTnT has not yet been elucidated [39]. It is believed that stretching and deformation of myocardium in LVH impairs the permeability of cardiomyocytes, leading to the release of cardiac troponins. On the other hand, the increased oxygen demand in hypertrophied LV with reduced coronary reserve may cause subclinical ischemia with or without macrovascular coronary artery disease [39]. Since all patients studied are asymptomatic, the established moderately high positive correlation between LVMI, LVRW and IVS and the values of the cardiac biomarkers suggest that their measurement can be used for preclinical diagnosis of LVH.

In conclusion, our data is based on 83% incidence of LVH in patients receiving dialysis. Pre- and post-dialysis values of cardiac biomarkers and hs CRP were increased in all patients just as it is manifested in those with LVH. There is a positive. moderate correlation between the degree studied biomarkers. Cardiac biomarkers correlate well with echocardiography parameters showing LVH. The study of cardiac biomarkers can be used as a screening for the presence of LVH in asymptomatic patients treated with hemodialysis.

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Clinical Science

Validation of the Schizophrenia Quality of Life Scale Revision 4 (SQLS-R4) Among Patients with Schizophrenia

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Abstract

BACKGROUND: The main goal of psychiatric care is not to be focused only on reducing psychopathological symptoms, but on improvement of the patient's quality of life.

AIM: To examine validation and reliability SQLS-R4 among patients with schizophrenia.

METHODS: The sample consisted of 61 outpatients with schizophrenia attending the Psychiatry Hospital "Skopje". Inclusion criteria for subject selection were: 1) age more than 18 years, 2) clinically stable (not acutely ill or has not been recently hospitalized at least for the past 3 months). They completed SQLS-R4 and SF36 questioners.

RESULTS: The internal consistency reliability was satisfactory for both the psychosocial and vitality domains (Cronbach's α = 0.928, 0.83). Most of the items were significantly correlated with own scale score (from 0.189 to 0.687). The average of the score for the psychosocial quality life was 39.9 ± 8.6 (sometimes), for the cognition and vitality was 26.5 ± 6.1 (sometimes) (SQLS-R4). There was moderate correlation between SF 36-energy with SQOLS - motivation and energy; SF 36-mental health correlation with SQOLS-psychosocial.

CONCLUSION: SQLS-R4 appears to offer excellent potential as an easily administered and patient acceptable assessment and monitoring measure of quality of life (QoL). However, a principle psychometric criterion crucial to the use and validity of the instrument concerns the underlying factor structure.

Introduction

Schizophrenia is a serious mental disorder characterized by loss of contact with reality-psychosis, hallucinations, delusions, abnormal thinking, irrational thinking, bizarre behaviour, restrictive range of emotions, motivation weakened and impaired social and work function, chronic mental illness that differently affect the behaviour, thought and emotion [1-3]. Nowadays, the main goal of psychiatric care is not to be focused only on reducing (the) psychopathological symptoms, but (one part of the main goal of psychiatric care is the) improvement of the patient's quality of life. Decreased quality of life is often an important cause or consequence of psychiatric illness, and needs to be included in a comprehensive treatment plan. Literature research reveals that mentally ill patients declare (a) lower overall quality of life compared to the general population [4-9]. People with schizophrenia are at increased risk for multiple and chronic social, cognitive, and behavioural deficits that may lead to inadequate health and self-care practices [7-10]. Quality of live may be defined as a person's sense of wellbeing and satisfaction with his/her life circumstances, as well as a person's health status and access to resources and opportunities [11-12].

Many instruments have been developed to measure HRQOL (health-related quality of life), but there is no consensus on the most appropriate scale

for measuring HRQOL in schizophrenia [13]. The instrument of choice for HRQOL measurement depends on the assessment purposes [14]. Generic instruments designed to be applicable across all diseases or conditions are likely to be useful in comparing different group of patients, while disease-specific measures have more potential in detecting treatment effects [15].

The hypotheses are: H1 - The SF 36 energy dimension would be strongly associated with SQOLS motivation and energy dimension; and H2 - The SF 36 mental health dimension would be strongly associated with SQOLS psychosocial score.

The aim of this study is validation of the Schizophrenia Quality of Life Scale Revision 4 (SQLS-R4) among patients with schizophrenia. This study was designed to determine the reliability and validity of the Quality of Life Scale Revision 4 (SQLS-R4) for schizophrenic patients, and to compare SQLS-R4 with short-term change in SF-36 scores for patients.

Material and Methods

Subjects

Of the 70 people with schizophrenia who were approached, 61 (87.1%) agree to take part, sample of 61 outpatients with schizophrenia attending the Psychiatry Hospital “Skopje”. Inclusion criteria for subject selection were: 1) age more than 18 years, 2) clinically stable (not acutely ill or has not been recently hospitalized at least for the past 3 months).The mean age of the patients was 47.4 years (s.d. 9.0, min.-32, max-74) (Table 1 & Fig.1).

Measure

The internal consistency of the SQLS-R4 was determined by item to total correlations and Cronbach’s alpha coefficients [16, 17].

The SQLS-R4 was developed by Wilkinson et al. [18-20] for the measurement of HRQOL in people with schizophrenia. It comprises 33 items incorporated in two domains: psychosocial feelings (22 items) and cognition and vitality (11 items). All except four items are scored on a five-point Likert-type scale (0- never, 1-rarely, 2- sometimes, 3- often, 4- always), with the exceptional four items being reverse coded (0- always, 1-often, 2- sometimes, 3- rarely, 4- never). Individual domain and total scores are standardized by scoring algorithm to a 0 (best health status) to 100 (worst health status) scale, with higher scores indicating comparatively lower quality of life. All questionnaires were scored using a Likert-type format. The SQLS-R4 was completed by all patients within 5-10 min, the few who took longer expressed the need to think longer about their responses. The instrument was translated from English to Macedonian using the procedure of forward backward translation procedure. With intention to develop a Macedonian version of the instrument that is conceptually equivalent to the original version, the back-translated English version was compared and discussed.

The SF-36 consists of 36 items divided into 2 components: the Physical Component and the Mental Component. The final scores of each dimension ranges from 0-100, with the highest score corresponding to a better condition. The physical component includes the following dimensions: Physical Functioning (PF), Physical Role Functioning (RP), Bodily Pain (BP) and General Health (GH). The Mental Component is measured by the following dimensions: Vitality (VT), Mental Health (MH), Social Functioning (SF) and Emotional Role Functioning (RE). These eight dimensions also can be used to generate a physical and mental health summary score [21].

Statistical analysis

Patient characteristics were presented by using descriptive statistics. The construct validity was established by comparing scores of the SQLS-R4 subscales with those of the SF 36, by Spearman`s rank correlations. The level of statistical significance was defined less than 0.05. The statistical analyses were performed by using SPSS version 20.0

Results

Completed questionnaires were collected from 61 patients suffering from schizophrenia.

Table 1 shows the correlations of items with their scale totals, and the internal consistency reliability of the scales (that is the extent to which items in a scale reflect a single underlying dimension). Items were highly correlated with their own scale score (corrected to exclude the item being correlated). Internal reliability was assessed using Cronbach α statistic. The internal consistency reliability was satisfactory for both the psychosocial and vitality domains (Cronbach’s α = 0.928, 0.83), the deletion of any of the 33 items did not improve the Cronbach α value for booth domains.

For some items correlations were satisfactory for the psychosocial domains ranging from 0.506-0.687 and the vitality domains -0.456 - 0.686. Some items from the vitality subscale had correlations less than 0.39 (p23 = 0.381; p28 = 0.377; p7 = 0.333; p14 = 0.189). Most of the items were significantly

correlated with own scale score (from 0.189 to 0.687) (Table 1).

Table 1: Corrected item to total correlations (p) and internal reliability (Cronbach`s α) of scales

Scale and items	Corrected Item-Total Correlation	Cronbach's α
Psychosocial		0.928
P 3	.527	
P 4	.548	
P 5	.580	.
P 6	.563	
P 8	.535	
P 10	.630	
P 11	.541	
P 13	.687	
P 15	.529	
P 16	.640	
P 17	.528	
P 18	.568	
P 19	.506	
P 21	.602	
P 22	.611	
P 24	.607	
P 25	.554	
P 27	.517	
P 29	.662	
P 30	.634	
Cognition and vitality		0.83
P 1	.686	
P 2	.675	
P 7	.330	
P 9	.522	
P 12	.456	
P 14	.180	
P 23	.381	
P 20	.560	
P 26	.400	
P 28	.377	
P 31	.572	
P 32	.626	
P 33	.565	

Table 2 shows the average of the score for the psychosocial quality life was 39.9 ± 8.6 (sometimes), for the cognition and vitality was 26.5 ± 6.1 (sometimes) each scale has a range from 0-best possible health to 100-worst possible health.

Table 2: **Descriptive Statistics**

	N	Minim.	Maxim.	Mean	Std. Deviation	Skewness		Kurtosis	
						Statistic	Std. Error	Statistic	Std. Error
Quality life	61	28.00	94.00	66.4754	13.69502	-.467	.306	.612	.604
Psychosocial quality life	61	17.00	57.00	39.9508	8.57793	-.425	.306	.550	.604
Cognition vitality quality life	61	10.00	38.00	26.5246	6.05422	-.524	.306	.258	.604

Table 3 presents the demographic profile of the patients. The mean age of the patients sample was 47.4 years. A majority of patients were secondary school educated - 70.5%. In the study participated- 68.9% male and 31.15 female, 36.1%of the patients were living alone and majority of them - 68.9% were single, most of them were unemployed - 65.6%.

Table 3: Demographic profile of survey population

Gender	**%**	**N**
Male	68.9	42
Female	31.1	19
Age	**mean ± SD**	
	47.4 ± 8.9 y	
Education	**%**	***N***
Primary school	16.4	10
Secondary school	70.5	43
University	13.1	8
Marital status	**%**	***N***
Single	68.9	42
Married	31.1	18
Living arrangement,	**%**	***N***
With family or relatives	63.9	39
Living alone	36.1	22
Employment status,	**%**	***N***
Employed	34.3	21
Unemployed	65.6	40
Duration of illness	**mean ± SD**	
	18.1 ± 9.4 y	

Construct validity was assessed comparing results on the SF36 - The Short Form [36] Health Survey. The predict correlations were substantiate- SF 36-energy correlation with SQOLS- motivation and energy ρ=0.63 for $p<0.05$; SF 36-mental health correlation with SQOLS-psychosocial ρ=0.52 for $p<0.05$, it was moderate correlation (Correlation coefficients-Spearman) (Table 4).

Table 4: Correlations between the SQOLS and SF 36

	SQOLS-motivation and energy	SQOLS-psychosocial
SF 36-energy	ρ=0.63	
SF 36-mental		ρ=0.52

Discussion

The internal consistency reliability was satisfactory for both the psychosocial and vitality domains in our study were high (Cronbach's α = 0.928, 0.83). Cronbach's α was also high as those found in Taiwan [22], Malaysia [23] and UK study [18]. The internal consistency reliability for the psychosocial and vitality domains in Taiwan study [7] were very similar - Cronbach's α = 0.92 and 0.84; and in Malaysia study [23] - Cronbach's α = 0.95 and 0.85.

The characteristics of the SQOLS-R4 scale were good. Assessment of the internal consistency revealed significantly high correlations of items with their scale total except for four items.

Similar findings were also found in the validation of the SQOLS-R4 in the Japan study by Kaneda Y. et al [24] and Malaysia study by Nur Akmar Taha et al [23]**.** In Malaysia study [23] validity was supported by correlations between domains measuring related constructs of the SQO LS-R4 and SF-36 (ρ - 0.65 to 0.67), the correlations which was obtained in our study confirms (ρ - 0.63 to 0.52) findings. As suggested by Kuo et al. [22], these results may be indicative of those items being interpreted

differently from the vitality construct in the Asian customs. Alternatively, because of the study design, kind of patients (patients with various degree of symptom severity), kind of symptoms (negative and positive, depressive, cognitive) and sample size. The sample size for both studies in the Taiwan and Japan was relatively smaller than study in Malaysia and our sample size was smallest than other tree study. It was hypothesized that significant correlations between scores would be found for the SF-36 with all dimensions of the SQOLS. These hypothesized associations were indeed found.

Construct validity was explored by correlation of the scales of the SQLS with established psychiatric self-report measures and the SF-36. Results suggest that the measure is addressing areas related, but not identical, to those of previously existing measures. The SQLS was developed to be a valid and feasible questionnaire for self-completion that addresses the perceptions and concerns of people with schizophrenia - except, of course, those too unwell to complete the questionnaire. Its main use is likely to be in clinical trials and the evaluation of clinical interventions. Evidence is presented in this report to suggest that the SQLS has desirable properties in terms of reliability and validity, and we have found the measure to have excellent acceptability and feasibility in practice.

The background characteristics of the study patients were: most of them were male; a majority of them were aged approximately 47 years with mean illness duration of 18 years; marital status-single, unemployed, and education status-secondary school.

In conclusion, quality of life is a major area of concern for patients with chronic schizophrenia. The schizophrenia quality of life scale revision 4 (SQOLS-R4) appears to offer excellent potential as an easily administered and patient acceptable assessment and monitoring measure of quality of life. The questionnaire was found to be acceptable total patients and feasible for use in a routine clinical setting.

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Appendix

Items (p) in the SQOLS

1. Lack energy to do things
2. Can t be bothered to do things?
3. Worry about future
4. Feel lonely
5. Feel hopeless
6. Feel panicky
7. able to carry out daily activities
8. Take things people say the wrong way
9. Find hard to concentrate
10. difficult to mix with people
11. Feel down
12. Feel I can cope
13. Feel very mixed up
14. Sleep well
15. Feelings go up and down
16. Concerned won't get better
17. Worry about things
18. Feel people avoid me
19. Get upset thinkking about the past
20. Trouble remembering things
21. Feel cut off from the world
22. Feel uncomfortable with people
23. Has trouble thinking clearly
24. Has upsetting thoughts
25. Have suicidal thoughts
26. Feel happy
27. Feel depressed
28. Feel drowsy
29. Feel restlessness
30. Concerned about the social life
31. Feel tired
32. Feel physical weak
33. Feel like not leading a normal life

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Clinical Science

Thumb Reconstruction Using Foucher's Flap

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Key words: Foucher's flap; thumb; First DMCA; CMC joints; interosseus.

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Abstract

BACKGROUND: Extensive pulp defects of the thumb, with the exposure of tendon or bone, are challenging reconstructive problems. Surgical treatment includes the use of local, regional, and free flaps.

AIM: This paper is focused in Foucher's neuro vascular flap. First DMCA or Foucher's pedicle flap is a successful thumb reconstruction method, especially in patients not disturbed by its cosmetic appearance.

MATERIAL AND METHODS: The first dorsal metacarpal artery (FDMCA) arises from the radial artery in the first intermetacarpal space, just distal to the tendon of the extensor pollicis longus. Pulp area of the thumb is the area where Foucher's flap is more utilizable. This technique has other applications such as first web reconstruction, thumb lengthening, and following resection of tumors on the dorsum of the hand.

RESLTS: We have in study 7 cases with work related trauma in two years period of time, between 2012 and 2014. We had only one partial flap survival and all the other flaps survived entirely. We have also taken in consideration subjective satisfaction with a range score from 4 to 10, cold intolerance, flap area and donor site sensibility with a range score from low to medium to normal.

CONCLUSION: Careful pedicle discovery, secured elevation, pedicle strangulation prevention are very important for flap survival.

Introduction

Extensive pulp defects of the thumb, with the exposure of tendon or bone, are challenging reconstructive problems. Surgical treatment includes the use of local, regional, and free flaps.

Moberg flap, Littler neurovascular flap, Kutler's V-Y flaps, Foucher's flap, and free flaps including toe to thumb transfer are best surgical choices.

This paper is focused in Foucher's neuro vascular flap. The innerved first dorsal metacarpal artery flap from the dorsum of the index finger was first described by Hilgenfeldt. An island flap carried on a neurovascular pedicle consisting of the first dorsal metacarpal artery was first demonstrated by Foucher [1]. Despite a successful reconstruction, the thumb may never return to a pre-injury level of function. The patient and surgeon should be aware of this possibility.

First DMCA or Foucher's pedicle flap is a successful thumb reconstruction method, especially in patients not disturbed by its cosmetic appearance.

The first dorsal metacarpal artery (FDMCA) arises from the radial artery in the first intermetacarpal space, just distal to the tendon of the extensor pollicis longus. The artery divides into the radial branch to the thumb, the intermediate branch to the first web space, and the ulnar branch to the index finger. In 90% of cases, the FDMCA parallels the second metacarpal bone distally. In 10% of cases, it is found in the midline of the triangle that is formed by the first commissura. The FDMCA is in a superfascial (57%)

or subfascial (43%) location [1-3]. The outer diameter of the artery at its widest point is 1.0 ± 1.5 mm. In those without a palmar branch the diameter is less than 1 mm. A muscular artery is present in 40%. Flap pedicle is about 5-9 cm. The length of the flap varies from 2-4 cm.

Material and Methods

We have in study 7 cases with work related trauma in two years period of time, between 2012-2014. Two of the cases were presented in our emergency with partial distal thumb amputation and five other this distal open wounds with bone exposure.

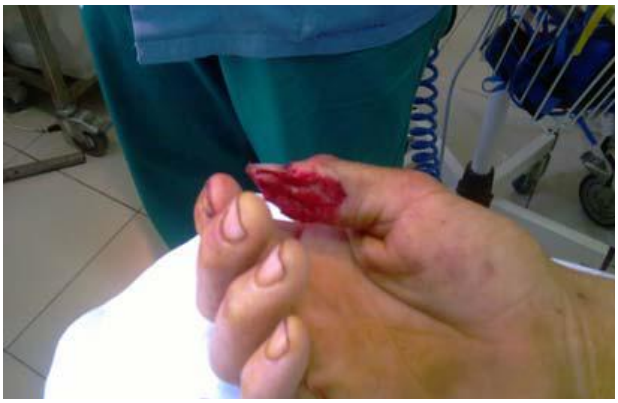
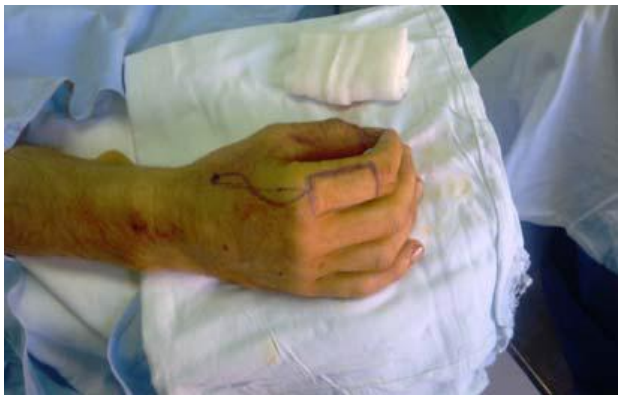
The patients mean age was 31 (range 17-48 years), and mean follow-up was 11 months (range 6-17). All the patients were males. Emergency surgery was performed in all patients with a time delay after injury of 11-25 hours. The minimum defect was 14 x 19 mm and the maximum 20 x 38 mm. Pedicle length was 51-93 mm. We had only one partial flap survival and all the other flaps survived entirely. We have also taken in consideration subjective satisfaction with a range score from 4 to 10, cold intolerance,flap area and donor site sensibility with a range score from low to medium to normal.

Results

Selecting the most appropriate technique for thumb reconstruction depends on multiple factors, including the following: Level of injury; Status of the remaining hand; Presence or absence of the thenar musculature; Age, occupation, overall health, and functional demands of the patient.

Pulp area of the thumb is the area where Foucher's flap is more utilizable. In cases of traumatic injury to the thumb, radiographs should be obtained to determine the presence of fracture and to assess the quality of the IP, MCP, and CMC joints.

The patient is placed in a supine position, and the arm is positioned on the arm table. The operation is done in plexus block or under general anesthesia. A tourniquet (250–300 mmHg) is applied at the upper arm. After debridement of the thumb defect, the skin flap is outlined. The flap is harvested from the dorsal aspect of the index finger, including the first dorsal metacarpal artery and a branch of the superficial radial nerve as a pedicled flap. First DMCA is identified at the emerge point between the two heads of the first dorsal interosseous muscle in te index finger.

a)  b) 

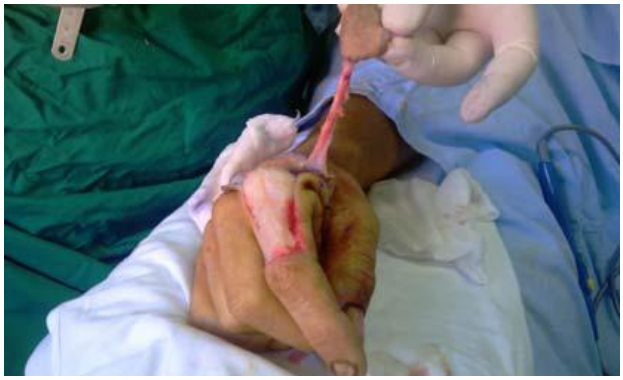
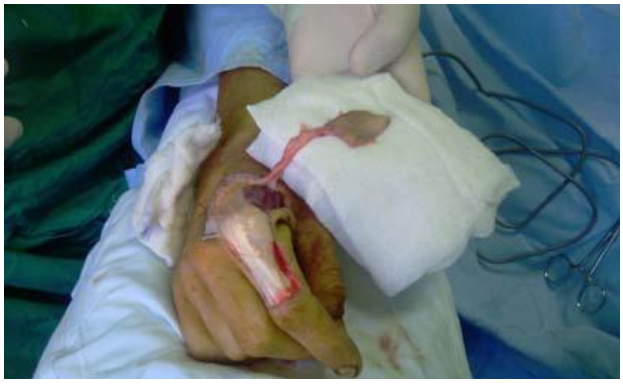
c)  d) 

Figure 1: a) and b) Case display [a 25 years old boy with a work-related injury]; c) and d) flap elevation

The fascia is cut and the periosteum is then stripped off the second metacarpal bone on the radial side.The nutrient branch to the metacarpal head is identified and tied up. The flap is elevated, leaving the paratenon intact. The pedicle includes the fascia of the first dorsal interosseus muscle, the dorsal veins, and the sensory branch of the radial nerve. Then ,the flap is placed over the defect taking.The donor area is covered by an antecubital full-thickness skin graft.A lot of attention should be taken to the pedicle positioning in order to prevent strangulation [4-6].

The predicted outcome of surgery generally favors reconstruction when an amputation has occurred distal to the MCP joint and has therefore left the first web space, as well as the thenar muscles (including their insertions), preserved.

This technique has other applications such as first web reconstruction, thumb lengthening, and following resection of tumors on the dorsum of the hand [5, 7].

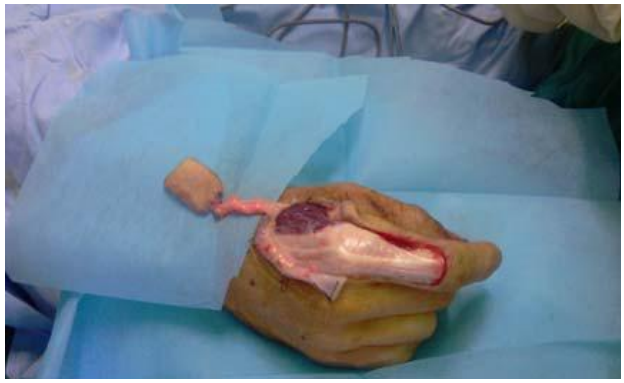
Individual results of the seven patients with thumb reconstruction using Foucher's flap are shown in Table 1.

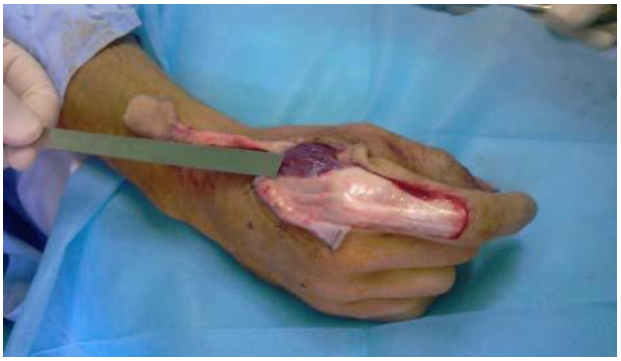
Table 1: Individual results of the seven patients with thumb reconstruction using Foucher's flap

Patient	1	2	3	4	5	6	7
Sex	Male	Male	Male	Male	Male	Male	Male
Age	25	28	48	17	36	43	39
Surgery time after truauma	11 hours	16 hours	21 hours	25 hours	18 hours	22 hours	15 hour
Flap survival	Total	Total	Partial	Total	Total	Total	Total
Pedicle measured	68mm	65mm	51 mm	72mm	80mm	93 mm	85mm
Cold intolerance	No	No	Jes	No	No	No	No
Satisfaction	10	9	8	9	10	10	9
Flap sensibility	Normal	Normal	Medium	Normal	Normal	Normal	Medium
Donor site sensibility	Medium	Normal	Medium	Normal	Normal	Normal	Medium

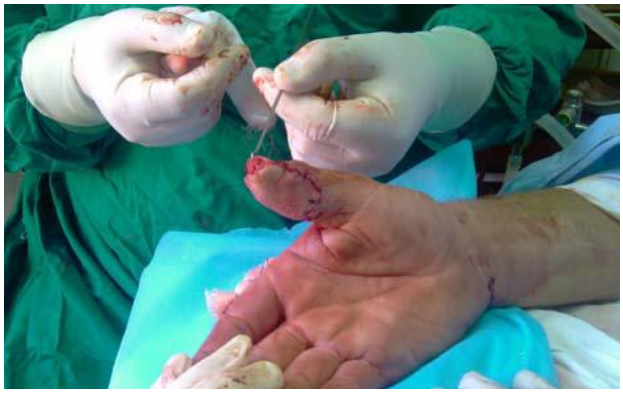
Details of the patient with thumb reconstruction using Foucher's flap are shown in Fig. 1 and Fig. 2 [a 25 years old boy with a work-related injury].

a) b)





c)



d)

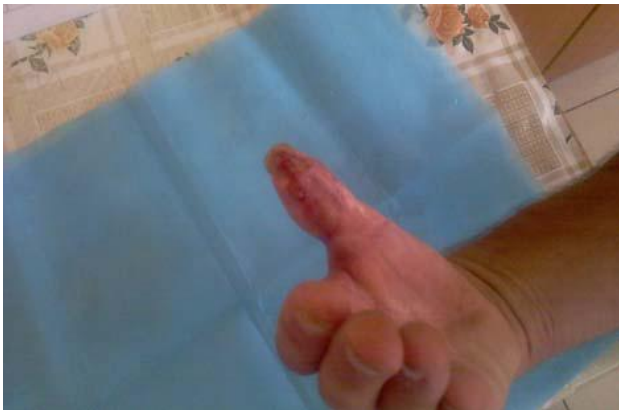


Figure 2: a) and b) pedicle length is measured; c) wound closure; d)after three weeks

In conclusion, Foucher's flap is a reliable choice in thumb reconstruction surgery. The results are very good and complications are rarely seen. Careful pedicle discovery, secured elevation, pedicle strangulation prevention are very important for flap survival.

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Clinical Science

Echocardiography as a Predicting Method in Diagnosis, Evaluation and Assessment of Children with Subvalvar Aortic Stenosis

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Key words: congenital heart disease; aortic stenosis; subaortic membrane; hypertrophic cardiomyopathy; heart surgery.

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Abstract

BACKGROUND: Obstruction to the left ventricular outflow of the heart may be above the aortic valve (5%), at the valve (74%), or in the subvalvar region (23%). These anomalies represent 3 to 6% of all patients with congenital heart defects (CHD), and it occurs more often in males (male-female ratio of 4:1).

AIM: The purpose of this study was to determine the sensitivity and specificity of transthoracic echocardiography in diagnosis of discrete subaortic membrane, to determine convenient time for surgical intervention, and for identifying involvement of the aortic valve by subaortic shelf.

MATERIAL AND METHODS: A retrospective review of the medical records and echocardiograms of 18 patients [14 male (77%) and 4 female (23%)] with discrete subaortic membrane, aged 11 month to 12 years, with mean age of 5 years and 3 month, diagnosed at the Pediatric Clinic in Prishtina, during the period September, 1999 and December, 2010 were done.

RESULTS: Four patients, in neonatal age were operated from critical coarctation of the aorta and, initial signs of congestive heart failure were presented. 2 of them were operated in Belgrade, Serbia and 2 in Lausanne, Switzerland.

CONCLUSION: In all presented patients bicuspid aortic valve was noted, but none of them subaortic membrane was registered.

Introduction

Obstruction to the left ventricular outflow of the heart may be above the aortic valve (5%), at the valve (74%), or in the subvalvar region (23%). These anomalies represent 3 to 6% of all patients with congenital heart defects (CHD), and it occurs more often in males (male-female ratio of 4:1). Valvar and subvalvar stenosis may be associated with other CHD such as pulmonary stenosis (PS), anomalies of the mitral valve, patent ductus arteriosus (DAP) and, defects of the interatrial septum. A variety of lesion can obstruct the subaortic outflow tract, with or without a coexisting ventricular septal defect. Obstruction can be produced by hypertrophy of the ventricular septum, as seen in hypertrophic cardiomyopathy, by anomalous tissue tags derived from the membranous septum or the leaflets of the atrioventricular valves or by anomalous attachment of the tension apparatus of the atrioventricular valve [1, 2]. When the ventricular septum is intact, the most common lesion is the so-called subvalvar ridge which presents the fibrous shelf that encircles the outflow tract in diaphragmatic fashion. The shelf tends to be a discrete structure, although its position can vary with regard to its proximity to the valvar leaflets. Discrete subaortic stenosis represents a form of subvalvar aortic stenosis characterized by a membranous ridge proximal to the aortic valve. Diagnosis of discrete

subaortic stenosis in neonatal period is rare and, lesion more commonly presents as an acquired defect in early infancy or childhood. Two thirds of these patients have associated cardiac lesions, such as ventricular septal defect, DAP or coarctation of the aorta (CoA) [3-6]. Risk of late diagnosis, consequence as a post-operative aortic insufficiency, the potential for recurrent stenosis, and surgical management of discrete subaortic stenosis have traditionally fairly conservative due to the unpredictable rate of progression, postponing resection unit a certain left ventricular outflow tract gradient has been reached.

The most commonly described sequela in patients with subaortic stenosis is aortic regurgitation, which have been estimated to occur to some degree in 30 % to 50 % of pediatric patients and as many as 80 % of adult patients. Damage of aortic valve and subsequent regurgitation is thought to be secondary to the subvalvar high-velocity systolic jet produced by the outflow tract obstruction [3, 4]. Earlier diagnosis and surgical treatment of discrete subaortic stenosis is now preferred by many surgical centers in an effort to avoid consequences such are left ventricular hypertrophy, sudden death, aortic insufficiency or endocarditis. Increasing left outflow tract gradient is a well-accepted indication for surgical treatment of discrete subaortic membrane, this indication may overlook the shelf's progressive nature to grow and encroach on the leaflets of the aortic valve, potentially impairing future valve function. Since the morbidity and mortality for discrete subaortic membrane resection is negligible, resection may be indicated at the time of echocardiographic diagnosis to minimize aortic valve impairment [7, 8].

Transthoracic echocardiography is used to diagnose and follow of progression of all types of left outflow tract obstruction, especially discrete subaortic membrane; however, its ability to demonstrate the extent of discrete subaortic membrane encroachment onto the aortic valve is unclear.

The purpose of this study was to determine the sensitivity and specificity of transthoracic echocardiography in diagnosis of discrete subaortic membrane, to determine convenient time for surgical intervention, and for identifying involvement of the aortic valve by subaortic shelf.

Material and Methods

A retrospective review of the medical records and echocardiograms of 18 patients [14 male (77%) and 4 female (23%)] with discrete subaortic membrane, aged 11 month to 12 years, with mean age of 5 years and 3 month, diagnosed at the Pediatric Clinic in Prishtina, during the period September, 1999 and December, 2010 were done. At the same time, database and medical reports for 13 patients (72%) undergoing primary repair for discrete subaortic stenosis in different European and North America centers, as a consequence that in Kosova still don't exist cardiac surgery service; at the same time surgical reports of all operated children were analyzed. All operations were performed utilizing cardiopulmonary bypass. Access to the discrete subaortic membrane was achieved through a transverse aortotomy and, circumferential resection of the entire obstructing shelf was performed. 5 (27%) patients had isolated discrete subaortic membrane, 10 patients (55%) had CoA and 13 patients (72%) had bicuspid aortic valve, 2 (11%) patients had supravalvular aortic stenosis.

A three pediatric cardiologist executed minimum 3 echocardiograms in all children, with a mean interval of 6 month. None of the patients was receiving cardiovascular medications at the time of examination. All the echocardiograms were performed at rest, without sedation, and include M- and two dimensional modes, continual and color Doppler imaging. Used ultrasound systems were Acuson Aspen, Acuson Sequoia and Hewlett Packard Sonos 2000. The systolic function of the left ventricle was evaluated through the ejection fraction, using the M-mode measurements. The morphological aspect of the discrete aortic membrane, aortic valves and others heart structures was evaluated by the two-dimensional mode, while the severity of discrete membrane and aortic valves stenosis was determined by continual and color Doppler, according to the recommendations of the American Society of Echocardiography.

Results

Four patients, in neonatal age were operated from critical coarctation of the aorta and, initial signs of congestive heart failure were presented. 2 of them were operated in Belgrade, Serbia and 2 in Losana, Zvicerland. In all of them bicuspid aortic valve was noted but, none of them subaortic membrane was registered. During the next 2 years of following all of them subaortic membrane manifested, while by continual and color Doppler increasing turbulent flow was registered.

The mean group of children in our study (12 patients 66%) subaortic membrane was diagnosed during the routine echocardiogarphic examination for the heart murmur. 6 of them had aortic coarctation and, 6 other had bicuspid aortic valve with consequence of aortic stenosis. In 2 oldest patients in our group, diagnosis was decided at tertiary level for cardiac evaluation, after routine examination from school doctor where, high blood pressure was noted.

2 patients had additional non cardiac congenital anomalies, with one patient each having Down and Alagille's syndrome, a variant of Shone's syndrome.

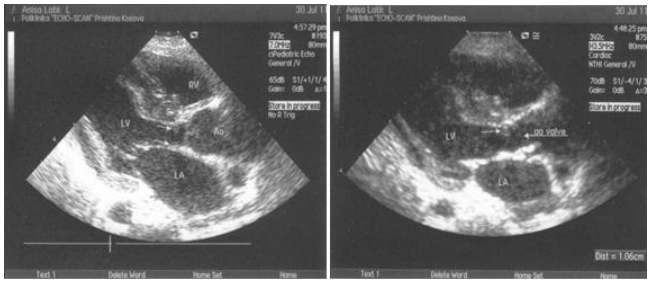

Figure 1: Transthoracic long-axis view in diastole (Fig 1a), and in systole (Fig 1b) shows subaortic shelf, and as a consequence, hypertrophy of the left ventricle and dilated ascending aorta

In all patients pre-operative trans thoracic echocardiography demonstrated increasing velocity in left outflow tract with e mean peak of 3.9 m/s plus or minus 1.1 m/s with a mean peak left outflow tract gradient of 60.8 plus or minus 19.3 millimeters of mercury. Trivial aortic insufficiency was noted in 8 patients (44 %), mild in 6 patients (33%) and absent was in 4 patients (22%).

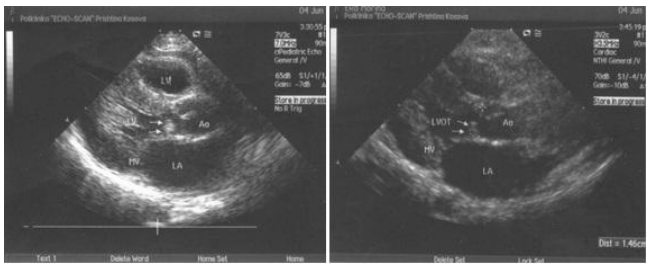

Figure 2: Transthoracic view of eight years old boy, previously operated from perimembranous VSD, showed subaortic membrane few millimeters under arotic valve, closing left ventricular outflow tract

A total 13 patients undergoing primary repair of the discrete subaortic membrane, where intra-operative evaluation revealed involvement of the aortic valve by a discrete subaortic membrane in 10 patients or 77%.

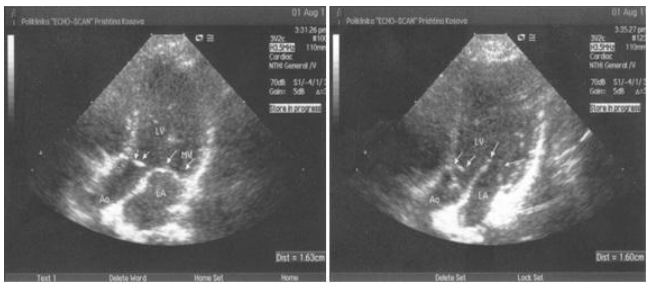

Figure 3: Long-axis view in diastole (Fig 1a), and in systole (Fig 1b) shows hyperechogenic subaortic shelf, hypertrophic left ventricle and dilated left atrium as a consequence of severe mitral regurgitation

Reviewing our echocardiograms reports and reports from the centers where surgery was done, showed that the involvement of the aortic valve preoperatively was identified in 4 patients (40%). There was a significant difference between involvements of the aortic valve predicted by pre-operative echocardiogram reading compared with the involvement of the aortic valve documented intra operatively. The sensitivity and specificity of pre-operative trans thoracic echocardiography to diagnose involvement of the aortic valve are 40% and 77%.

Discussion

A variety of lesion can obstruct the subaortic outflow tract and the lesion has been described in many ways and has been the subject of multiple investigations. Although often termed "membranous", almost always the lesion is a firm fibrous shelf that encircles the outflow tract in diaphragmatic fashion. Discrete subaortic ridges are not congenital; they are acquired lesion as a result of some hemodynamic abnormality, and are not usually found before the age of 9 months. The membrane usually extends from the anterior septum to the anterior mitral leaflet, causing the variable degree of obstruction to flow. Lesion is associated with other sequels as aortic regurgitation, infective endocarditis, left ventricular hypertrophy and recurrence after surgical resection [9-12].

Symptoms are uncommon with this subvalvar stenosis, even when the narrowing is severe; however, occasionally syncope and giddiness occur. In undiagnosed and presenting in middle life, congestive heart failure, dyspnoea and syncope have been described. Clinical manifestation and physical signs are similar to aortic valvar stenosis (carotid thrill, left ventricular apex and aortic ejection systolic murmur).

Echocardiography with Doppler ultrasound is the quickest and best way of making the diagnosis and differentiating the common from rarer forms. M-mode echo allows identification of the degree of left ventricular hypertrophy, the presence of septal hypertrophy and characteristic mid-systolic vibration of aortic valve. Cross-sectional examination in the parasternal long axis shows the characteristic ridge in the outflow tract. With two-dimensional echocardiography, these membranes are seen as a discrete linear echo in the left ventricular outflow tract perpendicular to the intreventricular septum. Because the membranes are parallel to the beam, recording these structures from the parasternal long-axis window may require the use of multiple transducer positions. In many cases, the membranes are detected more easily from the apical views, where the ultrasound beam is oriented perpendicular to the structure [13-15]. In older children or young adults, transesophageal echocardiography demonstrates this lesion more fully and it's a better way to identify

associated lesions of the mitral and aortic valves. Two-dimensional echocardiography distinguished discrete subarotic membrane form subaortic fibromuscular ridge or tunnel. Tunnel-type subaortic obstruction, rarely seen in adults, is characterized by diffuse thickening and narrowing of the left ventricular outflow tract with associated concentric left ventricular hypertrophy [16-18].

Doppler imaging plays an essential role in the evaluation of these patients. After the location and orientation of the jet are visualized with color flow imaging, continuous wave can be used to estimate the peak pressure gradient across the membrane. In the absence of aortic valve stenosis, this value correlates well with the catheterization-derived measure of obstruction. In the presence of multiple serial stenoses, Doppler imaging may overestimate the catheterization-measured gradient [19, 20].

Because of progressive nature of the disorder, and the presence of aortic regurgitation, surgery is indicated even when the gradient through left ventricular outflow tract is over 30 – 40 millimeters of mercury. There are several controversies regarding the optimal surgical management of these patients, including additional indications for shelf resection, the timing of resection, and the indication for septal myectomy. Additional indications for resection at many centers include the presence of co-existing cardiac lesions and evidence of new or progressive aortic insufficiency. The progressive nature of discrete subaortic membrane is unpredictable, thus the timing of surgical intervention is controversial [21, 22]. Some centers recommend earlier surgical intervention to avoid the development of the left ventricular hypertrophy and aortic insufficiency. Scraping a fibrotic shelf of the aortic valve is technically challenging and puts the aortic valve leaflets at risks of perforation. At the same time, it is difficult to imagine that this damage to the endothelium of the aortic leaflets does not change the natural history of the valve's function. There are several controversies regarding to the concomitant septal myectomy which aim is to achieve maximum relief of left ventricular outflow tract obstruction and perhaps reduce re-growth of the discrete subaortic membrane. Other centers reported little additional benefit with septal myectomy [23, 24].

Table 1: Baseline characteristics

Male	14 (77)
Age	11 m – 12 y
(Mean 5 y 3 m)	
Associated anomalies	13 (72)
Coarctation of the aorta	10 (55)
Bicuspid aortic valve	13 (72)
Supravalvular aortic stenosis	2 (11)
Surgical resection of subaortic membrane	13 (72)
Other surgeries performed during follow-up	
Coarctation repair	10 (55)
Supravalvular aortic repair	2 (11)
Konno procedure	3 (16)
Aortic valve replacement	2 (11)

N =18; values are: n, n (%), median (range).

This study demonstrates that the transthoracic echocardiography is the best noninvasive diagnostic procedure in diagnosis subvalvar aortic stenosis and estimation time for surgical intervention. At the same time echocardiography has either the sensitivity or specificity to demonstrate aortic valve encroachment by a discrete subaortic membrane. Some centers recommended transesophageal echocardiography as a routine method for following discrete subaortic shelf progression due to its invasive nature. From 13 our patients operated from subaortic membrane 10 of them had involvement of the aortic valve. 4 of them required re-operation after 3 - 6 years, where in 3 of them primary resection of membrane less than 2 years of age were done. All 4 reoperated patients had involvement of the aortic valve and after surgery moderate aortic insufficiency were manifested. In this series of 6 patients with moderate aortic insufficiency 2 patient developed late post-operative insufficiencies and aortic valve replacement were done. Since 4 patients in our study, who required re-resections, had the involvement of the aortic valve at the time of primary resection, the risk of next reoperation associated with the involvement of the aortic valve must be accounted for.

In conclusion, basing on the data of our study and recent published data from many centers we can conclude that:

- Transthoracic echocardiography is the best diagnostic procedure in diagnosis and assessment of the all forms of subaortic stenosis;
- Transthoracic echocardiography is not able exactly to demonstrate involvement of the aortic valve by discrete subaortic membrane and reliably to give prognosis of possible postoperative aortic insufficiency.
- Transesophageal echocardiography is more sensitive method in predication of the involvement of subaortic valve by discrete subaortic shelf but there are now still recommendations for improving this method as a routine modality.
- The increasing peak gradient of left ventricular outflow tract greater than 40 millimeters of mercury and age of patients above of 5 years are accepted as an indication for surgery. We can conclude also that the distance from discrete membrane to the aortic valve is an important predicting factor for involving the aortic valve by discrete membrane.
- Bicuspid aortic valve disease, coarctation of the aorta, and supravalvular aortic stenosis were associated with the need for aortic stenosis surgery. Careful clinical follow-up of this young population to monitor aortic valve

status continues to be warranted even after a successful surgical resection.

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Clinical Science

Prevalence of Diabetes Mellitus in Patients with Chronic Kidney Disease

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Key words: chronic kidney disease; diabetes mellitus; risk factors; prevalence.

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Abstract

BACKGROUND: Chronic kidney disease (CKD) became a new epidemic of the twentieth and twenty-first centuries. Diabetic nephropathy is one of the leading causes of end-stage renal failure as a result of the diabetes epidemic worldwide.

AIM: The aim of our study was to assess the prevalence of CKD in the Republic of Macedonia and its association with diabetes mellitus.

MATERIALS AND METHODS: The study was a part of a study conducted in 2006 in terms of screening for early detection of kidney disease. It was a cross-sectional study based on a random sample of patients aged > 20, consecutively consulting their primary physician for any cause. Fifty physicians throughout the country were included in the study. A total of 2637 patients have been analyzed based on integrity data. GFR was estimated using corrected values of serum creatinine and calculating kidney function by the Cockroft & Gault formula, adjusted for body surface using the Gehan & George formula. Patients with estimated glomerular filtration rate (eGFR) less than 60 ml/min were considered as having CKD. Blood pressure, body weight, height, serum creatinine, glucose, hemoglobin, hematocrit, urinalysis and medical history for presence of cardiovascular diseases or diabetes were also assessed.

RESULTS: The mean age of the subjects was 45.97 ± 16.55 SD and 17.97% were older than 60. Regarding gender, 44.14% were males. The prevalence of diabetes mellitus was 13.9%. Subjects with CKD (eGFR less than 60 ml/min) were 7.53% of the total. Subjects aged 60 or above, had 20 times higher risk of having CKD (eGFR less than 60 ml/min/1.73 m^2). Out of the total group of subjects, 13.9% had diabetes mellitus and they had 3.13 times higher risk of having CKD stage 3-5 (eGFR less than 60 ml/min/1.73 m^2) when compared to non-diabetics. The results showed that diabetes was significantly more associated with lower eGFR (less than 60 ml/min/1.73 m^2) in younger subjects (age less than 60) compared to older ones (odds ratio 3.29 versus 1.21).

CONCLUSION: Our study showed that chronic kidney disease is frequent in the Republic of Macedonia and is associated with older age and diabetes. Diabetes had a significantly stronger association with CKD at younger age.

Introduction

Chronic kidney disease (CKD) became a new epidemic of the twentieth and twenty-first centuries. At present, it is a global problem, mainly because a variety of risk factors are being involved in its etiology and pathophysiology. It is not surprising that diabetic nephropathy is one of the leading causes of end-stage renal failure having in mind the diabetes epidemic worldwide [1].

The prevalence of CKD differs among countries and ethnicities. It is of great importance for the policy makers to know the prevalence of CKD, as it is associated with high morbidity and mortality and high cost of renal replacement therapy. Guidelines

from the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) recommend estimating glomerular filtration rate and screening for albuminuria in patients with risk factors for chronic kidney disease, including diabetes, hypertension, systemic illnesses, age greater than 60 years, and family history of chronic kidney disease. The glomerular filtration rate (GFR), calculated by using a prediction equation, detects chronic kidney disease more accurately than does the serum creatinine level alone; the GFR also is used for disease staging. Current KDOQI guidelines recommend screening for kidney disease with a serum creatinine measurement for use in GFR estimation and analysis of a random urine sample for albuminuria [2].

The prevalence of CKD has increased dramatically in the period of 1999 to 2007 in different countries around the world, developed, as well as developing countries. It has reached epidemic proportions with 10–13% of the populations in Taiwan, Iran, Japan, China, Canada, India and the USA [3].

The aim of this paper was to estimate the prevalence of CKD in the Republic of Macedonia and the association of diabetes mellitus in patients with CKD not on renal replacement therapy.

Materials and Methods

This study is part of a study conducted in August/September 2006 in terms of screening for early detection of kidney disease (SKROBB). It was a cross-sectional study based on a random sample of patients aged $\geq$ 20, consecutively consulting their primary physician for any cause. Fifty physicians throughout the country were included in the study. A total of 3019 patients were included, but 2637 have been analyzed based on integrity data. The physicians were asked to fill in a questionnaire for demographic and medical history data. They were also required to measure blood pressure, body weight, height, serum creatinine, glucose, hemoglobin, hematocrit and urinalysis. Proteinuria (qualitatively, 1+ or more), and/or hematuria, and estimated glomerular filtration rate (eGFR) $\leq$ 60 ml/min per 1.73 m^2 have been considered as markers of renal damage or of chronic kidney disease. Serum creatinine has been measured by the use of Jaffe's method, and in order to minimize the differences among alkaline picrate methods regarding influence of protein on creatinine results, at least in 10% of all the samples of each laboratory creatinine has been measured at the reference laboratory at the Medical Faculty in Skopje. A correction factor for creatinine has been estimated in each laboratory in the country. The body surface area has been measured using the Gehan & George formula [4]. The eGFR has been estimated using the Cockroft & Gault formula (Calculated creatinine clearance = [(140-age) x 1.23 x BW(kg)/Scr] x 0.85 if female). Gender, age, presence of cardiovascular diseases, hypertension, diabetes, anemia, and obesity (BMI) has been considered as risk factors for CKD. Hypertension was defined as systolic blood pressure (SBP) $\geq$140 mmHg and diastolic blood pressure $\geq$ 90 mmHg or having medical history or being treated for hypertension [5]. Persons were considered diabetic if fasting blood glucose was more than 7.1 mmol/l, or having medical history or being treated for diabetes [6].

The prevalence of CKD was calculated after adjustment for age and gender to the general population (the population census in 2002 was taken; the total population aged $\geq$ 20 was 1,429,642).

Stage 1 and 2 CKD was considered on the basis of proteins in the urine (1+ or more) without evidence of urinary infection.

Statistical analysis has been conducted using the software program Statistica for Windows 10.0. Student-t test was used to compare the numerical data. Mantel-Haenszel Chi-square test was used to see the relationship between two dichotomous variables. A p-value < 0.05 has been taken as statistically significant.

Results

The study included 2,637 subjects based on integrity data. The mean age of the subjects was 45.97 ± 16.55 SD and 17.97% were older than 60. Regarding gender, 44.14% were males. The relevant demographic and clinical data were the following: prevalence of diabetes mellitus was 13.9%, hypertension had 36.37% of subjects, actual smokers were 28.02%%, previous smokers and non-smokers were 12.49% and 59.08% respectively. According to ethnicity, Macedonians were 74.42%, Albanians 17.23%, Roma 5.67% and others 2.68%. Anemia (hemoglobin less than 110 g/l) had 8.52% of subjects, history of cardiovascular diseases 37.46%, subjects living in urban area were 83.63%, and rural 14.42 %.

Table 1: Prevalence of CKD stages estimated by Cockroft-Gault (adjusted by age/gender)

CKD stage	% male	Per million males	% females	Per million females	% total	Per million total
Stage 1	3.97	39.758.00	3.2	32.445.00	3.57	35.673.4
Stage 2	2.11	21.132.2	3.51	35.129.8	2.89	30.103.0
Stage 3	5.5	54.969.7	8.4	84.357.0	7.1	71.384.9
Stage 4	0.24	2385.5	0.27	2677.6	0.25	2548.6
Stage 5	0.07	717.4	0.26	2622.9	0.18	1781.8
Total					**13.99**	

Table 1 shows the prevalence of CKD stages in the investigated population adjusted by age and

gender to the general population. The population census in 2002 has been used. Stages 1 and 2 were defined by presence of proteins in the urine (1+ or more).

Table 2 shows that subjects older than 60 years have a 20 times greater risk of having CKD (eGFR less than 60 ml/min/1.73 m^2) compared to those being less than 60 years of age.

Table 2: Association of age and low eGFR

Age	eGFR ≥ 60 ml/min/1.73m^2	eGFR < 60 ml/min/1.73m^2
≥ 60 years	395	175
< 60 year	2008	43
Total	2403	218

Mantel-Haenszel = 478.43; (95% CI: 14.6-29.4); OR = 20.7; p < 0.001.

Table 3 shows the presence of diabetes in different levels of eGFR. Those having established CKD, eGFR less than 60 ml/min/1.73 m^2 have higher percentage of diabetics compared to those with eGFR above 60 ml/min/1.73 m^2.

Table 3: Presence of diabetes mellitus (DM) in subjects according to the level of eGFR

Egfr Ml/Min/1.73m^2	Presence of DM	Absence of DM	Total	No Data	Total	%
≥90	126	1431	1557	30	1587	7.9%
60-89	166	632	798	18	816	20.3%
30-59	64	140	204	3	207	30.9%
15-29	2	5	7	0	7	28.6%
<15	0	4	4	0	4	0
Total	358 (13.9%)	2212	2570	51	2621	

Table 4 shows that those having diabetes have 3 times higher risk of having CKD (eGFR less than ml/min/1.73 m^2) compared to those who do not have diabetes.

Table 4: Association of diabetes and lower eGFR (less than 60 ml/min/1.73 m^2)

Presence of DM	GFR ≥60 ml/min/1.73 m^2	GFR <60 ml/min/1.73 m^2
Yes	292 (81.6%)	66 (18.4%)
No	2063 (93.3%)	149 (6.7%)

Mantel-Haenszel = 54.9 (95% CI 2.28-4.28), OR- 3.13 p < 0.001.

After correction for age, it appeared that subjects aged less than 60 and having diabetes, have greater risk for having lower eGFR (less than 60 ml/min/1.73 m^2) compared to subjects with diabetes that are older than 60 (Table 5). It indicates that diabetes is significantly more associated with lower eGFR in younger subjects. Age is a confounding variable. In order to see whether age (below/above 60) will have an impact upon a subject with diabetes to have lower eGFR (less than 60 ml/min/1.73 m^2), a statistical analysis using 3 x 2 table (2 x 2 x 2) was performed, and it showed a Chi-square of 5.991, degrees of freedom 1, and p = 0.0144. This result confirms that the difference in odds ratios between 3.29 and 1.1 is significant. Thus, we can conclude that age significantly predisposes the subject with diabetes towards having lower eGFR.

Table 5: Association of diabetes with lower eGFR after correction by age

DM	Age < 60		Age ≥ 60	
	GFR ≥ 60	GFR < 60	GFR > 60	GFR < 60
Yes	172 (94.5%)	10 (5.5%)	120 (68.2%)	56 (31.8%)
No	1757 (98.3%)	31 (1.73%)	306 (72.2%)	118 (27.8%)

For age less than 60: For age above 60: Mantel-Haenszel – 11.458, p < 0,001 Mantel-Haenszel 0.959; p = 0.3274; 95% CI 1.58-6.83; 95% CI 0.826-1.77; OR-3.29; OR-1.21.

Discussion

Data on the epidemiology of CKD in predialysis stages exists only in a small minority of countries in Europe. So, there is lack of information on prevalence of CKD in the different stages in different countries [7]. The available studies from the USA, Europe, Australia, and Asia showed that the prevalence of CKD is about 9–13% in the general population. The incidence and prevalence of patients with CKD including end-stage renal disease (ESRD) have doubled in the past 10 years in the USA and it is increasing not only among adults in the United States but also worldwide [8-10]. The incidence and prevalence of CKD increase markedly at older age [9, 11]. For example, in the Framingham Heart Study, the risk of developing stage 3 CKD was 2.36 times higher for each 10 years older age [11]. Additionally, the prevalence of stage 1 or 2 and stage 3 or 4 CKD among US adults has been reported to be 3.3 and 54 times higher, respectively, for adults ≥70 versus 20 to 39 years of age [9]. Our study also showed a strong correlation of age and CKD (20 times greater risk of having CKD if a subject is 60 years of age or older).

In the study of Islam T. et al, for adults 20 to 49, 50 to 69 and ≥ 70 years of age, the prevalence ratios (95% confidence interval) of stage 3 or 4 CKD associated with diagnosed diabetes mellitus were 3.01 (1.35 – 6.74), 1.61 (1.15 – 2.25), 1.40 (1.15 – 1.69), respectively, p-trend = 0.067; and 2.67 (0.53 – 13.4), 1.35 (0.69 – 2.63), 1.08 (0.78 – 1.51), respectively, for undiagnosed diabetes mellitus (p-trend = 0.369) [10]. Our study also showed strong correlation of diabetes mellitus and CKD, Subjects had 3 times higher risk of having CKD stage 3-5 if being a diabetic. Age in diabetic subjects was a confounding variable for having CKD in our study. Subjects with DM and older than 60 had 1.2 higher risk of having CKD, compared to those with DM and younger than 60 (who had 3.3 times higher risk of having CKD). In the study of Islam [12], although not statistically significant, there was also a trend towards

lower prevalence ratios of stage 3 or 4 CKD at older age for diagnosed (p-trend = 0.067) and undiagnosed diabetes mellitus (p-trend = 0.369).

This study has two limitations. The study population were patients consecutively consulting their general physician for any reason, and not subjects from the general population. Therefore, a possible selection bias must be considered. The high number of patients with diabetes mellitus in the study population points out to this notion. Secondly, stage 1 and 2 CKD were defined by qualitative measurement of protein in the urine, and not by albuminuria or albumin/creatinine ratio. Therefore, the presence of CKD stage1 and 2 may not be completely accurate. Hence, to show the association of age and diabetes with CKD, we take into consideration only CKD stage 3-5 (eGFR less than 60 ml/min/1.73 m^2).

In conclusion, our study showed that chronic kidney disease is frequent in the Republic of Macedonia and is associated with risk factors as older age and diabetes. Diabetes mellitus is associated with stage 3 to 5 CKD across the full adult age range. But, this association was statistically significantly stronger at younger age.

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Clinical Science

Effects of Hormone Replacement Therapy on Insulin Resistance in Postmenopausal Diabetic Women

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Abstract

BACKGROUND: Insulin resistance (IR) is closely associated with diabetes mellitus. On the other hand, increased visceral fat in menopause is also associated with IR, which makes postmenopausal diabetic women in a big risk for cardiovascular diseases. There are conflicting reports about the effects on hormone replacement therapy (HRT) on IR.

AIM: The aim of the study was to investigate the effects of HRT on IR.

METHODS: A total of 40 postmenopausal women with type 2 diabetes were enrolled and followed for 12 months. Half of them were assigned to take HRT, while the other half made the control group. Fasting plasma glucose (FPG) and insulinemia were measured in both groups at baseline and after 12 months. IR was represented by Homeostatic model assessment for IR (HOMA-IR).

RESULTS: HRT was associated with significant decrease in HOMA-IR, FPG and insulinemia in the examined group. There was no significant reduction in FPG and no significant increase in insulinemia levels and HOMA-IR values in control group after 12 months.

CONCLUSION: HRT was associated with statistically signifficant increase of insulin sensitivity. Larger clinical trials will be necessary to understand whether HRT may improve insulin resistance and glucose homeostasis in women with diabetes, especially when given shortly after entering menopause.

Introduction

Postmenopausal estrogen therapy and estrogen plus progesterone hormone replacement therapy (HRT) alleviate symptoms of menopause and attenuate bone loss [1]. Moreover, several observational studies suggest that use of estrogen replacement therapy decreases the risk of coronary heart disease [2-4] and lowers overall mortality rates [5, 6].

The menopause transition, as well as the early postmenopausal period, is associated with an increase in total and central obesity [7-9]. Increased visceral fat is associated with insulin resistance [10], and this preferential storage of abdominal fat may contribute to cardiovascular disease and diabetes in postmenopausal women. Estrogen and HRT may improve fat distribution in postmenopausal women by preventing the increase in central body fat [7, 11-13]. However, the evidence concerning the effects of HRT on glucose homeostasis is controversial. Estrogen replacement therapy has been reported to have no effect on insulin sensitivity in postmenopausal women [14-16] and to improve carbohydrate metabolism in individuals with type 2 diabetes [18, 19]. In contrast, nondiabetic women taking estrogen alone were more insulin-resistant than women not on HRT or women taking estrogen and HRT [19]. Differences in the study population, type and route of administration of hormone therapy, and method of measuring insulin sensitivity may explain the disparate results of estrogen replacement on glucose metabolism.

Individuals with insulin resistance require

increasing levels of insulin to maintain normal glucose levels and are likely to progress to type 2 diabetes mellitus [20]. Insulin resistance is often measured by using the homeostasis model assessment–insulin resistance (HOMA-IR) equation, which is a function of the product of fasting insulin and glucose levels [21]. Fasting insulin levels are the major indicator of insulin resistance and are increased by obesity and decreased with higher degree of physical activity [22]. Despite the potential advantages of measures of insulin resistance, the prognostic value of such measurements has not been extensively evaluated. Estimation of insulin resistance using either levels of insulin, HOMA-IR, glucose, or the ratio of triglycerides and high-density lipoprotein-cholesterol (TG/HDL-C) could potentially improve on cardiovascular risk stratification [23].

Insulin resistance and hyperinsulinemia are clinically important since the effects of insulin have been shown in the formation of atherosclerotic plaques, [24] and, hence, increased risk of hypertension and atherosclerosis has been attributed to insulin resistance in postmenopausal women. In these women, fasting glucose and insulin levels decrease with HRT [25]. It has been postulated that insulin resistance could decrease with HRT use [26]. Different studies resulted in different conclusions; estrogen and progesteron were shown to decrease insulin sensitivity in one study [27], but were reported not to affect insulin sensitivity in others [27, 28].

For this reason, we aimed to study the effects of HRT on IR by the HOMA - IR

Patients and Methods

We prospectively studied 40 women in natural menopause with type 2 diabetes. Diabetes was diagnosed using the criteria of the World Health Organization, at least 2 years before entering the study. In order to maintain their glucose levels in an acceptable range, women with type 2 diabetes were on dietary management alone (two patients) or taking oral anti-diabetic drugs that consisted of metformin and sulfonylureas (38 patients). Each diabetic patient received a diabetic diet of 1300 ckal/day. The women were instructed not to change their diet. None of them were taking insulin. None of the women were taking anti-lipidaemic, corticosteroid or anti-convulsant therapy. The anti-diabetic medications were left unchanged during the study.

Menopause was confirmed by the absence of menstruation for at least 12 months and by high serum levels of FSH (> 30 mIU/ml) and low serum levels of estradiol (E_2) (< 20 pg/ml). The subjects had not received HRT previously. Gynaecological examination and mammogram were normal in all subjects.

Half of the subjects (20 women) were assigned to take HRT (DM – HRT) group. The other half (20 women) made the control group, not taking HRT (DM – non HRT group). The randomisation of the subjects has been done upon base of willingness and motivation to cooperate. Subjects in the DM - HRT group had been taking oral HRT consisting of 17β - estradiol (E2) 1 mg and DRSP (drospirenone) 2 mg - Angeliq®, Schering AG, Germany, 1 tablet daily for 12 months. Subjects in DM – non HRT group were followed the same way as examined group. Exclusion criteria in both groups included *1*) hypertension (systolic blood pressure ≥ 140 or diastolic blood pressure ≥ 90 mmHg); *2*) anemia; 3) various degrees of renal insufficiency; 4) evidence of significant liver disease; and 5) hysterectomy or a history of recent surgery and who demonstrated significant chronic alcohol intake, were also excluded. All patients in this study gave written informed consent.

This work was approved by the local medical ethics committee and all participants gave informed consent before the onset of study.

All of the participants enrolled in our study have been contacted by telephone in three months + interval in order to discover any adverse effect of HRT. All metabolic and physical examinations were performed at the onset of the study and then again after 12 months of receiving HRT. Blood samples were taken after a 12 h fast. HbA_1C was determined on an Cobas c 111 analyzer using commercial kits supplied from Roche Diagnostic Gmbh (Switzerland), and glucose levels were determined in an Beckman Analyzer 2 automated analyser by using commercial kits supplied from Analox Instruments Ltd, London (UK). Insulinemia was determined in an Elecsys 2010 analyzer using commercial kits from Roche Diagnostics Gmbh (Switzerland).

Assessment of IR

In primary analysis, we evaluated degrees of IR by homeostatic model assessment for IR (HOMA-IR) using the formulas HOMA-IR = glucose × insulin/22.5. A higher HOMA-IR value indicates greater IR.

Statistical analysis

Statistical analysis was carried out with descriptive statistics, t - test for related samples and t - test for independent samples. The data are expressed as means ± SEM. Statistical significance was set at $P < 0.05$. Data were analysed using Statistica, version 10.0 (StatSoft).

Results

All of the women who were enrolled into the investigation completed the study. The mean age of the subjects was 49.3 ± 0.34 and 48.5 ± 3.1 years, and their mean body mass index (BMI) was 27.27 ± 3.32 and 28.3 ± 2.4 kg/m^2 in the DM-HRT and DM non-HRT groups respectively. High school educated was 58.3 % and 54.2 %. The baseline characteristics of the subjects are given in Table 1. Two subjects in the DM-HRT group and one subject in the DM non-HRT group complained about abnormal vaginal bleeding such as metrorrhagia and four patients reported breast tenderness in the DM-HRT group. Other adverse effects were not seen

Table 1: Baseline characteristics of postmenopausal women by HRT status

	Women on HRT (*n* = 20)	Women not on HRT (*n* = 20)	P
Age (years)	49 ± 3.34	48.5 ± 3.1	N/S
BMI (kg/m^2)	27 ± 3.32	28.3 ± 2.4	N/S
Fasting plasma glucose (mmol/l)	7.8 ± 0.86	8.0 ± 0.9	N/S
HbA1C (%)	7.6 ± 0.54	7.9 ± 0.5	N/S
Insulinemia(μU/ml)	12.2 ± 3.41	12.3 ± 3.2	N/S
HOMA – IR (μU/ml-mmol/l)	4.23 ± 1.7	4.31 ± 1.8	N/S

N/S for P value – not statistically significant

There was no statistically significance between two groups at baseline. HRT was associated with statistically significant decreases in serum fasting glucose, HbA1C, insulinemia levels and HOMA – IR values in the DM - HRT group. There was no significant reduction in glucose levels and HbA1C together with no significant increase in insulinemia levels and HOMA-IR values in the DM non-HRT group throughout 12 months.

Table 2: Effects on HRT on Fasting Plasma Glucose (FPG), HbA1C, Insulinemia & HOMA - IR

	Women on HRT (*n* = 20)	P value	Women not on HRT (*n* = 20)	P value	P* value
FPG (mmol/l) Baseline 12 months	7.8 ± 0.86 6.9 ± 0.6	p< 0.001	8.0 ± 0.9 7.8 ± 1.1	P=0.66	P*< 0.0001
HbA1C % Baseline 12 months	7.6 ± 0.54 7.2± 0.43	p<0.001	7.9 ± 0.5 7,7 ± 0.4	p=0.477	P*< 0.0001
Insulinemia (μU/ml) Baseline 12 months	12.2 ± 3.41 10.4 ± 2.92	p<0.001	12.3 ± 3.2 13.1 ± 3.7	p= 0.08	P*<0.0001
HOMA – IR (μU/ml-mmol/l) Baseline 12 months	4.23 ± 1.7 3.18 ± 1.4	P<0.001	4.31 ± 1.8 4.54 ± 1.7	P=0.69	P*<0.0001

P < 0.05 statistically significant for all postmenopausal women included in adequate group at baseline and after 12 months; P* < 0.05 statistically significant for group comparison at 12 months.

Regarding group comparison after 12 months, there was statistically significance noted in all examined parameters. Namely, there was statistically significant increase of FPG, HbA1C, insulinemia and HOMA - IR between DM – HRT group and DM – non HRT group after 12 months. The changes in serum fasting glucose, HbA1c levels, fasting insulin levels and HOMA-IR are given in Table 2.

Discussion

The results of the current study indicate that use of HRT in postmenopausal women contributes to the variability in insulin sensitivity observed in diabetic postmenopausal women. Specifically, women taking oral estrogen plus progesterone have higher glucose utilization and insulin sensitivity than women who were not on HRT. Similar to our findings, several studies suggest that estrogen replacement therapy improves glucose homeostasis [29-31]. HRT - treated women had significantly lower fasting insulin levels than women not taking HRT [32]. Glucose utilization by the hyperinsulinemiceuglycemic clamp had a tendency to increase, and HbA1c significantly decreased after 3 months of oral estradiol therapy in postmenopausal women with type 2 diabetes and moderate hyperandrogenicity [33]. The dose of therapy may be important such that low doses of conjugated equine estrogen improved insulin sensitivity by ITT, but higher doses resulted in deterioration of insulin sensitivity [34].

Other studies report that estrogen therapy does not affect carbohydrate metabolism [14-16, 35-37). In the Postmenopausal Estrogen/Progestin Interventions Trial [14], women were given estrogen alone or one of three estrogen/ progestin regimens for 3 years. Fasting insulin levels decreased nonsignificantly in women on active treatments, and changes in 2-h insulin did not differ between treatments or with the treatments. Furthermore, estrogen replacement therapy did not change HbA1c and insulin area under the OGTT curve in patients with diabetes [35, 36] or change glucose tolerance or glucose uptake by the hyperinsulinemiceuglycemic clamp in healthy postmenopausal women [15, 16]. Additional reports conclude that estrogen therapy may, in fact, worsen glucose homeostasis [32–35]. Randomized trials of postmenopausal women indicate that 6–18 months of HRT increase fasting insulin levels [33] and decrease insulin sensitivity using either the intravenous glucose tolerance test (IVGTT) [34] or the insulin tolerance test (ITT) [35].

Differences in the study population (healthy versus diabetic postmenopausal women), type of therapy (estradiol versus the combination of estradiol plus progestin), and method of measuring insulin

sensitivity (fasting insulin concentrations, OGTT, ITT, IVGTT, glucose clamp) may explain the disparate results of HRT on glucose metabolism. Moreover, the route of estrogen administration may contribute to discrepancies in findings such that transdermal therapy may [15] or may not affect glucose metabolism [36, 37]. However, because none of the women in our study were using transdermal estradiol therapy, this does not contribute to the differences in glucose utilization observed.

The mechanism by which estrogen treatment may alter insulin action in humans is not completely understood. Similar to our results, animal studies would suggest that estradiol maintains or improves insulin sensitivity. Estrogen has been shown to increase glucose transport and glucose utilization in muscle cells of animals [38, 39]. Estrogen regulates insulin-induced glucose transport [40] through glucose transporter translocation in rat skeletal muscle [41]. Moreover, in oophorectomized rats, there is a reduction in insulin-stimulated translocation of GLUT4 to the plasma membrane as well as a reduction in glycogen synthase protein expression in skeletal muscle, which contributes to a decrease in whole-body insulin sensitivity [41]. We are unaware of any studies examining skeletal muscle glucose transport in postmenopausal women on or not on HRT. Based on the literature, which suggests that GLUT4 levels do not vary between normal lean glucose-tolerant and obese diabetic subjects [42], we would not expect differences in skeletal muscle GLUT4 protein between women taking estrogen, on HRT, or not on HRT. However, it is possible that glycogen synthase, GLUT4 translocation, and/or other early steps in the insulin-signaling pathway change are altered with the use of estrogen or estrogen plus progesterone in women. In addition, another potential mechanism of estrogen on insulin sensitivity could be mediated through estrogen's effect on peripheral vascular reactivity [43, 44].

In conclusion, our results show that women taking oral HRT are more insulin sensitive than non-hormone users. Additional studies with longer duration and more subjects are needed to determine the cellular mechanisms that could account for the differences in insulin sensitivity between postmenopausal women who are taking estrogen, on combined hormonal therapy, or not on HRT.

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Clinical Science

Evaluation of Anesthesia Profile in Pediatric Patients after Inguinal Hernia Repair with Caudal Block or Local Wound Infiltration

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Abstract

AIM: The aim of this study is to evaluate anesthesia and recovery profile in pediatric patients after inguinal hernia repair with caudal block or local wound infiltration.

MATERIAL AND METHODS: In this prospective interventional clinical study, the anesthesia and recovery profile was assessed in sixty pediatric patients undergoing inguinal hernia repair. Enrolled children were randomly assigned to either Group Caudal or Group Local infiltration. For caudal blocks, Caudal Group received 1 ml/kg of 0.25% bupivacaine; Local Infiltration Group received 0.2 ml/kg 0.25% bupivacaine. Investigator who was blinded to group allocation provided postoperative care and assessments. Postoperative pain was assessed. Motor functions and sedation were assessed as well.

RESULTS: The two groups did not differ in terms of patient characteristic data and surgical profiles and there weren't any hemodynamic changes between groups. Regarding the difference between groups for analgesic requirement there were two major points - on one hand it was statistically significant $p < 0.05$ whereas on the other hand time to first analgesic administration was not statistically significant $p = 0.40$. There were significant differences in the incidence of adverse effects in caudal and local group including: vomiting, delirium and urinary retention.

CONCLUSIONS: Between children undergoing inguinal hernia repair, local wound infiltration insures safety and satisfactory analgesia for surgery. Compared to caudal block it is not overwhelming. Caudal block provides longer analgesia, however complications are rather common.

Introduction

Prophylactic analgesia with local anesthetics is an attractive concept, especially in pediatric practice, because the evaluation of pain can be very challenging in young children [1]. In contrast to opioids, local anesthetics can be administered safely, and in recent guidelines regional anesthesia is accepted as the cornerstone of post-operative pain relief in the pediatric patients [2]. Although regional anesthesia holds a good safety record overall [3], the global experience with pediatric regional anesthesia is still quite low; even the most commonly performed procedure, caudal block, represents only 2.5% of all central neuraxial blocks performed [4]. Determining the risk-benefit ratio is rather difficult for techniques that are relatively rarely performed. Wound infiltration can produce reliable analgesia for superficial skin surgery. Infiltration itself is extensively used by pediatricians, surgeons and emergency physicians for skin laceration repair or minor superficial surgery [1]. Several studies have compared the local anesthesia

so far, including: ilioinguinal and iliohypogastric nerve block plus subcutaneous injection by the surgeon against the caudal anesthesia [5-7]. But, to our knowledge there is still no study comparing the local wound infiltration by itself and caudal anesthesia. The aim of this study was to evaluate anesthesia and recovery profile in pediatric patients after inguinal hernia repair with caudal block (CB) or local wound infiltration (LWI).

Methods

The study was approved by the Institutional Review Board of and was registered at Clinical Trials.gov (registration number: NCT02620566). This randomized double-blinded study was conducted at a single tertiary medical centre ("Mother Teresa") in Skopje, Republic of Macedonia between September and December 2015. After obtaining written information consent from parents, we enrolled a total of 80 children aged 6 months to 7 years of ASA physical status I or II, undergoing unilateral hernia repair. Exclusion criteria included a history of developmental delay or mental retardation, type I diabetes, known or suspected coagulopathy, known allergy to any local anesthetic, known congenital anomaly of the spine, or signs of spinal anomaly or infection at the sacral or inguinal region.

For all the patients included in this trial (n = 80), a standardized anesthetic protocol was used. No premedication was administered. Anesthesia was induced with 2–3 mg/kg of propofol or 8% of sevoflurane in 100% oxygen. Standard monitors including: electrocardiography, noninvasive arterial pressure, pulse oximetry, carbon dioxide, and gas analyzer were applied during induction and maintenance of anesthesia. The airway was established by using a laryngeal mask airway (LMA). Anesthesia was maintained with sevoflurane, and depth of anesthesia was adjusted accordingly with a goal of 80–120% baseline arterial pressure and 4.7–6 kPa end-tidal carbon dioxide ($EtCO_2$). Spontaneous breathing was maintained during surgery. After completion of surgery, the LMA was removed, and the child was sent to a post-anesthetic care unit (PACU) so long as there was no compromise in airway or hemodynamic instability per operatively.

According to the analgesic method used, all patients were allocated to two groups. Group C (caudal) (n = 40) and Group L (local wound infiltration) (n = 40). Enrolled children were randomly assigned to either Group C or Group L according to a computer-generated randomization table. For caudal blocks, Group C received 1 ml/kg of 0.25% bupivacaine (maximum volume 20 ml); Group L received 0.2 ml/kg 0.25% bupivacaine (maximum volume 4 ml) applied as local wound infiltration. An investigator who did not participate in the care of the enrolled children prepared all study medications according to group assignment. Another investigator, performed local infiltration or caudal blocks in all patients.

After induction of anesthesia, children from Group C were placed in a left lateral decubitus position. After the sacral cornua and hiatus were identified, the location of needle entry site was marked. Then a 5 cm short beveled 22 G block needle was inserted into the sacral epidural space. An aspiration test was conducted to exclude intravascular placement before injection administration.

The patients allocated in Group L received local infiltration of the surgical area with 0, 25% bupivacaine 10 min before skin incision. The infiltration technique was standardized as follows: the subcutaneous tissue in the proposed area was infiltrated with 0.25% bupivacaine before incision. A 16-mm 26-gauge needle was inserted in the center of the area, and the medial and lateral parts of the proposed skin incision were infiltrated. With the needle still in central position, a fan-shaped application was administered under the external abdominal fascia.

Surgery was initiated ten minutes after performing the caudal block or local infiltration. The caudal block or local infiltration is considered to have failed if the patient moved his or her limbs, had an increased heart rate, had an increase in mean arterial pressure, or both of more than 15% compared with baseline during the surgery. In such instances, the patient is to be withdrawn from the study and treated with 1–2 μg/kg of fentanyl.

Another investigator who was blinded to group allocation provided postoperative care and assessments. Postoperative pain was assessed using the Children's Hospital of Eastern Ontario Pain Scale (CHEOPS, 0–10) [8] and the Faces Legs Activity Cry Consolability tool (FLACC, 0– 10) [9] at 15 min, 30 min and 1, 2, and 3 h after operation. A child with a score of more than 4 on both CHEOPS and FLACC received 0.5 μg/kg of fentanyl i.v. for rescue analgesia.

Motor function was assessed using the following scale: 0, no motor block; 1, able to move legs; 2, unable to move legs. Assessment of sedation was done with objective score based on eye opening: 0- spontaneously, 1- on verbal stimulation, 2- on physical stimulation. The presence of other adverse events was evaluated as well including: bradycardia, hypotension, respiratory depression, wound infection, fever, wound dehiscence, retching, vomiting, agitation, or urinary catheterization. Hypotension and respiratory depression were defined as 80% of baseline arterial pressure and ≤ 95% of pulse oxygen saturation, respectively. The decision to place a urinary catheter for urinary retention and the evaluation of micturition

were made by an urologist. Analgesia on ward was provided with oral acetaminophen (15 mg/kg). The time for first supplemental oral acetaminophen demand (first acetaminophen time) was defined as the time from the end of surgery to the first registration of more than 4 on both CHEOPS and FLACC by the investigator.

Twenty-four hours after surgery, reports on delayed side-effects and demands for rescue acetaminophen from the child were gathered. The investigator, who was blinded to the treatment group, documented these data with the medical records. Children were discharged from the hospital after 24 h if they met the following discharge criteria: conscious, hemodynamically stable, tolerating oral intake, voiding, walking in an appropriate manner for age, with the absence of retching, vomiting, and other side-effects [10].

Statistical analysis Statistical analysis was performed by using SPSS 17.0. Data were expressed as mean and ± standard deviation and statistically analyzed using Student's t-test, the Mann–Whitney rank-sum test and the test of Difference. A value less than 0.05 were considered as statistically significant for all tests.

Results

In this trial 80 patients were choose eligible for examination but for several reasons they were excluded (Fig. 1). A total of 60 subjects were enrolled in the study and six in total were excluded. Four subjects (one in Group C and three in Group L) were with inadequate caudal block/local infiltration and required additional analgesia in operation theatre.

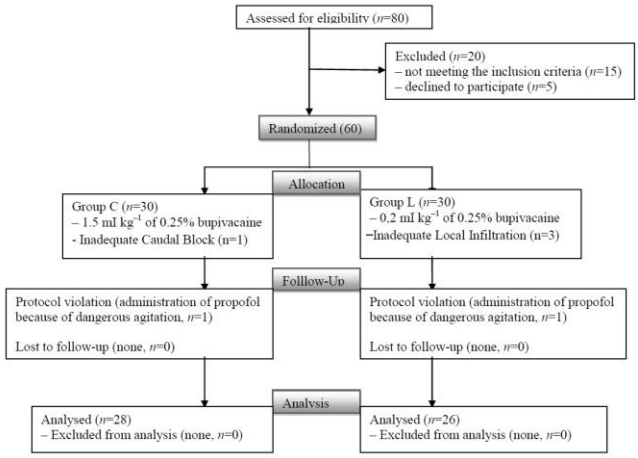

Figure 1: Consort diagram. C: Caudal Group, L: Local Infiltration Group

Two subjects (one in Group C and one in Group L) were excluded because attending anesthesiologists administered propofol to treat agitation that could not be controlled by fentanyl administration. Therefore, these six subjects were all excluded from the study.

Table 1: Patient demographic data and other details (mean ± SD)

	Group Caudal (n = 28)	Group Local (n = 26)
Gender (M/F)	23/5	21/5
Age (years)	3.6 ± 1.8	3.4 ± 1.9
Weight (kg)	14.6 ±4.4	16.5 ± 4.6
Duration of Anesthesia (min)	52.7 ± 9.0	53.5 ± 8.1
Duration of surgery (min)	34.9 ± 8.2	36.8 ± 8.1
Fluids (ml)	44.6 ±11.4	49 ± 10.9

The two groups did not differ in terms of patient characteristic data and surgical profiles as well as hemodynamic changes between groups (Table 1, 2).

Table 2: Hemodynamic changes between groups (mean ± SD)

	SBP		DBP		HP		SaO_2	
Times points	Local (n = 26)	Caudal (n = 28)	Local (n = 26)	Caudal (n = 28)	Local (n = 26)	Caudal (n = 28)	Local (n = 26)	Caudal (n = 28)
T0	102 ± 9.3	103.4 ± 8.3	53.1 ± 7.2	55.6 ± 4.7	138.9 ± 31.5	149.3 ± 14.3	98.3 ± 0.6	98.3 ± 0.7
T1	95 ± 9.5	94.9 ± 8.3	49.6 ± 5.9	49.7 ± 4.4	114.3 ± 22.7	118.3 ± 13.9	99.3 ± 0.4	99.5 ± 0.5
T2	95.2 ± 9.1	92.7 ± 8.3	50.1 ± 6.2	48.3 ± 5.5	114.3 ± 15.6	117.8 ± 14.0	99.4 ± 0.4	99.6 ± 0.4
T3	93.7 ± 8.9	91.6 ± 8.8	47.3 ± 6.3	47.3 ± 6.4	111.8 ± 14.6	113.1 ± 14.1	99.4 ± 0.5	99.5 ± 0.4
T4	98 ± 9.7	94.2 ± 9.2	52.2 ± 6.1	49.4 ± 7.1	119.5 ± 18.8	122.4 ± 14.3	98.8 ± 0.4	99.3 ± 0.4

To – Baseline value; T1 – After induction in anesthesia; T2 – incision; T3 – surgery; T4- end of operation; HR- Heart rate; SBP – Systolic blood pressure; DBP – Diastolic blood pressure; SaO_2%–Peripheral oxygen saturation.

The incidence of rescue fentanyl in the PACU and acetaminophen on ward was significantly lower in children who received caudal block compared to those who received local wound infiltration (Table 4). Two of 28 in the caudal group and nine of 26 in the local group received fentanyl rescue analgesia in PACU. Only one in 28 of the caudal group received acetaminophen on ward and neither one of 26 from the local group received acetaminophen on ward. The difference between groups for analgesic requirement is statistically significant $p<0.05$. Time to first analgesic administration was not statistically significant $p=0.40$.

Table 3: Postoperative analgesic profile in the groups

		CaudalGroup (n = 28)	Local Group (n = 26)
No need of analgesic		25	17
N0 of subject with oral analgesic		3	9
One dose of analgesic		3	9
More than one dose of analgesic for 24 hour		0	0
Rescue fentanyl at PACU		2	9
Acetaminophen on WARD		1	0
Time to first analgesic requirement (min)	Min	15	15
	Max	840	90
	mean ± SD	325 ± 449	55 ± 30
Complications	Vomiting	4	0
	Agitation	1	0
	Urinary retention	1	0
	Motor block	1	0
Analgesia	Opoids	2	9
	Acetaminophen	1	0

Pain scores using CHEOPS and FLACC assessed at the PACU were significantly lower in the caudal group. As for sedation, it was similar in both groups and motor function was better in local group; all subjects had good motor function; in caudal group one of 28 was not able to move the legs and two were able to move their legs and 26 haven't got motor dysfunction (Table 3).

There were significant differences in the incidence of adverse effects in caudal and local group including: vomiting (14.29% vs. 0%), delirium (3.5% vs. 0%), and in one subject from caudal group an urologist decided on urinary catheterization (3.5% vs. 0%). Vomiting was well controlled by a single dose of antiemetic. Delirium was controlled with single dose of midazolam. Adverse effects were not noted in the Local group. The time from when the patient entered the recovery room to when they met the discharge criteria did not differ in both groups; all subjects were discharged after 24 hours.

Table 4: Post anesthesia assessment in the groups in Post Anesthesia Care Unit

Assessments	Group Caudal (n = 28)	Group Local (n = 26)
CHEOPS	2.8 ± 1.6	4.2 ± 1.8
FLACCS	2.6 ± 1.4	3.8 ± 1.9
Post-operative sedation	0.3 ± 0.7	0.4 ± 0.4
Motor function	1.3 ± 0.5	0.0 ± 0.0

(CHEOPS = Children Hospital of Easter Ontario Pain Scale); (FLACCS= Face Legs Activity Cry Consolability scale)

Discussion

To our knowledge, this study is the first to examine the effects of local infiltration alone without ilio-inguinal and ilio-hypogastric nerve block on pain management after pediatric hernia repair surgery. We demonstrated that a single dose of local infiltrated bupivacaine 0.25% 0.2 ml/kg compared with caudal block does not reduce postoperative pain; and compared to caudal block, local wound infiltration is safe in terms of adverse events. In the review article of Martin Jöhe for regional anesthesia in neonates, infants and children, local infiltration can produce reliable analgesia and is widely used by pediatricians, surgeons and emergency physicians [1]. In the same article Jöhe mentions that ultrasound is not essential for performing a CB. On the contrary, it may make a simple procedure complicated and more prone to infection, however it can help in cases of suspected anomalies at palpation and also for teaching purposes [1]. In our study we tried to use the LWI anesthesia alone for hernia repair in children. We did not use ultrasound and failure rate was 3.3% for CB and 10% for LWI. There are studies comparing CB versus LWI but all of them include ilio-inguinal, ilio-hypogastic nerve block and local infiltration [11]. We identified 7 studies in total. Both interventions were performed after surgery in two studies [12-14]; however the other 4 studies performed caudal preoperatively and infiltration postoperatively [5, 13, 15, 16] and only one study performed both techniques preoperatively [17]. All included hernia surgeries only except for Lafferty and colleagues (only orchidopexy) [13]. All used bupivacaine in concentration of 0.25% for CB and 0.25%–0.5% for LWI. The volume ranged from 0.7 to 1.0 ml/kg (CB) and from 0.2 to 0.7 ml/kg (LWI). Only Conroy and colleagues used epinephrine along with bupivacaine [16]. Variations of the infiltration techniques involved infiltration of the wound site through the skin and infiltration of fascia or aponeurosis before closure. No study used image guidance. We performed both interventions preoperatively, CB with 1 ml/kg – 0.25% pure bupivacaine and LWI with 0.2 ml/kg – 0.25% also pure bupivacaine.

Since 1992 Ejlersen compared the efficiency of pre-incision and pos-incision wound infiltration with Lidocain 1% on the postoperative pain of adult patients with inguinal herniotomy. The demand for additional analgesics occurred earlier in those who received Lidocaine infiltration after incision. The pre-incisional infiltration group also had fewer patients requiring supplemental analgesic [18]. The findings of our study and the findings of Ejlersen suggest that the inhibition of peripheral sensitization may be of major importance in impeding the development of acute pain and explain why prevention is important in handling operative pain. There are no firmly established dosage schemes for either technique and each has reasonable alternatives. A 1 ml/kg dose of bupivacaine for CB is widely employed, simple and safe [19]. The best method of local anesthesia is unknown. Varieties of techniques have been used and include wound instillation, wound infiltration and local neural blockade of ilio-inguinal, ilio-hypogastric and genitor-femoral nerves [20]. The optimal concentration of bupivacaine is not known; also which combination of local blocks are optimal needs to be determined. For this study we chose a simple, yet effective technique, which we believe to be popular and clinically relevant.

There are several limitations to the present study. First we can't close the eyes of the investigator who performed the intervention, which means that potential bias exists. There is also lack of long postoperative follow-up to evaluate whether there are other late-onset complications. Number of investigated subjects is low.

In conclusion, between children undergoing inguinal hernia repair, local wound infiltration insures safety and satisfactory analgesia for surgery. Compared to caudal block it is not overwhelming. Caudal block provides longer analgesia, however complications are rather common.

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Clinical Science

The Impact of Treatment with Beta-Blockers upon Mortality in Chronic Heart Failure Patients

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Key words: heart failure; beta-blockers; mortality; combined outcome; relative risk reduction.

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Abstract

BACKGROUND: Besides the conventional therapy for heart failure, the diuretics, cardiac glycosides and ACE-inhibitors, current pharmacotherapy includes beta-blockers, mainly because of their pathophysiological mechanisms upon heart remodeling.

AIM: The study objective was to assess the cardiovascular mortality in the beta-blocker therapy group and to correlate it with the mortality in the control group as well as to correlate the combined outcome of death and/or hospitalization for cardiovascular reason between the two groups.

MATERIALS AND METHODS: The study included 113 chronic heart failure patients followed up for a period of 18 months. The therapy group received conventional therapy plus the target dose of beta blockers, and the control group received the conventional therapy only. The therapy group was divided in three separate subgroups in terms of the type of beta-blocker (Metoprolol subgroup, Bisoprolol and Carvedilol subgroup). To compare the mortality and the combined outcome, the RRR (relative risk reduction) and NNT (number needed to treat) were used, as well as the survival analysis by Kaplan-Meier.

RESULTS: The results showed the following: in regards of the cardiovascular mortality, the relative risk for death in the therapy group was 34%, which, though statistically not significant, is of great clinical significance. In regards of the combined outcome (death and/or number of hospitalizations) the results showed a RRR of 40% in the therapy group compared to the control group, which is statistically highly significant.

CONCLUSION: The study confirmed that patients with stable chronic heart failure, treated with optimal doses of beta-blockers, show a significant reduction of the risk from death as well as combined outcome (death and/or number of hospitalizations).

Introduction

Heart failure is a pathophysiological condition when the abnormal heart function results in a failure of the heart to achieve blood output adequate to meet the requirements of the tissue metabolism.

In condition of heart failure as a response to the heart dysfunction, several neuro-endocrine systems are activated, as well as the sympathetic nervous system. Norepinephrine and plasma-catecholamine levels are increased and correlate with mortality rate, but the density of myocyte beta-1 receptors is lowered in heart failure patients. Beta-blockers inhibit the activity of norepinephrine and enhance the density of beta-1 receptors. By lowering the heart rate they lower the oxygen demand, prolong the diastole, resulting in a better myocardial perfusion and less malignant arrhythmias. They protect the heart of the direct cardiotoxicity of the catecholamines [1, 2]. They also suppress several activated neurohumoral systems in heart failure: rennin-angiotensine system and endothelyne-1 system, a powerful vasoconstrictor [3].

Metoprolol and Bisoprolol are beta-1 selective, and Carvedilol is a potent antagonist of

beta-1, beta-2 and also alfa-1 receptors. It defers from the other beta-blockers with the effect upon the polymorphonuclear cells and its antioxidant activity [4].

Beta–blockers are recommended for treatment of all patients with stabile heart failure from ischemic or non-ischemic etiology and reduced left ventricular ejection fraction in NYHA Class II to IV, as a standard therapy, including ACE-inhibitors and diuretics.

Despite their long term benefit they can make an initial worsening of the symptoms, and therefore, we should start their application carefully, starting with a low dose and gradually raising it to the target level [5].

The aims of the study were: to assess mortality rate in the group treated with the target dose of beta-blockers (the therapy group), compared with the group on conventional therapy (the control group). To compare the combined outcome (mortality and/or number of hospitalizations from cardiovascular reasons) between the two groups; to compare mortality as well as the combined outcome, regarding different types of beta-blockers in the therapy group (Metoprolol, Bisoprolol and Carvedilol); and to compare the mortality of each of the subgroups from the therapy group with the mortality in the control group.

Material and Methods

One hundred and thirty five patients with verified heart failure were investigated in the Outpatient department of the Cardiology Clinic in a period of two years. Out of 135, 113 patients underwent a complete investigation, and 22 of them were excluded from the study due to a worsening of the condition in the titration period.

The follow up period of these 113 patients was 18 months. The minimum follow up period was 3 months. The patients were divided in 2 groups, statistically not different in age and gender. The first group was treated by conventional therapy and target dose of beta-blocker (the therapy group), and the second by conventional therapy only (the control group).

The therapy group was divided in 3 subgroups according to the type of beta-blocker: a subgroup with Metoprolol, Bisoprolol and Carvedilol.

Inclusion criteria: Age 40-70 years; Verified stable chronic heart failure: clinically according to the classification of the NYHA Functional Class from II – IV, and by echocardiography with a confirmed ejection fraction (EF%) of 45% or less.

The patient was required to be stable more than one month before included in the study.

The investigation included: complete laboratory analyses every 3 months, electrocardiogram once a month, one and two-dimensional transthoracic echocardiography every 3 months.

We compared mortality and the combined outcome between the two groups. To simplify the combined outcome we quantified this parameter by scoring other parameters taking part in it: (1) Number of hospitalizations (each hospitalization is scored by 1); and (2) number of acute attacks of chronic heart failure (each attack is scored by 1).

Table 1: Clinical and laboratory parameters for the total patient population (control and therapy group)

Variable	n/M ± SD
Gender: men	91 (80.5%)
women	22 (19.5%)
Age	57.35 ± 8.6
Weight (in zero time) - kg	76.18 ± 11.6
Weight (end of follow up) kg	70.81 ± 12.3
BMI (in zero time) kg/m^2	26.11 ± 2.8
BMI (end of follow up)	24.4 ± 12.5
Htc (in zero time) vol%	0.39 ± 0.05
Htc (end of follow up)	0.37 ± 0.05
Scr (μmol/l)	84.53 ± 8.4
Alb (g/l)	44.01 ± 3.1
Total. lipids (g/l)	8.87 ± 1.3
HDL (mmol/l)	1.13 ± 0.2
LDL (mmol/l)	3.51 ± 0.7
Triglicerids (mmol/l)	1.53 ± 0.5
Na (mmol/l)	141.7 ± 2.6
K (mmol/l)	4.65 ± 0.4
ECG-zero time	1.66 ± 1.15
ECG-end of follow up	1.82 ± 1.17
EF % (zero time)	36.79 ± 6.6
EF % (end of follow up)	37.3 ± 8.3
ΔEF%	1.17 ± 6.8
NYHA-FC(zero time)	3.27 ± 0.7
NYHA-FC(end of follow up)	2.55 ± 0.9
NYHA score	0.35 ± 0.55
Number of hospitalizations	1.0 ± 1.26
Number of attacks of AHF	0.57 ± 0.98
SBP (mmHg)	98.45 ± 15.9
DBP (mmHg)	65.25 ± 9.7
Diagnosis: Ischemic	53 (46.9%)
Non-ischemic HF	60 (53.1%)
Mortality	15 (13.2%)

The study was clinical, prospective, interventional and controlled. We investigated 38 variables and compared the data of the 2 groups at the beginning of the follow up (the zero time) and at the end of follow up, using Student t-test for numerical and Chi square test for nominal parameters. To compare the variables of interest between the three subgroups in the therapy group, we used ANOVA-one way test. We calculated the relative risk reduction and the number needed to treat to prevent the outcome in one patient (RRR and NNT) for the variables of the primary objective (mortality and the combined outcome-mortality and number of hospitalizations from cardiovascular causes). We made an analysis of the complete survival of all the groups and compared the survival between the control and therapy group and all the therapy subgroups separately with the Kaplan-Meier method. A p value of < 0.05 was taken to be statistically significant.

Results

Out of a total of 113 patients with chronic heart failure with NYHA-FC II–IV in a stable clinical condition, 60 were with a non-ischemic and 53 with ischemic heart failure. Ninety one of them were men and only 21 women (81.5% and 19.55% respectively), with a mean age of 57.3 ± 8.6 years. During the follow up the total mortality was 15 (13.2%).

The total patient population is divided in 2 groups, control and therapy group. The therapy group is divided in 3 subgroups, Metoprolol, Bisoprolol and Carvedilol.

Table 2: Comparison of the parameters between the group of patients who died and the survived ones

Parameters	Survived n=98 M ± SD	Dead n=15 M ± SD	P =
Weight - zero time (kg)	77.86 ± 12.55	76.46 ± 9.93	0.68
Weight - end of follow up	72.54 ± 11.74	69.33 ± 11.65	0.32
Htc - zero time (vol%)	0.396 ± 0.5	0.402 ± 0.4	0.64
Htc end of follow up	0.377 ± 0.5	0.366 ± 0.4	0.49
Scr (μmol/l)	84.47 ± 8.41	88.96 ± 6.11	0.49
Alb (g/l)	44.28 ± 2.67	43.56 ± 4.73	0.39
Tlip (g/l)	8.82 ± 1.22	8.84 ± 1.24	0.96
HDL(mmol/l)	1.22 ± 0.89	1.14 ± 0.22	0.74
LDL (mmol/l)	3.47 ± 0.63	3.47 ± 0.66	0.98
Tg (mmol/l)	1.55 ± 0.47	1.60 ± 0.55	0.70
Na (mmol/l)	141.82 ± 2.45	140.65 ± 3.52	0.10
K (mmol/l)	4.55 ± 0.43	4.80 ± 0.40	0.04
EF% zero time	36.39 ± 7.07	34.13 ± 5.57	0.23
EF% end of follow up	38.16 ± 7.86	31.53 ± 8.74	0.003
ΔEF%	1.74 ± 6.26	2.60 ± 8.82	0.02
N° Hospitalizations	0.86 ± 1.00	1.86 ± 1.50	0.001
No. AHF	0.40 ± 0.71	1.60 ± 1.63	0.000004
NYHA-FC zero	3.16 ± 0.71	3.93 ± 0.25	0.00007
NYHA-FC end	2.33 ± 0.75	3.93 ± 0.25	0.00000
NYHA score	0.25 ± 0.50	1.00 ± 0.37	0.000000
SBP (mmHg)	104.83 ± 15.21	82.30 ± 10.40	0.000000
DBP (mmHg)	68.95 ± 9.04	54.44 ± 6.69	0.000000
Age	57.20 ± 8.72	58.33 ± 7.79	0.637

When comparing the parameters in the total population group between the survived and the patients who died, at zero time and at the end of the follow up, there was a statistically lower ejection fraction at the end of the follow up in the group of the patients who died, as well as lower ΔEF%, higher number of hospitalizations, more frequent attacks of acute heart failure, higher NYHA-FC at the beginning and at the end of the follow up and also higher NYHA-score at the end of follow up, but regarding systolic and diastolic blood pressure, the values were statistically lower in the group of patients who died.

Table 3: Comparison of outcome between the control and the therapy group

a) Mortality

CER	*EER*	RR	95%CI	RRR%	X^2	NNT
6/34 0.17	9/79 0.113	0.66	0.21 - 1.96	34%	R=0.37	17.5

b) Combined outcome

CER	EER	RR	95%CI	RRR%	X^2	NNT
29/34 0.85	41/79 0.51	0.6	0.33 - 1.13	40%	R=0.0008	3.03

CER - Control event rate; EER - Experimental event rate; RR - Risk ratio; RRR - Relative risk reduction; NNT - Number needed to treat.

CER = the number of patients who died divided by the number of the patients in the control group.

EER = the number of patients who died divided by the number of the patients in the therapy group.

95%CI (confidence interval) is the interval (the borders) between the events.

CER= n (events)/total n EER= n (events)/total n

RR= EER/CER RRR %= (CER-EER)/CER x 100

NNT= 1/(CER-EER)

Regarding mortality rate of the patients with the target dose of beta-blockers, compared to the control group, there wasn't any statistically significant difference (Chi square test), but the RRR%, although the value of 34% was also statistically not significant, it had a substantial clinical value.

Regarding the combined outcome (mortality and/or hospitalization), the therapy group showed a statistically significant improvement ($p < 0.0008$). RRR% was 40% and NNT was 3.03, which indicates that if we treat 3 patients with a target dose of beta-blockers, we might prevent the combined outcome in one patient.

Table 4: Comparison of outcome between the control group and the Metoprolol subgroup

a) Mortality

CER	EER	RR	95%CI	RRR%	H^2	NNT
6/34 0.17	4/29 0.13	0.76	0.2 - 3.04	24%	0.68	25

b) Combined outcome

CER	EER	RR	95%CI	RRR%	X^2	NNT
29/34 0.85	20/29 0.68	0.80	0.38 - 1.72	20%	0.0007	5.8

The mortality in the 3 therapy subgroups did not show a statistically significant difference when compared to the control group, but the combined outcome (mortality and/or hospitalizations) in the 3 subgroups, separately, when compared to the control group, showed a statistically significant improvement.

Table 5: Comparison of outcome between the control group and the Bisoprolol subgroup

a) Mortality

CER	EER	RR	95%CI	RRR%	X^2	NNT
6/34 0.17	3/24 0.12	0.70	0.16 - 3.12	30%	0.58	20

b) Combined outcome

CER	EER	RR	95%CI	RRR%	X^2	NNT
29/34 0.85	14/24 0.58	0.68	0.3 - 1.56	32%	0.022	3.7

Regarding RRR% and NNT, there was a major improvement for both outcomes in all the 3 subgroups of beta blockers, but the Carvedilol subgroup showed highest values for RRR% (mortality

- 56%, and the combined outcome - 38%) (Table 4, 5, 6).

Table 6: Comparison of outcome between the control group and the Carvedilol subgroup

a) Mortality

CER	EER	RR	95%CI	RRR%	X^2	NNT
6/34 0.17	2/26 0.076	0.44	0.08 - 2.34	56%	0.22	11

b) Combined outcome

CER	EER	RR	95%CI	RRR%	X^2	NNT
29/34 0.85	14/26 0.53	0.62	0.28 - 1.43	38%	0.0079	3.1

Discussion

Our study did not show a statistically significant difference for cardiovascular mortality when comparing conventional therapy with treatment with beta blockers (p = 0.37), but there was a 34% risk reduction for mortality which, on the other hand, is of clinical relevance. Our results for mortality in patients with heart failure when treated with beta blockers are similar to those in the Cibis II study (treatment with Bisoprolol) [6]. Our therapy group showed a significant improvement in the combined outcome (p = 0.0008), RRR 40% and NNT 3.03, which is consistent mostly with the US Carvedilol Study where RRR% for the combined outcome was 38% [7-9].

In the analysis of the mortality and the combined outcome between the control group with each therapy subgroup separately, it appeared that the Metoprolol subgroup did not have a significant difference compared to the control group for mortality (p = 0.68) and RRR was 24%, but for the combined outcome, there was a statistically significant improvement (p = 0.0007), which is consistent with the MCD Study for Metoprolol where the mortality rate was also not significant (p = 0.69) [7].

The Bisoprolol group also did not show a statistically significant difference for mortality compared to the control group (p = 0.58), RRR was 30%, but it showed a significant difference for the combined outcome (p = 0.02) and was RRR 32%. This result differed from the CibisII study where the difference for the mortality was statistically significant, but CibisII study included larger number of patients and lasted longer (two and a half years) [6].

The Carvedilol subgroup compared to the control group did not show a significant difference in mortality (p = 0.220), but RRR was highest among all the subgroups, 56%, and there was also a significant reduction in the combined outcome (p = 0.007), RRR 38%, which was consistent with the US Carvedilol Study where the RRR for the combined outcome was 63% (p = 0.001) [8, 9].

The meta-analysis of Chatterjee (BMJ, 2013) on the effect of beta-blockers showed that they reduced the mortality in heart failure patients, but as a result of their class effect. No specific beta blocker showed predominant effect upon risk reduction of mortality in chronic heart failure patients [9, 10].

In conclusion, our study confirmed that patients with stable chronic heart failure, treated with optimal doses of beta-blockers, had a significant reduction of the risk from death as well as the combined outcome (death and/or number of hospitalizations). No specific beta-blocker surpasses the effect of the other beta-blockers in the treatment of chronic heart failure considering the risk reduction.

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Clinical Science

Refractory Epilepsy-MRI, EEG and CT scan, a Correlative Clinical Study

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Abstract

OBJECTIVES: Refractory epilepsies (RE), as well as, the surgically correctable syndromes, are of great interest, since they affect the very young population of children and adolescents. The early diagnosis and treatment are very important in preventing the psychosocial disability. Therefore MRI and EEG are highly sensitive methods in the diagnosis and localization of epileptogenic focus, but also in pre-surgical evaluation of these patients. The aim of our study is to correlate the imaging findings of EEG, MRI and CT scan in refractory symptomatic epilepsies, and to determine their specificity in detecting the epileptogenic focus.

METHODS: The study was prospective with duration of over two years, open-labelled, and involved a group of 37 patients that had been evaluated and diagnosed as refractory epilepsy patients. In the evaluation the type and frequency of seizures were considered, together with the etiologic factors and their association, and finally the risk for developing refractory epilepsy was weighted. EEG and MRI findings and CT scan results were evaluated for their specificity and sensitivity in detecting the epileptogenic focus, and the correlation between them was analyzed.

RESULTS: Regarding the type of seizures considered in our study, the patients with PCS (partial complex seizures) dominated, as opposed to those with generalized seizures (GS) ($D=1.178$, $p < 0.05$). Positive MRI findings were registered in 28 patients (75.7%). Most of them were patients with hippocampal sclerosis, 12 (42.8%), and also they were found to have the highest risk of developing refractory epilepsy (RE) (Odds ratio = 5.7), and the highest association between the etiologic factor and refractory epilepsy ($p < 0.01$). In detecting the epileptogenic focus, a significant difference was found ($p < 0.01$) between MRI and CT scan findings, especially in patients with hippocampal sclerosis and cerebral malformations. There was a strong correlation between the MRI findings and the etiologic factor ($R = 1$), and for CT scan and etiologic factor an $R=0.75$ correlation. There was a significant difference between imaging methods MRI/CT ($p < 0.1$), and CT/EEG ($p < 0.05$) in detecting the etiologic factor, and little difference was noticed between findings of EEG/MRI.

CONCLUSION: Our study confirms that for an accurate diagnosis of refractory epilepsy in patients, a combination of neuroimaging and neurophysiologic methods is required. MRI showed to be highly sensitive in detecting the etiologic factor in RE patients, whereas EEG was sensitive in localization of the epileptogenic focus, with high correlation between these two methods. An early diagnosis of these patients is very important in having a better therapeutic response and prognosis for them.

Introduction

Refractory epilepsies are a specific group that pose a great challenge for diagnosis and treatment to physicians because of their low therapeutic benefit with all the AED [1]. The distribution of these is more often noticed in patients with detectable structural lesion, cerebral tumors, infections, vascular malformations, and hippocampal sclerosis. This group of patients is particularly important because they are under risk of psychosocial disability, irreversible and/or prolonged illness, and pose a serious therapeutic problem. Surgically correctable syndromes are a group of intractable epilepsies, where early surgical treatment reassures reduction of seizure frequency, and prevention of disability [2, 3]. Hippocampal sclerosis as a prototype of these is detected in 40-70% of surgically correctable syndromes [4]. Others, such as small structural lesions, glial tumors, congenital malformations, or anomalies in migration are less often detected.

Magnetic resonance imaging is a mandatory method in defining the syndrome in these epilepsies, precise localization of epileptogenin focus, and pre-surgical evaluation. MRI enhances the detection of low grade gliomas, cavernomas, focal cortical displasia, and disembrioplastic tumors [5]. Compared to CT scan, MRI has 80%-100% sensitivity in detecting this structural lesion, whereas the CT scans only 1%. SPECT and PET scan also take a pertinent place in pre-surgical evaluation of these patients.

Other studies have shown however that the CT scan has specificity and sensitivity in detecting the epileptogenic macrofactor in about 60% of cases. Conventional EEG and video EEG are still the main neurophysiologic methods in detecting epileptic abnormalities, diagnosis of the type of epilepsy, and determining the epileptic focus. Besides medicament treatment, surgical treatment of refractory epilepsy can be of great help, especially in cases of surgically correctable syndromes. The interventions that are most commonly used are partial lobectomies. The outcome and prognosis in patients with hippocampal sclerosis, DNT, cavernomas, is very good. In that sense 59% of the patients have good remission after surgery, 19% have reduction of seizures, and 2% remain unchanged. The early identification of refractory epilepsies, structural lesions and epileptogenic focus are crucial for their successful surgical treatment and further prognosis.

The aim of the study is to determine the specificity and sensitivity of neurophysiologic and neuroimaging methods, in detecting the epileptogenic focus of patients with refractory epilepsy, as well as to analyze the correlation between them.

Material and Methods

The study was prospective, open-labeled, with duration of two-years, and involved 37 patients previously diagnosed as refractory epilepsies. Patients were classified by age, type of seizures, and frequency was determined with the Bohen seizure frequency scoring system.

Nevertheless, the type of seizures, underlying structural lesions as etiologic factors, frequency of seizures, as well as correlation and association of the type of seizures and etiologic factors were evaluated. A conventional EEG was performed by standard 10-20 SI, and underlying epileptiform abnormalities were specified. The MRI was performed with the use of standard sequences, sagital, axial and coronal, T1 and T2, including FLAIR. In the CT scan standard techniques and sequences (16-18 transaxial sequences) were used, and contrast was used where required. Also where there was a certain need, coronal and sagital reconstructions were made. The findings of MRI, EEG and CT were evaluated in detecting various etiologic factors, as well as the correlation with the epileptogenic lesion. The sensitivity and specificity of these methods in detecting the epileptogenic focus was determined.

The statistical program STATISTICA for Windows was used for data elaboration. For testing the significance of difference among specific parametars, the Fisher exact test was used, Kolmogorov Smornov test, and Man Whitney U test. Odds ratio was used to examine risk for certain etiologic factors. Spearman coefficient of correlation was used to determine the relationship between some parameters. Values of $p < 0.05$ were considered as significant and 0.01 as highly significant.

Results

A group of 37 patients diagnosed as refractory epilepsy at the age 2-57 (14 male, 23 female) were included in the study. The evaluated frequency of seizures showed that 24 patients had weekly seizures and 13 were with daily seizures.

Table 1: Patients with refractory epilepsy and the type of seizures

Type of seizures	Patients (No)	Patients (%)
Partial simple seizures (PSS)	5	13.5
Partial complex seizures (PCS)	23	62.1
Generalized seizures (GS)	9	24.3

The distribution of the type of seizures was evaluated, and 23 patients were found to have partial complex seizures PCS (62.1%), whereas 9 (24.3%) had generalized seizures, and simple partial seizures were registered in 5 patients (13.5%). The presence of PCS is dominant and significant as for $D = 0.178$ and $p < 0.05$ (Table 1).

Table 2: MRI findings in patients with refractory epilepsy

MRI findings	Patients (No)	Patients (%)
Normal findings	9	24.3
Pathological findings	28	75.7

Positive (abnormal) MRI findings were registered in 28 patients (75.7%) (Table 2).

Table 3: Frequency of seizures in different etiological factors

Etiological factor	Patients No	Frequency of seizures % (weekly)	Frequency of seizures % (daily)	Score Bohen
Cerebral malformation	5	40	60	44
Post cerebrovascular accident	3	33,3	66.6	26
Vascular malformation	2	100		16
Perinatal trauma	1	100		8
Tumors	2		100	18
Hippocampal sclerosis	12	50	50	102
Posttraumatic	2	100		16
Postinflamation, demielinisation	1	100		8
Total	28	53.5	46.4	

The highest Bohen score was found in patients with hippocampal sclerosis (102), cerebral malformations (44) and postcerebrovascular illness (26) (Table 3).

The Table 4 shows distribution of different etiological factors. Twenty eight patients had pathological findings, in which those with hippocampal sclerosis 12 (42.8%) and cerebral malformations 5 (17.8%) dominated, whereas traumatic lesions, vascular malformations and tumors were found in 6 patients. Hippocampal sclerosis and cerebral malformations dominated with 60.7% of all patients with cerebral lesions. The assessment of association of the etiologic factor and RE, showed that in all factors except in hippocampal sclerosis there was no significant association, whereas the association of hippocampal sclerosis patients was significant for $p < 0.01$, Odds ratio = 5.7, showing the risk for refractory epilepsy in patients with hippocampal sclerosis.

Table 4: Type of etiological factors in patients with RE

Etiological factor	Patients No	Patients %	X^2	Fisher	p	Sig./n.sig.	Odds ratio
Trauma	2	7.14		*	p>0.05	n.sig.	0.31
Cerebral malformation	5	17.8		*	p>0.05	n.sig.	1.0
Post cerebrovascular accident	3	10.7		*	p>0.05	n.sig.	0.97
Vascular malformation	2	7.14		*	p>0.05	n.sig.	1.76
Perinatal trauma	1	3.5		*	p>0.05	n.sig.	
Tumors	2	7.14		*	p>0.05	n.sig.	1.15
Hippocampal sclerosis	12	42.8	18.29	102	p<0.01	sig.	5.76
Toxical							0.32
post infection	1	3.5		8	p>0.05	n.sig.	0.47
Total	28	100					

The comparison of positive etiologic findings on CT and MRI was shown in Table 5. MRI had positive findings in all 28 patients with positive etiology (100%), and the CT scan was positive in 11 patients (39.2%). A significant difference between these two methods, $p < 0.01$ was detected. The difference of MRI and CT findings versus various etiologic factors has shown considerable difference in hippocampal sclerosis patients ($p < 0.01$), and cerebral malformation ($p < 0.05$), where in other etiologies there was no significant difference between the two imaging methods ($p > 0.05$). The highest correlation found was the one between MRI findings and the etiologic factor ($R = 1$), and the correlation of CT scan findings and the etiologic factor ($R = 0.75$).

Table 5: CT scan and MRI findings in patients with RE

Etiological factor	Patients No	Patients with positive MRI No %		Patients with positive CT No %	
Trauma	2	2	7.14	1	3.5
Cerebral malformation	2	2	7.14	1	3.5
Post cerebrovascular accident	3	3	10.7	3	10.7
Perinatal trauma	1	1	3.5	1	3.5
Tumors	2	2	7.14	1	3.5
Hippocampal sclerosis	12	12	42.8	3	10.7
Anomal.migrat.	5	5	17.8	1	3.5
Post infection	1	1	3.5	0	0
Total	28	28	100	11	39.2

The distribution of diagnostic evaluation with MRI, CT and EEG has been shown in Table 6. There is significant difference between the results of MRI/CT ($p < 0.01$) and for CT/EEG ($p < 0.05$). No significant difference was registered between the results of MRI and EEG, $p > 0.05$. Positive findings on MRI were found in 75.6% from all 37 patients included in our study; positive results on CT were registered in 29.7% of the patients and positive findings on EEG were registered in 70.2% of the patients.

Table 6: MRI, CT and EEG findings in patients with RE

Patients n=37					
Positive MRI (%)	Positive CT (%)	Positive EEG (%)	MRI/CT	MRI/EEG	CT/EEG
75.6	29.7	70.2	p<0.01	p>0.05	p<0.05

MRI, CT and EEG findings in patients with hippocampal sclerosis and cerebral malformations are shown in Table 7. The results from imaging methods in these two etiologies showed significant difference between the findings of MRI/CT ($p < 0.01$), CT/EEG $p < 0.05$, and no significant difference was found between the MRI/EEG results ($p > 0.05$).

Table 7: MRI, CT and EEG findings in patients with hippocampal sclerosis and cerebral malformation

Patients n=17					
Positive MRI (%)	Positive CT (%)	Positive EEG (%)	MRI/CT	MRI/EEG	CT/EEG
100	23.5	70.5	p<0.01	p>0.05	p<0.05

Discussion

Our study as well as similar ones of other clinical studies confirms MRI to be highly sensitive and a specific method in detection of cerebral structural abnormalities (micro and macroetiolocical factors) in RE patients. It is especially important for diagnosis of those etiologic factors where CT does not give good results such as hippocampal sclerosis, cortical dispasia and low grade tumors. Results from analyzed types of seizures in RE patients showed highest presence of PCS in 62.1%, and the evaluated frequency showed more patients to have weekly seizures (68.8%) versus those with daily seizures (35.2%). The highest frequency of seizures was registered in patients with hippocampal sclerosis, cerebral malformations and tumors. Hence, the resistance to therapy may be probably the result of poor diagnosis, low therapeutic response or the epileptogenicity of the etiologic factor. In finding the etiologic factor, MRI and CT scan showed significant difference. Hippocampal sclerosis (42.8%) and cerebral malformations (17.8%) were with highest incidence in RE patients. The risk for PCS is 5 times higher in patients with hippocampal sclerosis. Highest difference in MRI and CT findings was in patients with hippocampal sclerosis and cerebral malformations [6, 7]. Maximal correlation of MRI and etiologic factor was

found as for R=1. In the EEG epileptiform abnormalities were found in 70.2% of patients. The evaluation of correlation/difference between imaging methods and EEG showed significant correlation between MRI/EEG, and difference of CT/MRI results also of the EEG/CT results. The imaging techniques confirm with high sensitivity the anatomic lesion in RE patients, and are the first diagnostic step, but not always do they determine the epileptogenic foci. Thus a clinical and electrophysiological correlation should also be done.

Other studies have shown that EEG and intracranial EEG in the pre-surgical evaluation of RE patients, detect the epileptogenic focus with sensitivity of 60-96% specially in temporal epilepsy, and they correlate positive with the clinical semiology and imaging techniques in 73% of cases [8].

In conclusion, the diagnosis of refractory epilepsies requires a correlation of neurophysiologic and neuroimaging techniques. Having in mind the fact that there is a significant difference in the sensitivity of CT and MRI in diagnosis of various etiologic factors, correlation with EEG is important for the diagnosis and classification of RE and localization of the epileptogenic focus. This will furthermore impact the prognosis of these patients, by and large.

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Clinical Science

Evaluation of Maternal Complications in Severe Preeclampsia in a University Hospital in Tirana

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Key words: severe preeclampsia; eclampsia; HELLP syndrome; stroke; pulmonary edema; maternal outcome.

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Abstract

BACKGROUND: Preeclampsia is a hypertensive multisystem disorder of pregnancy that complicates up to 10% of pregnancies worldwide and is one of the leading causes of maternal and perinatal morbidity and mortality.

AIM: To evaluate maternal complications associated with severe preeclampsia.

METHODS: This is a retrospective cross-sectional study conducted in the UHOG "Koço Gliozheni", in Tirana. Primary outcomes evaluated: maternal death, eclampsia, stroke, HELLP syndrome, and pulmonary edema. Secondary outcomes: renal failure, admission in ICU, caesarean section, placental abruption, and postpartum hemorrhage. Fisher's exact test and Chi-squared test were used as statistical methods.

RESULTS: In women with severe preeclampsia we found higher rates of complications comparing to the group with preeclampsia. Eclampsia (1.5% vs. 7.1%, P < 0.001), HELLP syndrome (2.4% vs. 11.0%; P < 0.001), stroke (0.5% vs 1.9%, P = 0.105) pulmonary edema (0.25% vs. 1.3%, P = 0.0035), renal failure (0.9% vs. 2.6%, P = 0.107), admission in ICU (19.5% vs. 71.4%, P = 0.007), caesarean section rates (55.5% vs. 77%, P = 0.508), placental abruption (4.3% vs. 7.8%, P = 0.103) and severe postpartum hemorrhage (3.2% vs. 3.9%, P = 0.628).

CONCLUSION: Severe preeclampsia is associated with high rates of maternal severe morbidity and early diagnosis and timely intervention can prevent life treating complications.

Introduction

Preeclampsia is a hypertensive multisystem disorder of pregnancy that complicates up to 10% of pregnancies worldwide and is one of the leading causes of maternal and perinatal morbidity and mortality [1]. Hypertension in pregnancy should be defined as a systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, based on the average of at least 2 measurements, taken at least 15 minutes apart, using the same arm [2]. The diagnostic criteria of preeclampsia used to be de novo hypertension after 20 weeks of pregnancy and proteinuria (exceeding 300 mg of total protein in a 24-hour urine collection) [3]. Recently, Royal College of Obstetricians and Gynecologists (RCOG) have proposed another mode of proteinuria assessment, using spot urine measuring the protein to creatinine ratio. According to the fact that preeclampsia is considered a multi-system disorder, the diagnostic criteria such as hypertension and proteinuria alone is not sufficient for clinical practice. Hence, this disease can be presented in several ways and it is necessary to asses all the symptoms and signs that are suggestive for the presence of the disease. The above mentioned facts has led to widening of the preeclampsia definition including: de novo hypertension after 20 weeks' gestation and new onset of one of the following: a) proteinuria as defined above; b) renal insufficiency (creatinine > 0.09 mmol/L, or oliguria; c) liver disease (elevated transaminases and/or severe right upper quadrant or epigastric pain); d) neurological problems, convulsions

(eclampsia), hyperreflexia with clonus, severe headaches, persistent visual disturbances; e) hematological disturbances: thrombocytopenia, DIC (Disseminated Intravascular Coagulation), hemolysis; or f) fetal growth restriction [4].

According to American College of Obstetrics and Gynecology (ACOG) diagnostic criteria, the diagnosis of severe preeclampsia includes severe hypertension (systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg, or both), neurological disturbances (such as headache, visual disturbances, and exaggerated tendon reflexes), epigastric or right upper quadrant pain, oliguria (less than 500 mL in 24 hours), pulmonary edema, cyanosis, impaired liver function, thrombocytopenia or intrauterine growth restriction (IUGR) [5].

Preeclampsia and eclampsia account for 10–15% of maternal deaths worldwide [6]. A majority of deaths in developing countries result from eclampsia, meanwhile in developed countries result from the complications of preeclampsia (HELLP syndrome, eclampsia, DIC, renal failure, pulmonary edema) [7]. HELLP syndrome occurs in 0.1%-0.6% of all pregnancies and in 4%-12% of patients with preeclampsia. HELLP syndrome typically occurs between week 27 of gestation and delivery, or immediately postpartum in 15%-30% of cases [8, 9]. Eclampsia refers to the occurrence of new-onset, generalized, tonic-clonic seizures or coma in a woman with preeclampsia. Despite advances in detection and management, eclampsia remains a common cause of maternal morbidity and death.

Disseminated intravascular coagulation (DIC) was defined as platelet count < 100 000/mm^3, plasma fibrinogen <3 g/L, and fibrin degradation products > 40 mg/dL [10]. The prevalence of DIC in pregnancy ranges from 0.03 to 0.35 percent in population-based studies [11].

Acute renal failure was diagnosed in the presence of oliguria or anuria in association with a creatinine clearance ≤ 20 mL/min or serum creatinine ≥ 2 mg/dL. Pulmonary edema is a rare and serious problem that complicates about 3 % of cases of severe preeclampsia [7].

The objective of this study is to evaluate major maternal complications associated with preeclampsia and severe preeclampsia.

Material and Methods

This is a retrospective case control study conducted in the University Hospital of Obstetrics and Gynecology (UHOG) “Koço Gliozheni”, in Tirana. To collect the data for this study we used the medical records of deliveries from January 2009 until December 2013.

This study was approved by Institutional Review Board of UHOG “Koço Gliozheni”. Written informed consent was not obtained from pregnant women involved in this study, because it was a retrospective research and it was not possible to get the informed consent from every patient. The data collected for this study are anonymous.

The standard inclusion criteria in the study were pregnant women diagnosed with preeclampsia that had delivered in this hospital after 24 weeks gestation during the period mentioned above, despite the number of the babies, fetal presentation and mode of delivery.

The calculation of gestational age was made based on the first day of the last menstruation period (LMP – 13% of cases), on the early ultrasound examination (before 13 weeks gestation – 11% of cases) or based in the combination of both criteria (LMP and first ultrasound examination – 76% of cases).

The exclusion criteria in this study were: pregnancies with confirmed fetal lethal anomalies, pregnancies with missing data necessary for the study, pregnancies with inaccurate gestational age.

The variables collected from the medical records were: maternal age, parity, first day of the last menstruation (LPM), gestational age at the moment of severe preeclampsia diagnosis**,** gestational age at delivery, highest diastolic/systolic BP, medical treatment (antihypertensives, anticonvulsants, corticoids) as well as labor and delivery – spontaneous, induced or caesarean section (see Table 1).

Table 1: Maternal variables included in the study

Maternal variables
Maternal age
Parity
First day of the last menstruation (LPM)
Gestational age at the moment of severe preeclampsia diagnosis
Gestational age at delivery
Highest diastolic BP, Highest systolic BP
Highest proteinuria level
Medical treatment (antihypertensives, anticonvulsants, corticoids)
Labor and delivery – spontaneous, induced, caesarean section

The maternal outcomes measured were:

Primary outcomes that include: maternal mortality, eclampsia, stroke, HELLP syndrome (hemolysis, elevated liver enzymes and low platelets), and pulmonary edema. Pulmonary edema was assessed based on clinical findings and chest radiography.

Secondary outcomes that include: renal failure, admission in ICU (Intensive Care Unit), caesarean section, placental abruption, and

postpartum hemorrhage. Renal failure was diagnosed when oliguria or anuria in association with an elevated serum creatinine level ≥ 2 mg/dL.

All the data were collected in excel format and were checked for their completeness and accuracy. The evaluated variables were compared between the preeclampsia group and the severe preeclampsia group.

The statistical analysis was made using SPSS program. Differences between groups for categorical variables were examined with Fisher's exact test. In the situations with large numeric data of the variables we have used the "Chi-squared" test.

Results

The total number of deliveries for this 5 years period (January 2009 until December 2013) was 21,795. After a careful investigation of medical records, were identified 1274 cases hospitalized with hypertensive disorders, of which 897 were diagnosed with preeclampsia.

Based on the exclusion criteria we found 27 pregnancies with confirmed fetal lethal anomalies, 99 pregnancies with missing data necessary for the study (in the medical records there were not all the available data for the variables included in the study), 28 pregnancies with inaccurate determination of gestational age (the patient doesn't know the LMP and/or hasn't done an ultrasound examination in the first trimester).

After the application of the exclusion criteria, a total of 743 cases with preeclampsia were included in this study. Based on ACOG classification criteria (2013) for the severity of the diseases we found 154 cases with severe preeclampsia (0.7% of the total births of the study period and 20.7% of all cases with preeclampsia).

Taking into consideration the fact that our study extends in a 5-year period, the population available to this study do not allow for analysis of maternal mortality. For this reason, regardless of maternal mortality is considered as the most important parameter in the evaluation of maternal outcome, referring to the small sample of this study, it was impossible the statistical processing of the results on this variable.

Eclampsia and HELLP syndrome are considered almost exclusively as unique complications of preeclampsia. For this reason the number of cases with eclampsia and HELLP syndrome is equal in both preeclampsia and severe preeclampsia groups.

Primary maternal outcomes

In Table 2 are presented the major maternal complications in cases with preeclampsia and severe preeclampsia (primary outcomes) and comparison was done between the two the groups for all the outcomes. In this comparison we noted a statistically significant difference for the presence of eclampsia (1.5% vs. 7.1%, for total preeclampsia group vs. severe preeclampsia group respectively; $P < 0.001$). The same significance was observed even for HELLP syndrome (2.4% vs. 11.0%; $P < 0.001$). Stroke and pulmonary edema were more frequent in the severe preeclampsia group but this difference was not statistically significant ($P = 0.105$ and $P = 0.0035$ respectively).

Table 2: Primary maternal outcomes in severe preeclampsia and in total cases with preeclampsia

Primary maternal outcomes	Total preeclampsia n (%)	Severe preeclampsia n (%)	*P* value
Eclampsia	11/743 (1.5%)	11/154 (7.1%)	*P* < 0.001
HELLP syndrome	18/743 (2.4%)	18/154 (11.0%)	*P* < 0.001
Stroke	4/743 (0.5%)	3/154 (1.9%)	*P* = 0.105
Pulmonary edema	2/743 (0.25%)	2/154 (1.3%)	*P* = 0.0035

Secondary maternal outcomes

In Table 3 are presented the rates of secondary outcomes for both groups. In the comparison between the two groups we noted a statistically significant difference for the admission in ICU (19.5% vs. 71.4%, for total preeclampsia group vs. severe preeclampsia group respectively; $P = 0.007$).

Table 3: Secondary maternal outcomes in severe preeclampsia and in total cases with preeclampsia

Secondary maternal outcomes	Total preeclampsia n (%)	Severe preeclampsia n (%)	*P* value
Renal failure	7/743 (0.9%)	4/154 (2.6%)	*P* = 0.107
Admission in ICU	145/743 (19.5%)	110/154 (71.4%)	*P* = 0.007
Caesarean section	413/743 (55.5%)	118/154 (77%)	*P* = 0.508
Placental abruption	32/743 (4.3%)	12/154 (7.8%)	*P* = 0.103
Severe PPH*	24/743 (3.2%)	6/154 (3.9%)	*P* = 0.628

*Severe Postpartum hemorrhage (> 1000 mL).

The rate of renal failure resulted higher in women with severe preeclampsia than in women with preeclampsia, but the difference was not significant (0.9% vs. 2.6%, $P = 0.107$). The same results we had even for caesarean section rates (55.5% vs. 77%, $P = 0.508$), placental abruption (4.3% vs. 7.8%, $P = 0.103$) and severe postpartum hemorrhage (3.2% vs. 3.9%, $P = 0.628$).

Discussion

Pre-eclampsia constitutes a major source of morbidity and mortality worldwide. Overall, 10%–15% of maternal deaths are directly associated with severe

preeclampsia and eclampsia [6]. Although maternal mortality is a very important parameter in the evaluation of maternal outcome, referring to the small sample study, we considered impossible the statistical processing of the results on this variable.

Maternal complications generally correlated with the severity of preeclampsia. In our study we found that 1.5% of all preeclamptic women and 7.1% of all severe preeclamptic women were complicated by eclamptic seizures. In a previous study, Liu et al. demonstrated that 9.34% of cases with severe preeclampsia had eclampsia [12], which is slightly higher than our results.

Our study showed that, 2.4% of cases with preeclampsia and 11% of cases with severe preeclampsia had HELLP syndrome. Also Murphy and Stirrat found that 21% of cases with severe preeclampsia had HELLP syndrome which is almost the double of our result (21% vs. 11%) [13].

In our study we found stroke in 0.5% of cases with preeclampsia and 1.9% of cases with severe preeclampsia. Comparing to the literature in the PIERS study CNS complications were found 7 from 2020 cases with preeclampsia (0.35%), similar to our result for the preeclampsia group [14].

As well, our study we found pulmonary edema in 1.3% of cases with severe preeclampsia. This result is higher than the results found by Yildirim et al. (5/903 cases, 0.6%) [15], and lower than the results found in Tuffnell et al. (25/1087 cases, 2.3%) [16]

In our study cesarean delivery rate in severe preeclampsia group was 77 %. Similar to our study we found increased rates of caesarean section in even in the studies of Liu et al. [12**Error! Reference source not found.**] and Murphy and Stirrat [13] (87.3 and 80 % respectively). In our study 7.8 % of cases with severe preeclampsia presented placental abruption which is less than the rate of 15% reported by Murphy and Stirrat [13].

Yildirim et al. in their study found renal failure in 14 of 903 cases with severe preeclampsia (1.6%) while in our study we found renal failure in 4 of 154 cases (2.6%) [15].

We concluded that severe preeclampsia is associated with high rates of maternal severe morbidity. Early diagnosis and timely intervention can prevent life threating complications from this disorder and can improve maternal outcomes.

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Splenectomy in Patients with Sickle Cell Disease in Tabuk

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Key words: sickle cell disease; splenectomy; pediatrics; spleen sequestration; acute chest syndrome; blood transfusion.

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Abstract

BACKGROUND: Sickle cell disease is a common genetic disease in Saudi Arabia; it is an autosomal recessive disorder characterized by production of abnormal hemoglobin S and is associated with high morbidity and mortality. Acute splenic sequestration is a life-threatening complication for this disease. Prophylactic splenectomy is the only effective strategy for preventing future life-threatening episodes.

AIM: The aim of this study was to study hospital records for all children aged 2 to 12 year old with Sickle cell disease who underwent splenectomy in Tabuk in Saudi Arabia.

METHODS: Records of 24 children (13 males, 11 females) who underwent splenectomy in surgery department of King Salman North West Armed Hospital, Tabuk, Saudi Arabia between 2008 and 2015 were reviewed retrospectively and analyzed for age, sex, indications for splenectomy, surgical technique, preoperative and postoperative length of stay, operative and postoperative complications, acute chest syndrome, painful crises, blood transfusion and fever (preoperative and postoperative).

RESULTS: We stressed on the information about the details of operation, the frequency of blood transfusion, fever, acute chest syndrome and painful crisis before and after operation.

CONCLUSION: Here we found that blood transfusion frequency decreased after splenectomy.

Introduction

Sickle cell disease (SCD) is an autosomal recessive disorder characterized by production of abnormal hemoglobin S and is associated with high morbidity and mortality. It is relatively common in Saudi Arabia as consanguineous marriage rates exceed 50% [1]. The reported prevalence for sickle-cell trait ranges from 2% to 27%, while up to 2.6% has SCD [2]. The spleen is one of the most common and early organs to be involved in SCD. It is commonly enlarged during the first decade of life then it undergoes progressive atrophy because of repeated attacks of vasoocclusion and infarction and these cause autosplenectomy. Sometimes splenomegaly persists into older age group or even into adulthood .Necessary splenectomy is done for a variety of reasons including acute splenic sequestration crisis, hypersplenism, massive splenic infarction and splenic abscess [3]. Splenic complications of SCD are associated with an increased morbidity and sometimes it may lead to mortality. To obviate this, There is a paucity of evidence to support that splenectomy, by whatever means, should be performed to improve survival and decrease morbidity [4]. Acute splenic sequestration is a life- threatening complication [5]. It is considered the second leading cause of death after infection in the first decade of life in patients with SCD. It has high mortality rates and in survivors there will be frequent recurrence of first attack [4]. Repeated episodes of splenic sequestration are common, happening in half of patients, especially within 6 months of the previous episode [6]. The only effective strategy for preventing future life-threatening episodes is the Prophylactic splenectomy which performed after an acute episode has resolved. Splenectomy may be performed with either celiotomy (open Splenectomy), or with minimal access approach (laparoscopic Splenectomy) [6, 7].

The aim of this study was to study hospital records for all children aged 2 to 12 year old with Sickle cell disease who underwent splenectomy in Tabuk in Saudi Arabia.

Materials and Methods

Records of 24 children (13 males, 11 females) who underwent splenectomy in surgery department of King Salman North West Armed Hospital, Tabuk, Saudi Arabia between 2008 and 2015 were reviewed retrospectively and analyzed for age, sex, indications for splenectomy, surgical technique, preoperative and postoperative length of stay, operative and postoperative complications, acute chest syndrome, painful crises, blood transfusion and fever (preoperative and postoperative). The patients' demographics are shown in Table 1.

Table 1: The patient's demographics

Age (mean ± standard deviation)	10 ± 4 (4 – 16)
Gender	
Male	13 (54.2)
Female	11 (45.8)

Preoperative diagnosis and indications for splenectomy were established in pediatric and hematology departments. All patients were prepared in surgery department for splenectomy and they were followed there after the procedure. Indications for surgery were spleen sequestration episode (once or multiple), hypersplenism, and symptomatic splenomegaly.

All patients received preoperative vaccination with polyvalent pneumococcal, meningococcal, and Haemophilus influenza vaccines before two weeks at least Patients were evaluated with ultrasonography (US) to determine the size of spleen , to rule out presence of concomitant gallstones, and to determine the presence of accessory spleens. The hematology policy in this center is: all patients with Hb level of less than 10 g/dL on admission were transfused preoperatively with packed erythrocytes to increase their Hb level to 10.

Routine antibiotic prophylaxis was started at the onset of surgery and continued for 3 days thereafter. Antibiotics of choice were a third-generation cephalosporin. Operation was performed under general anesthesia, all operation were complete splenectomy, 13 were opened (54.2%), and 8 were Laparoscopic (33.3%), while 3 were Laparoscopic turned to open.

Results

From 2008 to 2015, the records of 24 patients with SCD had splenectomy in North West armed forces hospital. Their age ranging from 4 to 16 years mean age was 10 years ± 4. Gender was 13 males (54.2%), 11 females (45.8%). Some data from records was missed, 10 patients 41.7% had Hb SS, 13 patients 54.2% Had Hb Sβ thalathemia, 1 patient with missed HB electrophoresis.

Table 2: The technique details of operation

Variable		Frequency	Percent
Type of procedure	Complete	24	100.0
Technique of procedure	Laparoscopic	8	33.3
	Open	13	54.2
	Laparoscopic turn to open	3	12.5
Concurrent procedures	None	17	70.8
	Cholycystectomy	7	29.2

Indications of splenectomy were; two patients with single spleen sequestration episode (8.3%), 13 patients with multiple spleen sequestration episode (54.2%), three patients with hypersplenism (12.5%), three patients with multiple spleen sequestration episode & hypersplenism (12.5%), one patient with single spleen sequestration episode & hypersplenism (4.2%). The indications for two operations were missed The age at operation ranged from one year six month old to twelve years old with Median age 4 with 10 patients aged 3 years old and less and nine patients aged 6 years old and more.

Table 3: Hb electrophoresis

Variable	Median	Inter quartile range	
		Percentile 25	Percentile 75
Hb electrophoresis A1	.00	.00	2.60
Hb electrophoresis A2	3	2	3
HB electrophoresis S	70.70	61.00	79.40
Hb electrophoresis F	20.30	16.10	29.00

Hundred percentages of the patients underwent complete splenectomy. Thirteen patient underwent open splenectomy representing 54.2% of the patients, eight patents underwent laparoscopic splenectomy representing 33.3% while 3 patients had laparoscopic splenectomy that turned to open. Seven patients had cholycystectomy together with splenectomy. Twenty one patient needed blood transfusion during the operation (87.5%) and 3 patients needed no blood transfusion.

After the operation the median hospital stay was 4 days ± 1 compared to 2 days ± 1 preoperative. The patients Hemoglobin before the operation was 9 ± 1 gm/ dl (before blood transfusion), while after the operation the median was 11 ± 2. The median platelet count preoperative was 278*103 ± 118, the number increased to 363*103 ± 391 post-operatives. The frequency of ICU admission from acute chest syndrome was only one patient (4.2%) preoperative

while the frequency of ICU admission from acute chest syndrome was 6 (25%) post- operative. Wilcoxon signed-rank test was used for comparison between pre and postoperative variables.

Blood transfusion pre and postoperative are shown in Figure 1. The median rate of blood transfusion per year preoperative is 4.5 with the 25th quartile 2.0 and the 75th quartile 9.6, while median rate of blood transfusion for 1st year postoperative and 2nd year post- operative is 0, the 25th quartile is 0 and the 75th is 1 (P value < 0.001).

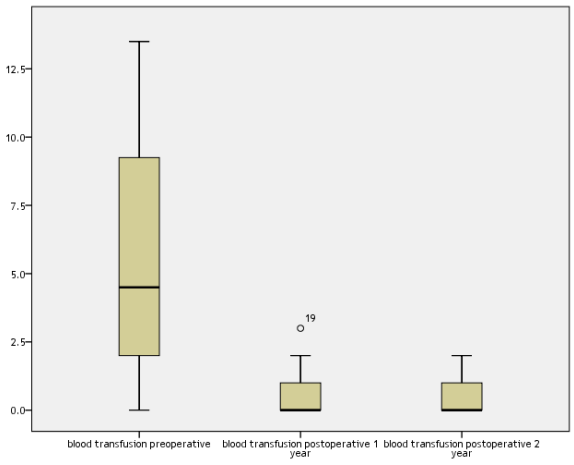

Figure 1: Blood transfusion pre and ostoperative

Regarding fever, preoperative the median number of fever episodes preoperative is 0.75, the 25th quartile is 0 and the 75th quartile is 2. One year postoperative the median increases to 1, the 25th quartile remains 0 and the 75th quartile is 1. 2nd year post- operative the median drops to 0, the 25th quartile is 0 and the 75th quartile is 1 (P = 0.382).

The episodes of painful crisis preoperative median is 1, the 25th quartile is 0 and the 75th quartile is 2, one year postoperative the median remains one , the 25th quartile is 0 and the 75th quartile is 4.25, the 2nd year postoperative the median remained 1, the 25th quartile is 0 and the 75th quartile is 2.25 (P value = 0.479). Results are shown in Figure 2.

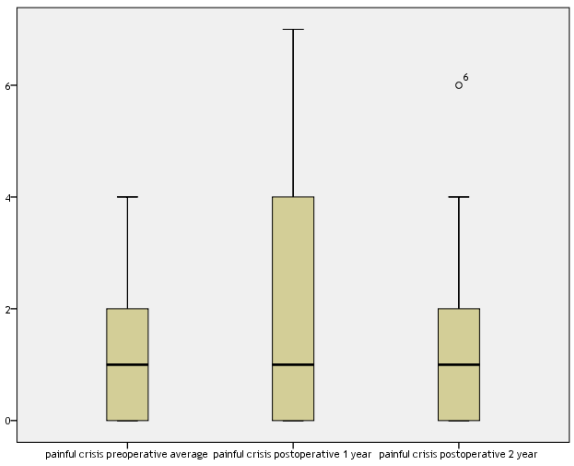

Figure 2: Painful crisis pre and postoperative

Fridman test for repeated measures as in comparison between variables pre, 1 year postoperative, and 2 year postoperative.

Discussion

Sickle cell disease (SCD) is a generic term referring to a group of disorders characterized by red blood cell deformity. Sickle cell disease is characterized by recurrent vasoocclusive episodes associated with accelerated hemolysis [8].

Splenectomy is one of the most common procedures required for patients with SCD [9]. The major indications for splenectomy in sickle cell children include splenic sequestration episodes and severe hemolysis secondary to suspected hypersplenism [3, 9-12]. Nowadays with the advances of minimally invasive surgery, LS is believed by many to be a gold standard [9, 13].

Acute chest syndrome is an important cause of mortality and morbidity in children with SCD [14] (21). Acute chest syndrome is characterized by basal pulmonary infiltrates that always involve the lung on the side of the surgery and either remains localized to that lung or progresses to involve both lungs [15].

In our study the indication of spleenectomy in most cases was recurrent splenic sequestration episodes and to a lesser extent hypersplenism or both, other studies in addition to Recurrent acute splenic sequestration crisis and hypersplenism found also splenic abscess, splenomegaly with a nonfunctioning spleen and massive splenic infarction to be less common indications [3].

In our study 100% of the patients underwent complete spleenectomy. 54.2% of the patients underwent open spleenectomy, while 33.3% of the patients underwent laparoscopic spleenectomy 12.5% had laparoscopic spleenectomy that turned to open. In other studies The ratio of laparoscopic vs open splenectomy was approximately 3:2 [8]. Since the first report of laparoscopic splenectomy in 1993 [1], the procedure has gained widespread acceptance and is considered safe and effective [16]. Seven patients (29.2%) had cholycystectomy together with spleenectomy. Twenty one patient needed blood transfusuion during the operation (87.5%) and 3 patients needed no blood transfusion. There was no mortality and post operative complications were ileus in one patient and fever with diarrhea in another patient (8.4%), in other studies Twenty-eight (21%) of our the patients had splenectomy and cholecystectomy There was no mortality, but 8 (6%) developed postoperative complications [8].

In our study the median hospital stay after the operation was 4 days ± 1 compared to 2 days ± 1

preoperative. Other studies have found the mean length of postoperative stay for laparoscopic procedures to be 3 days, which is similar to [3, 11, 13]. Laparoscopic splenectomy has been shown to confer a clear benefit of shorter hospital stay when compared to open splenectomy in sickle cell children [17].

In the current study the patients Hemoglobin before the operation were 9 ± 1 gm/ dl, while after the operation the median was 11 ± 2. The median platelet count preoperative was 278 ± 118, the number increased to 363 ± 391 post operative.

The median rate of blood transfusion per year preoperative was 4.5 with the 25th quartile 2.0 and the 75th quartile 9.6, while median rate of blood transfusion for 1st year postoperative and 2nd year post operative is 0, the 25th quartile is 0 and the 75th is 1 (P value < .001).

Lesher and collegues have found in a previous study there was a 38% decrease in the number of units transfused during 0 to 6 months postsplenectomy and a 45% decrease during 6 to 12 months postsplenectomy [8]. Despite a decrease in the number of units transfused postsplenectomy, all hematologic parameters remained stable or improved. Hematocrit increased marginally and reticulocytes decreased, indicating a decreased red cell turnover after the splenectomy. These results taken together indicate that there was an improvement in the survival of the transfused red cells [8]. Another study showed that the postoperative hematocrit and reticulocytes significantly improved in children in Saudi Arabia who underwent splenectomy for hypersplenism [3]. Svarch et al [6] showed significant improvement in hemoglobin concentration (6.0 vs. 7.7; P = .01) with decrease in transfusion after partial splenectomy for acute splenic sequestration with concurrent decrease in transfusion requirement [18].

In our study The frequency of ICU admission from acute chest syndrome was only one patient (4.2%) preoperative while the frequency of ICU admission from acute chest syndrome was 6 (25%) post operative.in previous studies the rate of acute chest syndrome was 5 to 15% [16, 19].

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Report on von Willebrand Disease in Malaysia

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Key words: von Willebrand Disease; Malaysian report; Sociodemographic details; Laboratory profiles; Year 2011-2013.

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Abstract

BACKGROUND: Von Willebrand disease (vWD) is an inherited hemostatic disorder that affects the hemostasis pathway. The worldwide prevalence of vWD is estimated to be 1% of the general population but only 0.002% in Malaysia.

AIM: Our present paper has been written to disclose the statistical counts on the number of vWD cases reported from 2011 to 2013.

MATERIAL AND METHODS: This article is based on sociodemographic data, diagnoses and laboratory findings of vWD in Malaysia. A total of 92 patients were reported to have vWD in Malaysia from 2011 to 2013.

RESULTS: Sociodemographic-analysis revealed that 60% were females, 63% were of the Malay ethnicity, 41.3% were in the 19-44 year old age group and 15.2% were from Sabah, with the East region having the highest registered number of vWD cases. In Malaysia, most patients are predominately affected by vWD type 1 (77.2%). Factor 8, von Willebrand factor: Antigen and vWF: Collagen-Binding was the strongest determinants in the laboratory profiles of vWD.

CONCLUSION: This report has been done with great interest to provide an immense contribution from Malaysia, by revealing the statistical counts on vWD from 2011-2013.

Introduction

Malaysia is one of the wealthiest and most developed countries in Southeast Asia with widespread and systematic healthcare system. The healthcare sector in Malaysia is primarily operated by the Ministry of Health Malaysia (MOH), established under Malaysian government [1]. In reviewing the Malaysian Health Profiles, coronary heart disease, stroke and respiratory illness are the leading causes of death, as recorded by the World Health Organization (WHO) in 2011, among 50 major etiologies of deaths [2]. Hospitals in Malaysia are very much dedicated in providing the most current advanced interventions to patients to save their lives from the deadliest diseases and to promote quality treatments. Hereditary hematological disorders affect the blood, and the diagnosis of such cases is more complicated due to the involvement of genes in the deoxyribonucleic acid (DNA) that interrupt the working mechanisms of blood clotting factors. Based on the recent update from the National Blood Centre (PDN) of Malaysia, there are only a few men and women suffering from these diseases, leading to the conclusion that the impact of these diseases is relatively low among Malaysians. PDN was officially built under public sector to provide expert medical services and a foundation for research in the field of hematology. PDN has also received accreditation from National Association of Testing Authorities (NATA), Australia for ISO/ IEC 17025 and

specialization in 17 different fields related to blood-associated diagnoses, resulting in quality medical outputs [3]. There are a few inherited hemostatic disorder cases reported by PDN repetitively every year, including Hemophilia, Von Willebrand disease (vWD), Bernard-Soulier Syndrome, adenosine diphosphate (ADP) receptor defect, Glanzmann Thrombasthenia and Factors deficiencies. PDN reports reveal that Hemophilia and vWD are the two major hemostatic disorders recorded at a high frequency level nationwide.

A mutation in the von willebrand factor (vWF) gene is the main cause of vWD. The vWF gene supplies crucial signals to form vWF blood-clotting proteins, which trigger the formation of blood clots. vWF acts as a sticky glue to hold the blood clots together in preventing excessive blood loss. At the same time, abnormal blood clotting causes prolonged bleeding episodes in vWD. Although the public attention towards global health has grown quickly over the past half century, the understanding and awareness of Malaysians towards hereditary hemostatic diseases are still considered to be poor in comparison with other ASEAN countries.

More needs to be done to coordinate Malaysians in order to improve the consciousness about these inherited hemostatic disorders. A total of five hundred fifty-four cases of vWD were reported in the PDN hemorrhagic disorders registry from 1979 to 2013. Five hundred seventy-two vWD events were registered in Malaysia as of 2013. In Malaysia, many campaigns, colloquia and conferences have been organized, but to date, there are no updates or published evidence surveying the statistics of vWD over the last 3 years.

Our present paper aims to disclose the statistic counts or percentages on vWD in Malaysia and to provide the report for the past 3 years, from 2011 to 2013, regarding the age, gender, race, region and diagnosis (approved by MOH). This report is a contribution from Malaysia with the clinical description of vWD to the countries all over the world.

Material and Methods

Prior to commencing the study, ethical clearance was obtained from the Medical Research & Ethics Committee from National Medical Research Registry (NMRR). This study has been registered with NMRR and the research identification number is NMRR-13-873-17276. The vWD patient entries in the PDN registry are from various hospitals across Malaysia. Reports on the statistics were prepared between 2011 and 2013 and included selected demographic profiles (age, gender, ethnicity, state, region) and other data including vWD classification, year of diagnosis (2011-2013) and laboratory findings includes [Factor 8 (FVIII), vWF: Antigen (vWF: Ag) and vWF: Collagen-Binding (vWF: CB)]. In our analyses, we categorized patients into 5 different age categories: (0-4), (5-13), (14-18), (19-44) and (45 and above) years old.

Ethnicities were classified based on the major populations of Malaysia, which are Malay, Chinese, and Indian, followed by Kadazan and Dusun, that distinguished in the ‘others’ category. Patients were also identified by their native states: eleven states (Perlis, Kedah, Pulau Pinang, Perak, Selangor, Negeri Sembilan, Melaka, Johor, Pahang, Terengganu, Kelantan) and 3 federal states (Wilayah Persekutuan, Sabah, Sarawak). All of the above listed states are divided into 2 separate regions, Peninsular Malaysia and East Malaysia.

The classifications of vWD diagnosis were split into 3 different categories, listed as type 1, 2 and 3. Laboratory findings expressed in percentages (FVIII, vWF: Ag and vWF: CB) were assigned into 5 distinguished categories, below 10, 10-30, 31-60, 61-100 and above 100. The data points were presented in percentages by using cross-tabulation between the demographic data. The independent t-test was applied to compare the mean and standard deviation (SD) of the diagnosis type and the laboratory findings. The statistical analysis was employed using SPSS version 18.0 software. Detailed data on the number of vWD incidences in Malaysia were solely provided by PDN.

Results

Sociodemographic characteristics

Ninty-two patients between 1 and 52 years old who presented with vWD were recruited and diagnosed by PDN within 2011 to 2013. Out of 92 patients, 40% were males and 60% were females. The majority of patients in this 3 year period were recorded in 2011 with 46 patients (35% of males and 65% of females). In 2012, both genders registered in equivalent percentages. In 2013, the lowest number of cases was registered, with a total of 20 patients at a 2:3 ratio of males to females (Fig. 1A).

Cases of vWD were categorized into 6 different age classifications. Patients between 19-44 years old were most commonly diagnosed within these 3 years (41.3%), followed by patients 5-13 years old (33.7%). Only 4.4% of registered patients were above 45 years old (Fig. 1B). The ethnicity distribution of the 92 total patients presenting with vWD to PDN in 2011 to 2013 is depicted in Fig. 1C. The majority of patients (63%) were the main ethnicity of Malaysia, Malay. Ethnicities falling under the

categories of 'others,' Indian and Chinese represented 16.3%, 15.2% and 5.5% of the total, respectively.

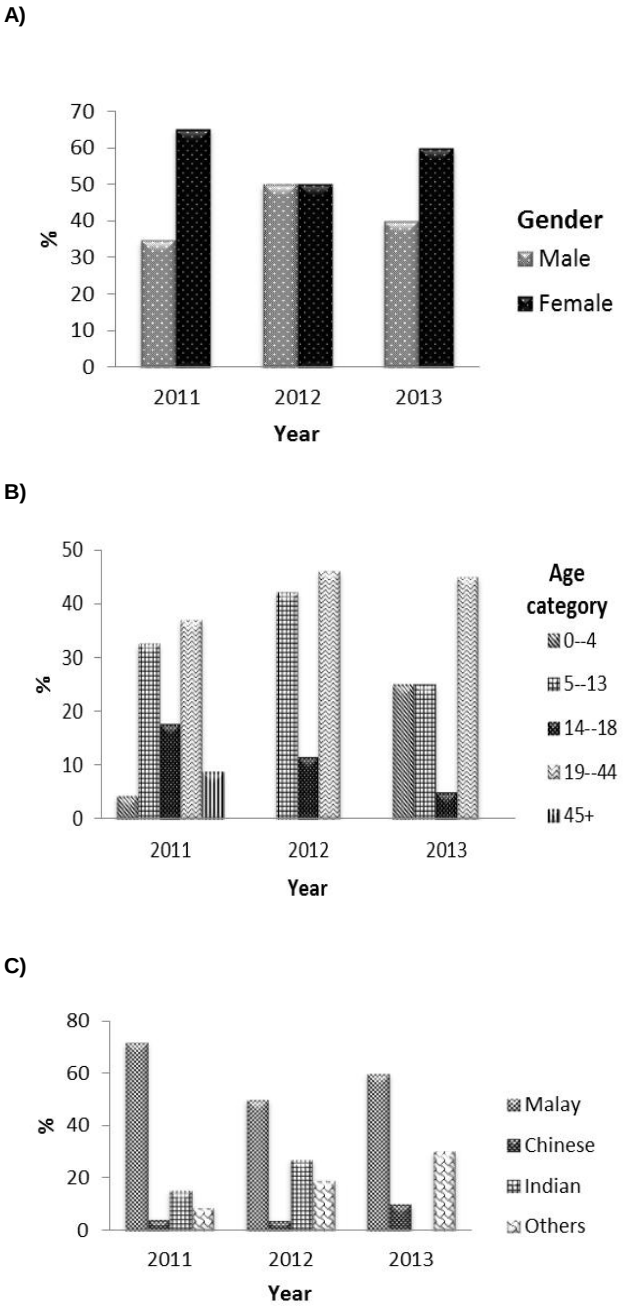

Figure 1: Percentages of gender, age category and ethnic distribution of vWD patients (n = 92) diagnosed in the PDN from 2011 to 2013, respectively. The analyses were expressed in percentages using descriptive statistics

States and Region

The majority of patients were from Sabah in the East Malaysia region, representing an overall percentage of 15.2%, followed by Kedah in 13.0% of patients, and Wilayah Persekutuan in 12.0%. Noticeably, only 1.1% of patients diagnosed in PDN were from the Negeri Sembilan state in the 3 consecutive years studied. Similarly, only 1 patient was considered to be of unknown origin (Fig. 2A). Eleven different states and 1 federal state comprise Peninsular Malaysia. The east coast region only consists of 2 distinct federal territories, Sabah and Sarawak. As for the overall analysis, most patients were from Peninsular Malaysia [74 (80.4%)], with only 17 cases from the East Coast region (18.5%). Although the total area of East Coast region represents approximately 61% of the total land area of Malaysia, the highest sum of vWD cases were recorded in the peninsular region, as it contains the most states (12) and is comprised of 22 million residents Fig. 2B.

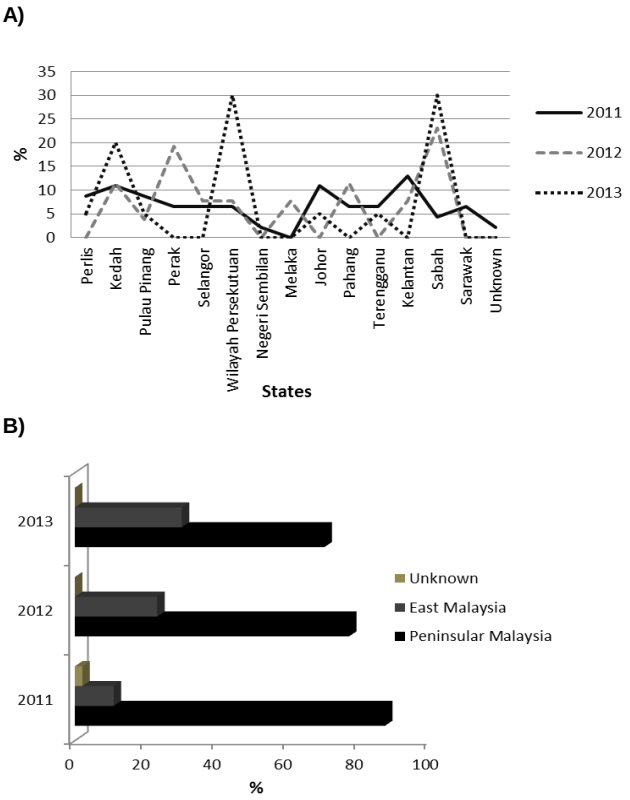

Figure 2: Frequency of vWD patient native states, federal territories and regions, depicted in percentages. The figures illustrate the data of the patients diagnosed in the PDN for 3 consecutive years from 2011 to 2013; n=92

Classifications of vWD; Laboratory findings; Year of diagnosis

The laboratory findings of each patient were depicted as percentages and the outcomes of each category were expressed as the mean (SD). It has been revealed that most patients are predominantly affected by vWD type 1 (77.2%) of patients throughout the 3 sequential years (2011-2013), with a mean (SD) value of 24 (7.23), as outlined in Fig. 3. The associations between types of vWD were strong factors influencing the laboratory findings. The highest prevalence recorded for all 3 distinct laboratory findings by groups of vWD patients was in the 31-60% group, with mean (SD) values as follows; FVIII: 11

(4.41), vWF:Ag: 13 (1.16) and vWF:CB: 13 (1.16). The next highest category was 10-30%, with mean (SD) values as follows; FVIII: 10 (7.64), vWF: Ag: 8 (6.56), vWF: CB: 8 (5.57).

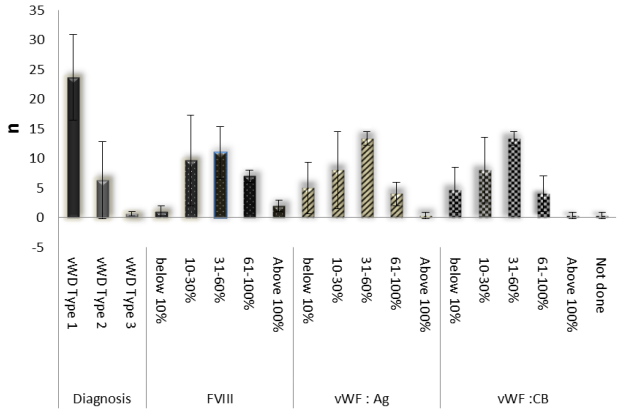

Figure 3: Classifications of vWD as Type 1, 2, and 3, and the laboratory findings of (FVIII, vWF: Ag, vWF: CB) based on the year of diagnosis (2011 to 2013). The outcome of the laboratory profiles classified into 5 different grades, below 10, 10-30, 31-60, 61-100, and above 100, is shown in percentages. The data were analyzed by the Independent t-test and are presented in means (SD); n=92

Discussion

The term vWD was coined by Finnish physician Eric Adolf von Willebrand in 1926. vWD is an inherited hemostatic disorder that affects the hemostasis pathway [1]. According to the recent annual global survey by the World Federation of Hemophilia updated in 2010, only 0.002% of vWD cases were reported among the population of 28 million Malaysians. Apparently, the Malaysian Statistical Department website has posted that the population of Malaysia has increased to 30 million, and that the counts now represent 0.043% of the world population. The worldwide prevalence of vWD is estimated to be 1%, and based on our recent analysis, vWD cases are estimated to be found in 0.002% of the total population [4]. PDN has diagnosed a total number of 554 cases of vWD from early 1979 to 2013. Five hundred seventy-two vWD cases have been officially diagnosed and reported in the Malaysian Health registry, which comprises 554 cases in the PDN, 6 cases from University hospitals and 12 cases diagnosed in state general hospitals.

vWD arises from a defect due to abnormal vWF, which disrupts platelet aggregation mechanisms. vWF, formed between endothelial cells, is a large multimeric protein that helps platelets adhere and aggregate in order to facilitate hemostasis [5]. Ninety-two cases of vWD were recorded in patients between 1 to 52 years old from 2011 to 2013. The numbers of female patients recorded was 20% higher than the number of males. We have categorized the age of the patients into 6 distinct categories. This age classification was adapted from a previous report on Hemophilia in Singapore described by Kwa *et al.,* [6]. The analysis showed that patients in the age category of 19-44 years old showed the highest number of registered vWD cases, with a total of 38.

Although the Malaysian population comprises multiple ethnicities, the majority of the Malaysian population was of the native ethnicity, often referred to as the Malays or Bumiputeras. The prevalence of vWD cases was highest in Malays (63%) and lowest in Chinese (5.5%). The ratio of the overall number of events based on ethnicity for both sexes standardized by age and relative to Malay are as follows: (Chinese: Malay; 1:11), (Indian: Malay; 1: 4.1) and (Others: Malay; 1:3.9). The Indian population lived in Malaysia are comparatively lower than the Chinese population in Malaysia, with only 7.1% Indians in the total Malaysian population. Indian descendant are recorded as the second most common ethnicity affected by vWD. Malaysia is well-known as a multiracial country consisting of 3 major ethnicities, Malay, Chinese, Indian and others. The ethnicities that fall under the 'others' category are Kadazan, Dusun, Iban, Minangkabau, Murut, Sikh, Bajau, Baba-Nyonya, Orang Asli, Dayak, Orang Ulu and Bidayuh. Based on our analysis, we found that of the 'others' category, only patients of the Kadazan and Dusun ethnicities has been diagnosed with vWD. Geographically, Malaysia is divided into 2 regions, Peninsular Malaysia and East Malaysia. Within these regions, Malaysia is divided again into 11 states and 3 federal territories. The majority of the vWD patients originated from Sabah, and this statement could be significantly correlated with the number of patients detected based on ethnicity. In Malaysia, the majority of the Kadazan and Dusun ethnicities originated from Sabah. Because other ethnic groups mostly lived in that state, together with the 3 major ethnic groups, this could explain why Sabah was reported to have the highest number of vWD cases.

vWD is an inherited disorder, so most of the patients diagnosed in PDN were from the same family. We also understand that patients who categorized under their native places were subject to change locations, because many people tend to migrate due to personal needs or for work purposes. Classification of vWD, including Type 1, Type 2A, 2B, 2 M, 2N, 3 and pseudo-type, based on the hereditary pattern, mechanism, prevalence in the general population, frequency of occurrence among vWD patients and bleeding tendency [7,8]. In Malaysia, among the majority of the 92 reported vWD patients, we encountered that the 97.9% of patients reported as having type 1 or 2 vWD, falling into the mild to moderate category. Subsequently, vWD type 3 occurred in only 2.1% of cases. The clinical assessment of a person who has identified as a vWD patient should be classified and diagnosed according to their family history and laboratory profile outcomes.

In medical practice, laboratory diagnosis could vary according to the patient's body and immune responses. Therefore, the laboratory profiles for vWD patients could be markedly different from the assumed and expected outcomes. These profiles are extremely dependent on the vWD classification, family history and response towards provided treatment [9-14].

In PDN, vWD patients were typically classified based on the 3 important laboratory findings: the assessment of FVIII, vWF: Ag and vWF: CB. These laboratory observations were categorized into 5 different measurement levels, as mentioned above. Patients with laboratory measurements below 10% are considered to have extremely low levels and are categorized as cases that are very severe and rare, while measurements between 10-30%, 31-60%, or 61-100% are assigned as severe to mild cases. Subsequently, levels above 100% are considered normal as type 1 vWD. In the 3 years studied, no mortality due to vWD was reported due to prolonged bleed. As for the overall view, no statistically significances were observed between gender, year of diagnosis or type of diagnosis.

It was reported by Mohsin *et al*., that many cases of vWD remain undiagnosed due to various types of clinical manifestations and perplexing laboratory analysis. Patients who have insufficient amounts of vWF, less than 20 IU/dL, are most likely to have vWF gene mutations with significant bleeding tendencies [11, 15]. There are actually two different types of bleeding: mucocutaneous bleeding and traumatic bleeding. Mucocutaneous bleeding normally affects the mucous membranes, which are the delicate tissues lining the body passages such as the nose, mouth, uterus, vagina, stomach and intestines. This type of hemorrhages frequently occurs upon epistaxis, gingival bleeding, menorrhagia, gastrointestinal bleeding and superficial ecchymosis (bruising). In contrast, traumatic bleeding occurs due to surgery, childbirth, larger injuries and tooth extractions.

There are two different types of treatment options that can be issued to vWD patients, desmopressin (DDAVP) and transfusion therapy, depending on the degree of severity and bleeding tendency. DDAVP is the standard form of therapy for patients experiencing Type 1 and 2A vWD. This drug mainly elevates the release of vWF and FVIII from endothelial cells and, in addition, increases the levels of vWF and FVIII:C by 3 to 5-fold. DDAVP can be administered intranasal and intravenously. In contrast, DDAVP is resistant and contradictory in patients with Type 2B vWD. This is due to their risk of developing thrombocytopenia. DDAVP has also been discovered to be an ineffective drug for Type 2N, 2 M and 3 vWD. Although antifibrinolytic (Tranexamic acid, epsilon-aminocaproic acid) and plasma-based drug substances have not been widely been used to treat Type 1 vWD, these compounds are the mainstay to treat patients with type 2 and 3 vWD.

Special cases that present with alloantibodies can be treated with recombinant FVIII and recombinant activated FVIII. Women undergoing heavy menstrual bleeding will usually be treated with oral contraceptive medications containing estrogen, which are more likely to be very effective in reducing the time frame of menorrhagia. For pregnant patients, they will be monitored accordingly to avoid excessive bleeding, especially during the initial postnatal weeks. Human-derived medium purity Factor VIII concentrates, which contain vWF, are available for patients undergoing surgical interventions with vWD complications. Humate P, Alphanate and Koate HP are also commercially available for prophylaxis of vWD. Platelet concentrates can be transfused for vWD patients categorized as having pseudo-type vWD, and blood transfusions can be given to prevent anemic and hypotensive cases among vWD patients [8, 11]. Many vWD patients in Malaysia only experience a mild form of this disease, which normally does not cause any serious hemorrhage. Patients identified under the severe category should seek emergency treatment to cease bleeding before it could become life-threatening. On the other hand, topical hemostatic agents such as thrombin, chitosan-derivatives, floseal, lyostypt have also been employed extensively to stop the hemorrhage from any injuries.

In conclusion, vWD is the second most common hemostatic disorder reported to occur in Malaysia, followed by Hemophilia. Our analysis has demonstrated important temporal changes in the demographics and the laboratory findings of vWD cases diagnosed in the PDN in Malaysia from 2011 to 2013. A total of 92 vWD cases were registered in Malaysia from 2011 to 2013. In Malaysia, most patients are affected by type 1 vWD. In reviewing the patient's sociodemographic characteristics, we noticed that most vWD cases occurred among females, patients of the Malay ethnicity and in the 19-44 year old age group. The East region and Sabah state registered the highest number of vWD cases. This report has been done with great interest to provide an immense contribution from Malaysia, by revealing the statistical review on vWD. Based on this report, it seems apparent that the occurrence of vWD could be increasing along with the population of Malaysia; therefore, it is important to address the clinical care treatments for vWD patients before the disease becomes life-threatening.

Acknowledgements

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Clinical Science

Metabolic Syndrome and Cardiovascular Risk Factors in Obese Adolescent

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Key words: cholesterol; cardiovascular diseases; body mass index; Blood glucose level; C-reactive protein.

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Abstract

BACKGROUND: Childhood and adolescent obesity is associated with insulin resistance, abnormal glucose metabolism, hypertension, dyslipidemia, inflammation, liver disease, and compromised vascular function. The purpose of this study was to determine the prevalence of cardiovascular risk factor abnormalities and metabolic syndrome in a sample of obese adolescent as prevalence data might be helpful in improving engagement with obesity treatment in future. The high blood lipid levels and obesity are the main risk factors for cardio vascular diseases. Atherosclerotic process begins in childhood.

AIM: This study aimed to investigate the relationship between obesity in adolescent and their blood lipids levels and blood glucose level.

METHODS: This study was conducted with 100 adolescents of both gender age 12-17 years and body mass index (BMI) greater than 95th percentiles and 100 normal adolescents as control group. The blood samples were collected from all adolescents after overnight fasting (10 hours) to analyze blood lipids (Total cholesterol, high density lipoprotein, low density lipoprotein) and hematological profile (Hemoglobin, platelets and red blood cell, C reactive protein and fasting blood glucose.

RESULTS: There were statistical difference between the two groups for red blood cells (P<0.001), Hemoglobin (P < 0.001) and platelets (P = 0.002), CRP (P = 0.02). Positive correlation was found between the two groups as regards total cholesterol (P = 0.0001), P value was positive for HDL (P = 0.005 and Atherogenic index P value was positive (P = 0.002). Positive correlation was found between the two group as regards fasting blood glucose (P = 0.001).

CONCLUSION: Saturated fat was associated with elevated lipid levels in obese children. These results reinforce the importance of healthy dietary habits since child-hood in order to reduce the risks of cardiovascular diseases in adulthood.

Introduction

Obesity has reached epidemic rate, both in develop and developing countries [1]. It is notably that overweight children and adolescents have higher possibility of becoming obese adults and represent health-related problems early in life including, diabetes, cardiovascular disease (CVD), anddyslipidemia. Dyslipidemia characterized by altered circulating levels of blood lipids and/or lipoproteins concentrations. Alterations in lipid profiles are associated with the development of atherosclerotic plaques which in turn have a relationship with high fat mass, in particular visceral fat [2]. The prevention of cardiovascular diseases must begin decades prior to the onset of symptoms to be effective. There are two types of preventions, the first one is preventing the development of risk factors and the second is managing those children with risk factors [3]. Due to scarcity of dyslipidemia longitudinal studies in childhood and adulthood cardiovascular diseases are needed. Study showed association between body mass index (BMI) in childhood as early as 7-13 years and heart failure in adulthood. Among the risk factors tracking young age, the obesity and cholesterol levels are the strongest ones [4]. Obesity related metabolic syndrome is associated with increase in the levels of a number of markers of inflammation especially CRP. This subclinical or low grade inflammatory state in simple obesity is associated with increased risk for cardiovascular

disease and diabetes

The environmental factors, such physical activity and dietary factors can influence the lipid levels and this relationship known also in adults.

Obesity has been described as an important marker of changes in cytokine concentrations and platelets count. Platletes, red blood cells (RBCs) and Hemoglobin are associated with cardio-respiratory conditions, oxidative metabolism, and cardio vascular events in obese children [5].

Subjects and Method

Written consents were taken from parents and children caregivers to participate in this study. The inclusion criteria were children with high body mass index (BMI). BMI ≥ 95th percentile according to age and gender that should undergo through biochemical tests. The exclusion criteria were overweight and obese children with any endocrine, renal, heart, or liver diseases. The inclusion criteria of children in this study agreed with the recent American Academy of Pediatric recommendation (fasting lipid profile screening for youths with BMI ≥ 95th percentile).

This study was conducted with 100 obese adolescents (40 girls and 60 boys). All adolescents had BMI ≥ 95th percentile, their ages were 12-17 years old and 100 control adolescents with the same age group, all children were free from chronic diseases. Adolescents were recruited from child health department clinic and from private clinic in Giza. Written consents were taken from parent and care givers of the students prior to study.

Biochemical Investigations:

Blood collection was performed 3ml from the cubital viens into a tube containing (EDTA) vacutainer after overnight fasting. RBCs, Hemoglobin and platelets counts were determined by fluorescence flow cytometry (Labquest , Labtest Diagnostica). Total cholesterol (TC), and high density lipoprotein (HDL), low density lipoprotein (LDL) were analyzed using the enzymatic colorimetric methods [6]. LDL was calculated usingFriedevald equation. C reactive protein was evaluated through a quantitative technique, value: 0.1-0.8 mg/dl considered as normal.

Blood glucose was done by clinical chemistry automatic analyser (Dade Behring).

Clinical assessment

A thorough physical examination was done in all subjects to exclude any significant systemic illness. Blood pressure was measured with a mercury sphygmomanometer after 20min of rest in supine position.

Anthropometric measurement

Anthropometric measurements were including weight, height, and waist, circumference.

Balanced scale, with the subject standing barefoot and wearing light clothing, weight was recorded in kilograms to the nearest tenth. Height was measured using a rural attached to scale and was recorded to the nearest 0.5 cm. Waist circumference was measured using a flexible, inextensible tap to the nearest 0.5.

Statistical analysis

It included Student's (t) test for comparison of mean, simple correlation analysis, and multiple regression using SPSS program of personal computer, all reported p-value were tow tailed, value <0.05 was considered significant.

Results

Anthropometric measurement and blood pressure of the studied groups are shown in the Table 1. All investigated parameters are significantly different between the control group and obese participants, except age.

Table 1: Anthropometric measurement and blood pressure of the studied groups

Parameter	Obese	Control group	P value
Age (Yrs)	11.5 ± 2.28	11.7 ± 0.73	0.404
BMI (Kg/m^2)	24.4 ± 2.82	17.5 ± 1.65	0.001
WC (cm)	77.7 ± 9.2	60.5 ± 4.52	0.001
Hip C (cm)	86.8 ± 8.67	78.7 ± 5.58	0.001
WHR	0.89 ± 0.79	0.76	0.001
SBP	116 ± 9	109 ± 8	0.001
DBP	72 ± 8	68 ± 6	0.003

Abbreviations: (BMI) body mass index, (wc) waist circumference,(HC) hip circumference, (WHC) waist hip circumference,(SBP) systolic blood pressure, (DBP) Diastolic blood pressure.

Comparison of biochemical parameters among two groups are shown in Table 2. All investigated parameters are significantly different between the control group and obese participants.

Table 2: Comparison of biochemical parameters among two groups

Parameter	Obese	Control group	P value
TC (mg/dl)	157 ± 32.39	144.4 ± 22.09	0.001
HDL (mg/dl)	36.4 ± 8.26	39.3 ± 6.27	0.005
LDL (mg/dl)	95.4 ± 26.77	88.3 ± 18.45	0.032
AI	2.62 ± 0.91	2.26 ± 0.45	0.002
FBG (mg/dl)	89.8 ± 9.86	81.6 ± 8.71	0.001

AI: atherogenic index; FBG: fasting blood glucose.

Blood indices and C reactive protein in obese and normal adolescent participants are shown in Table 3. All investigated parameters are significantly different between the control group and obese participants.

Table 3: Blood indices and C reactive protein in obese and normal adolescent

Variable	Obese	Normal	P value
RBCs (x^{12}/L)	5.2 ± 0.4	4.6 ±0.3	<0.001
Hemoglobin (g/L)	14.5 ± 1.1	12.9 ± 0.6	<0.001
Platelets ($x10^9$/L)	336.6 ± 64.4	292.7 ± 73.5	0.002
Leukocytes/ul (x^{12}/L)	14100 ± 100	12600 ± 100	0.07
CRP	0.9 ± 0.8	0.79 ± 0.8	0.02

CRP: C- reactive protein.

Discussion

In the present conditions that obesity is increasing at global level metabolic syndrome will always be in the top of medical problems. Our study is very actual as metabolic syndrome is very important for current medical practice due to a progressive increasing frequency and atherogenic risk [7]. Metabolic syndrome may affect most of the population and it may generate both vascular and metabolic complications [8]. The severity of inflammation in the metabolic syndrome measured by determining C-reactive protein and leukocytes is influenced by the number of criteria that make up metabolic syndrome [9]. Pro-inflammatory mechanisms can be considered as a base of increased cardiovascular risk.

Obesity related metabolic syndrome is associated with increase in the levels of a number of markers of inflammation especially CRP [10].This subclinical or low grade inflammatory state in simple obesity is associated with increased risk for cardiovascular disease and diabetes [11]. Detection of this systemic inflammation may help to identify children and adolescents at high risk for developing cardiovascular disease and diabetes later in the adulthood. In this study obese adolescent had higher systolic and diastolic blood pressure, P value < 0.001 for systolic blood pressure and P = 0.003 for diastolic pressure. As regards fasting blood glucose p = 0.001 between the two groups. Total cholesterol was higher in obese than normal P value = 0.001, HDL was higher in obese rather than normal P = 0.005 and atherogenic index was higher in obese group than normal P = 0.002.

It has been reported that BMI and waist circumference are strong predicator of central body fatness. The increase of body weight and adiposity, in particular central depots in childhood and adolescents are associated to change in the metabolic profile and cardiovascular problems even early in adult life [12].

The mean systolic and diastolic blood pressures differed significantly amongst the two study groups although none of the subjects were actually hypertensive. These results are consistent with some previous studies [13]. Our observation adds to the hypothesis that inflammatory state that occurs in obesity may contribute to elevation of blood pressure [14]. Elevated blood pressure and in particular the pulse pressure has been associated with an increased risk of cardiovascular disease and the underlying mechanism may be inflammation as demonstrated by elevated CRP levels [15]. Hypertension may also increase cardiovascular risk by causing chronic endothelial injury promoting structural and functional vascular alterations, especially in the microvascular network.

In the present study positive correlation were found between total cholesterol, in obese adolescent and normal (P= 0.04, P=0.03). Atherosclerosis and metabolic syndrome take long time to appear after middle age. This finding were similar to another study were obese adolescents had a significantly more atherogenic lipid profile. Other study stated that having a body mass index outside the normal range significantly worsen risk parameters for cardiovascular diseases in adolescents [16]. Some studies describe non alcoholic fatty liver disease in an early childhood due to dyslipidemia, which is a leading risk factor for cardio-vascular disease [17]. The inclusion criteria of children in this study agreed to the recent American Academy of Pediatric recommendation [10] (fasting lipid profile screening for youths with BMI ≥ 85th percentile on the CDC growth charts [18]).Due to the scarcity of dyslipidemia longitudinal studies in childhood and adulthood cardiovascular diseases, there are several speculations some studies found that obese children aged 7 and 13 year and the ratio of heart failures.

Furthermore, there is also an epidemiologic cardiovascular risk in children with dyslipidemia and tracking indicates that children maintain their percentile ranking over the time [19]. Among the risk factors tracking young age through adulthood, the obesity and cholesterol levels are the strongest ones [20]. The environmental factors, such physical activity and dietary factors can influence the lipids levels and this relationship is known in adults. However, there are few studies that evaluated the influence of dietary factors in childhood [21].

Growth acceleration in early life may be a predictor for obesity later on. BMI is an important parameter to determine obesity. There were very high positive correlation between anthropometric parameters in the two group (p = 0.00 for height, P = 0.03 for weight, P = 0.001 for BMI, and P = 0.001 for waist circumference), hip circumference (HC) P = 0.001.

Waist circumference is indicator for central obesity and should be added to every measurement

of obesity. Although imaging techniques can determine total body fat and its distribution reliably, anthropometric measurements remain important in clinical practice. Body mass index and waist circumference are reliable, save and non expensive method in clinical practice [22].

Childhood obesity become a serious public health problem, nutritional therapy plays an important role in its prevention and treatment. Healthy life style measures should be encouraged in this group of adolescents to prevent metabolic syndrome and cardiovascular complications in adulthood.

In conclusion, saturated fat was positively associated with elevated lipid levels in obese schoolchildren. This study suggests that number of cardiometabolic risk factors and metabolic syndrome are prevalent in obese adolescents. This observation might provide impetus to future strategies to treat pediatric obesity and to prevent or delay the appearance of cardiovascular disease and diabetes mellitus in future adult generation. These results reinforce the importance of healthy dietary habits since child-hood. The observation might also be used to encourage greater engagement among families.

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Cliical Science

Characteristic of the Oxidative Stress in Blood of Patients in Dependence of Community-Acquired Pneumonia Severity

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Key words: community-acquired pneumonia; modified proteins; malondialdehyde; blood plasma; erythrocytes.

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Abstract

BACKGROUND: At the present time the alternation of the oxidative metabolism is considered as one of the leading pathogenic mechanisms in the development and progression of community-acquired pneumonia (CAP). However the nature and direction of the oxidative protein changes in CAP patient's blood had been almost unexplored.

AIM: To define oxidative and modified proteins in erythrocytes and blood plasma of CAP patients.

MATERIAL AND METHODS: Blood plasma and erythrocytes obtained from: 42 patients with moderate severity pneumonia, 12 patients with grave severity pneumonia and 32 healthy volunteers. Content of advanced oxidation protein products, malondialdehyde and reactive carbonyl derivatives were estimated as indicators of the oxidative stress and oxidative damage of proteins.

RESULTS: In patients with grave severity the level of oxidative proteins and MDA in erythrocytes exceeded both: control values and similar meanings in CAP patients with moderate severity. The further growth of MDA in this group patients' blood plasma was observed, but the level of oxidative proteins decreased in comparison with those in CAP patients with moderate severity.

CONCLUSION: To sum up, our derived data show, that injury of erythrocytes' redox-status and blood plasma components plays an essential role in development and progression CAP.

Introduction

Nowadays the alteration of the oxidative metabolism (OM) is considered as one of the leading pathogenic mechanisms in the development and progression of community-acquired pneumonia (CAP). There were researches **concerning** the glutathione metabolism in CAP patients of young age, which helped to obtain the reliable **data proved decreasing activity of** glutathione in the blood against increasing activity of the superoxide dismutase and glutathione reductase. The same patients showed an increased index of peroxidation. Based on derived results the author concludes that leading **factors** in CAP belongs to disorder in immunity function and peroxide homeostasis [1]. The change in enzyme activity of antioxidant protection was found also in pilot research which had been made by Trefler S. et al. [2].

Reported that in CAP patients' blood plasma and erythrocytes the content of malondialdehyde (MDA) and isoprostane has been decreased, as the ratio of oxidized and reduced glutathione has being changed, along with this the correlation between degree of changing and pneumonia severity has been found out. It is suggested to use the indicators of lipid peroxidation as biomarkers to assess the severity and clinical outcome in CAP [3, 4].

The children with CAP showed the malondialdehyde content expansion simultaneously with decreasing of antioxidant summary activity and the level of zinc. At the same time children with focal CAP had more expressed mentioned indicators, that allowed the authors to recommend detaching of these children in risk group for lingering illness [5].

There was an evaluation of the hydrogen

peroxide and one of the lipid peroxidation indicators in expired air condensate of CAP patients. It was revealed that content of hydrogen peroxide in exhaled air was almost in 5 times higher than control values. Interestingly, on the 10th day of treatment, this figure was higher than control one in 3.3 times. At the same moment the level of malondialdehyde was higher than control values on 1st and 3d day of treatment. Authors suggested that the main source of the hydrogen peroxide were activated leucocytes, monocytes and macrophages. The basic conclusion of this research was ascertaining of the oxidative stress development in lungs at CAP patients [6].

Analysis of literature data showed, that the nature and direction of the oxidative protein changes in CAP patient's blood had been almost unexplored.

Protein oxidative modification – it is various kinds of post-translational modifications, which are caused by reaction of the amino acid residues with active oxygen and nitrogen forms. Moreover, protein oxidative modification might be determined by reaction with lipid peroxidation products, especially with reactive aldehyde [7]. The process of protein oxidative modification has biological significance, because, according to modern views, it is one of the most important mechanisms involved in cell signaling [8]. It is stated, that redox signaling is one of protection mechanisms against ischemic tissue lesion [9]. However, protein oxidative modification is often regarded as a negative component, since in conditions of oxidative stress the result is formation of large supramolecular complexes (aggregates), protein fragmentation etc. Eventually it causes the loss of functional protein activity that leads to severe metabolic disturbances [10].

The most stable products of the protein oxidative modification are reactive carbonyl derivatives. It should be noted, that carbonylation affects not only proteins, but also lipids and carbohydrates. The Semchyshyn H. M. [11] review described the currently known reactions of carbonyl derivatives formation in the organism, accumulation of which is positioned as a negative factor. There are new data about carbonyl derivatives (CD) ability to connect with a specific type of receptors, but this information needs to be verified. Advanced oxidation protein products (AOPP) present another type of oxidized protein. AOPP are formed from oxidative modification of albumin, but also the source might be fibrinogen or lipoproteins. It is suggested, that AOPP are formed with the participation of the myeloperoxidase and hypohaloids. AOPP structure is presented by dityrosine, carbonyl groups and cross-link. AOPP have quite distinct biological properties similar to advanced glycation end - products (AGEs) and can contact with AGE receptor (RAGE). It is believed, that the formation of AOPP might be increased in conditions of oxidative stress and inflammation [12]. AOPP have pro-oxidant and proinflammatory properties, as well as the ability to cause endothelial dysfunction [13, 14].

Membrane-bound hemoglobin is currently positioned as a variant of the modified protein. It is established, that membrane-bound hemoglobin is less protected by antiradical and antiperoxidant protection systems of red blood cells [15]. Hemoglobin is involved in the maintenance of red cells redox homeostasis. It is assumed, that hypoxia increases the proportion of membrane-bound hemoglobin, which in turn leads to erythrocyte membranes structural aberrations, redirection of calcium and potassium transportation, disruption of erythrocyte deformability [16].

Consequently, analysis of the literature data showed, that there are no researches about oxidative and modified proteins in erythrocytes and blood plasma of CAP patients, which was a reason enough for our investigation issue.

Material and Methods

Subjects

54 patients, at the age from 19 to 74 years old, with community-acquired pneumonia, treated in a specialized therapeutic and pulmonary department of city and regional hospitals of Karaganda have been studied. According to the design of study, the patients had been distributed in two groups: group (I) included the 42 patients with moderate serverity pneumonia and respiratory insufficiency grade (2), which was characterized by shortness of breath at rest, violation of restrictive capacity of lungs. The group (II) included 12 patients with severe pneumonia and respiratory insufficiency grade (2-3). Presence of infiltrative lung lesions, like tumors, diffuse connective tissue diseases, sarcoidosis, pulmonary tuberculosis, parasitic infestations and bronchiectasis were exclusion criterias of the study. Control group consisted of 32 healthy volunteers the same age as the group of CAP patients, who were without signs of any inflammation. All patients and healthy subjects have received the full information on probable inconveniences and complications at the blood sampling before giving their consent to participate.

42.6% patients with community-acquired pneumonia were smokers having smoker's index more than 21 years. Half of these patients had smoking history more than 15 years. At the time of our study, more than 90% of patients stopped smoking for two days or more. More than 79% of patients were citizens of industrial towns, where they were living more than 10 years at the **time** of onset of disease.

The aspects of disease were presented not only by classic symptoms of coughing, fever ranging from high values to subfebrile fever (from 37° C to 40°

C), shortness of breath but also by absence of symptoms of cough and respiratory failure. However, the first days of scant symptoms of pneumonia in some patients were limited to an expanded x-ray picture of inflammatory infiltration and changes in blood parameters. Furthermore, in a subsequent period spanning from 3 to 5 days after hospital admission of these patients, manifest clinical respiratory failure had been observed. The syndrome of cough in 14.8% of patients did not arise during the entire period of monitoring in hospital. In 75.9% of patients, location of pneumonic infiltrates at the basal segments of the right or left lung had been revealed, that allow diagnosing the 1-3 segmental pneumonia in the lower lobes. Affected region, which occupies the entire share or two shares, was detected in 22.2% of cases.

In 25.9% of the patients suffered from pneumonia, localization of disease had been revealed in the upper and / or secondary lobes. Bilateral pulmonary involvement, detected by X-ray, with the involvement of more than two segments occurs in 29.6% of patients. Thus, in our study, the pneumonia was a fairly extensive infiltrative lesion of the lung tissue. This provided significant intoxication syndrome, pyrexia and respiratory failure of patients.

The age of patients with bilateral pulmonary involvement was 49 ± 5.7 years old. Lower lobe pneumonia was typical for patients at the age from 31 to 72 years old. The sputum of all patients, without exception, had been examined for Mycobacterium tuberculosis.

Peculiarities of pneumonia in young people whose age does not exceed 32 years (n = 17) and averaged 28 ± 4.3 years were noticed. These patients had a prolonged clinical course and significant negative clinical and radiological improvement at the first week of inpatient stay in hospital. These patients did not belong to socially disadvantaged, did not have any previous alcohol incidents or habitual alcoholic intoxication. They never had cases of pneumonia or upper respiratory tract infections in their anamnesis. In the case of these patients, pneumonia started as a focal, then progressed in a short time and became a polysegmental or shared with the increase of intoxication and respiratory failure, which required the strengthening of antibiotic: prescription of the second antibiotic, increasing the dose and / or frequency of antibacterial substance injection.

The group of patients suffered from severe pneumonia (n = 12) involved persons at age from 21 to 63 years old, including, 4 men and 8 women. More than 75% of these patients were presented by young and middle age persons – from 27 to 42 years old. Area of pneumonia lesions covered one share and a number of other segments of the share. According to the distribution and localization it was bilateral pneumonia. Radiological improvement within two weeks after hospitalization was low, despite the reduction in symptoms of respiratory failure. For a long time it was mentioned the leukocytosis in the peripheral blood with significant prevalence of numbers of granulocytes in WBC differential and shifting of basilar cell, in spite of the significant positive improvement of ES (erythrocyte sedimentation) indicator.

Radiological examination

Community-acquired pneumonia was diagnosed based on the presence of pulmonary infiltrates, radiologically verified. Conclusion of radiologist (survey radiography of the chest, lateral X-rays of the lungs, made with X-ray apparatus, equipped with a digital decoder, CT scan of the lungs) was a prerequisite for the diagnosis of pneumonia. Dynamical X-Ray control (digital X-ray photography, computed tomography) was performed in the period from 7 to 10 days.

Blood sampling and Biochemical analysis

Blood was collected from the cubital vein (3 ml/sample) and was drawn into vacutainer tubes containing heparin. Erythrocytes were separated from plasma by centrifugation and washed for three times with physiological saline. Investigations of blood plasma and red blood cells were done within 1-2 hours after its collection.

AOPP were evaluated by Witko-Sarsat's method [17]. The results were expressed in μmol/l. AOPP are formed from oxidative modification of albumin and/or fibrinogen. To define the primary type of proteins involved in AOPP forming, AOPP were measured both in blood plasma and in blood serum. MDA content in blood plasma was defined with colorimetric thiobarbituric acid method [18], units of measurement – nmol/ml.

Metabolic status of erythrocytes was assessed by the level of MDA – lipid peroxidation cytotoxic catabolite, which was evaluated using the colorimetric thiobarbituric acid method [19]. During calculation molar extinction coefficient (1.56×10^5 M^{-1} cm^{-1}) was used; units of measurement – μpmol/ml. The level of CD was measured by R.L. Levine's protocol [20]. Measurements were performed using a UV -VIS Spectrophotometer Model PD-303UV.

Comparisons of the results obtained between patients and control participants were performed using non-parametric Mann-Whitney U-test (STATISTICA 7.0).

Bacteriological examination

Bacteriological examination of sputum and/or bronchial washings showed that **in** 79.6% of patients, *Str. Pneumonia*e played etiologic role in the

development of pneumonia, *E. coli*, *Klebsiella* were revealed in 13% of patients. Bacteriological examination of patients did not give a positive result, as at the stage of outpatient treatment, these patients received a series of broad-spectrum antibiotics: cephalosporin, fluoroquinolones and macrolides.

Patients follow up

From the first hours of inpatient stay, immediately after the X-ray verification of the diagnosis of pneumonia, patients received empirical antibiotic therapy (protected aminopenicillins), which was subsequently adjusted based on the results of bacteriological research. At the same time, the patients received the substances for pulmonary surfactant reinforcement and for the purpose of sputum control. Detoxication therapy included intravenous drip infusions of mixtures of electrolyte solutions in combination with vitamins.

All patients had been recovered or dismissed from the hospital with stabilization of condition to aftercare and post-hospital rehabilitation under the medical control in out-patient and/or home conditions. Length of stay in hospital was ranged from 11 to 27 days.

Results

It is evident from Table 1 data, that there is fixed reliable growth of CD in blood erythrocytes in proportion to CAP aggravation in 1.92 and 2.2 times respectively, in comparison with control.

Table 1: The indicators of oxidative stress in red blood cells of patients in dependence of CAP severity (X ± SD)

Groups	Carbonyl derivatives	Malondialdehyde	Membrane-bound hemoglobin
Control	8.24 ± 1.67	1.09 ± 0.005	12.63 ± 0.90
Patients with moderate severity of CAP	16.29 ± 1.31*	0.74 ± 0.06*	12.45 ± 1.59
Patients with severity CAP	17.97 ± 0.8*	1.82 ± 0.24*	14.34 ± 1.37

* - validity of differences with the control, p<0,05

At the same time in CAP patients with moderate severity the content of MDA was significantly decreased by 47% compared with control, whereas in CAP patients with grave severity this value, on the contrary, was considerably increased by 67%. There is a low tendency of membrane-bound hemoglobin content to enlarge in CAP patients' erythrocytes with grave severity.

It is evident from Table 2 data, that the content of reactive CD was significantly increased in CAP patients' blood plasma. In CAP patients with moderate severity the level of CD was significantly higher than control in 1.85 times, in CAP patients with grave severity – in 1.4 times.

Table 2: The indicators of oxidative stress in blood plasma of patients in dependence of CAP severity (X ± SD)

Groups	Carbonyl derivatives	Advanced oxidation protein products	Malondialdehyde
Control	0.39 ± 0.08	0.21 ± 0.02	0.76 ± 0.10
Patients with moderate severity of CAP	0.72 ± 0.067*	0.8 ± 0.09*	0.91 ± 0.07*
Patients with severity CAP	0.55 ± 0.05*	0.5 ± 0.08*	1.12 ± 0.16*

* - validity of differences with the control, p < 0.05.

There was a reliable increasing of AOPP content in CAP patients' blood plasma. Thus, in CAP patients with moderate severity AOPP level was higher than control in 3.8 times, in CAP patients with grave severity – in 2.38 times. Analysis of the AOPP content in the blood serum in both groups showed a similar meaning of this value that allowed suggesting the fibrinogen as the main source for AOPP.

The level of MDA was also considerably increased in the CAP patients' blood plasma. Thus, in CAP patients with moderate severity MDA level was higher than control in 19.7%, in CAP patients with grave severity – in 47% in comparison with control values.

Analyzing the data, it is possible to make the following conclusion: there is a fixed elevation of the oxidative proteins in erythrocytes, simultaneously with increasing concentration of MDA in CAP patients with moderate severity. A similar trend of increased oxidative proteins and MDA was observed in CAP patients with grave severity.

In patients with grave severity the level of oxidative proteins and MDA in erythrocytes exceeded both: control values and similar meanings in CAP patients with moderate severity. There is a detected trend to membrane-bound hemoglobin extend in CAP patients' erythrocytes with grave severity. The further growth of MDA in this group patients' blood plasma was observed, but the level of oxidative proteins decreased in comparison with those in CAP patients with moderate severity.

Discussion

Mainly CAP had pneumococcal etiology. It is established, that the hydrogen peroxide is used with pneumococcus as the virulent factor of epithelial cells lesion in the airways [21]. Generation of the hydrogen peroxide leads to the local oxidative stress development. The activation of neutrophils and other effector cells is also accompanied by local oxidative stress with excess generation of active oxygen forms. Active oxygen forms in the process of gas exchange

migrate through the alveolar-capillary membrane and are able to induce the oxidative stress development in the erythrocytes [22].

The main reason for the intraerythrocytic oxidative stress development is oxidative destruction of the hemoglobin [23]. As the evidence of this thesis accuracy there is data about enlargement of CD content in erythrocytes. We suggest that more sensitive to the pro-oxidant action is not the cytosolic fraction, but membrane-bound hemoglobin one.

It is determined that after the damage of membrane-bound hemoglobin the hemochromes are formed and the process of heme degradation starts. Formation of hemochromes is accompanied by generation of superoxide anions and hydrogen peroxide; degradation of heme led to releasing of the most powerful pro-oxidant – free iron [16]. The result is activation of peroxidation (MDA accumulation), carbonyl stress development (increase of reactive CD), that was established by our researches.

In turn, the development of intracellular oxidative stress in erythrocytes leads to red blood cells' membrane injury and releases the hemoglobin into blood plasma that causes redox reaction disruption. Another consequence of intracellular oxidative stress is the metabolic alternation and gas transport dysfunction of erythrocytes themselves [15]. On the one hand, it contributes to the hypoxemia development. On the other hand, diffusion of active oxygen forms from erythrocytes to endothelial cells of the lungs leads to additional attraction of leucocytes and contributes to the inflammatory process persistence. The principal possibility of such process is shown in the A.Huertas'study [24].

Accumulation of reactive CD, AOPP and MDA in patients' blood plasma illustrates the oxidative stress transition from local to systemic pattern. As it was obtained in our researches, the main **source of** MDA production in CAP patients' plasma is fibrinogen. It can be assumed that the blood plasma albumin is responsible for the reactive carbonyl derivatives formation. There are a number of adverse consequences due to increase of CD and AOPP in blood plasma, including further activation of neutrophils [25], which contributes to the inflammatory process persistence in CAP. Some decrease of CD and AOPP in CAP patients' blood plasma, in our opinion, should be considered as an unfavorable sign, as it shows substrates insufficiency for their formation in conditions of oxidative stress intensification (progressive growth of MDA). The main source for reactive CD and AOPP formation are albumin and fibrinogen. The increase of oxidative albumin forms proportions testifies the decreasing antioxidant activity of blood plasma that contributes to high level of oxidative stress maintaining. The oxidative albumin is able to activate neutrophils. The predominance of oxidative fibrinogen form is a potential threat to hemostasis, including platelets [26] and erythrocytes aggregation.

To sum up, our derived data show, that injury of erythrocytes' redox-status and blood plasma components plays an essential role in development and progression CAP mechanisms. It will very useful to estimate effectiveness of CAP treatment and possible diagnostics of CAP severity based on oxidative stress indicators.

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Case Report

Spontaneous Regression of an Incidental Spinal Meningioma

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Key words: meningioma; spinal cord; tumour regression.

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Abstract

AIM: The regression of meningioma has been reported in literature before. In spite of the fact that the regression may be involved by hemorrhage, calcification or some drugs withdrawal, it is rarely observed spontaneously.

CASE REPORT: We report a 17 year old man with a cervical meningioma which was incidentally detected. In his cervical MRI an extradural, cranio-caudal contrast enchanced lesion at C2-C3 levels of the cervical spinal cord was detected. Despite the slight compression towards the spinal cord, he had no symptoms and refused any kind of surgical approach. The meningioma was followed by control MRI and it spontaneously regressed within six months. There were no signs of hemorrhage or calcification.

CONCLUSION: Although it is a rare condition, the clinicians should consider that meningiomas especially incidentally diagnosed may be regressed spontaneously.

Introduction

Meningiomas are the most common non-glial tumors of the brain and spine and they account for approximately 13-20% of all primary intracranial neoplasms [1]. They are benign tumors usually arising from the meningothelial cells or arachnoid cap cells which reside in the arachnoid layer covering the surface of the brain [2]. They may occur intracranially or within the spinal canal and less than 10% ever cause clinical symptoms. The majority of meningiomas are sporadic, although several associations exist with 10% of multiple meningiomas associated with neuro fibromatosis type 2 [3].

In literature it has been previously reported that regression of meningioma can be seen due to hemorrhage, calcification and drug withdrawal [4-7]. However, spontaneous regression of meningioma is rarely observed [8].

Here we report a case of incidentally diagnosed spinal meningioma which regressed within six months without any hemorrhage or drug withdrawal.

Case Report

A 17-year-old male patient with a history of dizziness and an incidentally diagnosed meningioma was admitted to our neurosurgery clinic. His medical history was unremarkable and he wasn't taking any kind of treatment. His neurological examination was completely normal. The routine laboratory investigation revealed no pathology. The cervical MRI showed an extradural, cranio-caudal contrast

enchanced lesion at C2-C3 levels of the cervical spinal cord. The lesion had a length of 38.8 mm with 11.3 mm a sagittal diameter. It appeared as an isointense mass on the T1-weighted sequence, slightly hyperintense on the T2 sequence. The lesion showed contrast enhancement on a postgadolinium T1-weighted sequence as well. There is a slight compression towards the cervical spinal cord. These imaging characteristics strongly favored the diagnosis of a spinal meningioma (Figure 1A,B,C).

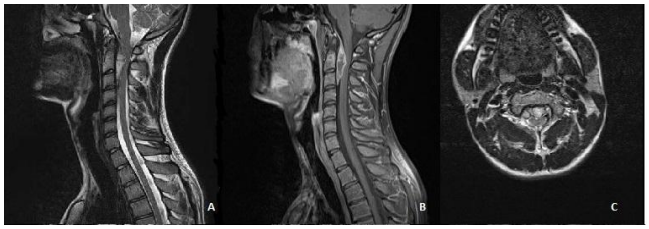

Figure 1: The cervical MRI findings sagittal T2 weighted (A), T1 weighted sagittal (B) and axial (C) images with contrast enhancement showing extradural, cranio-caudal lesion concominant with meningioma at C2-C3 levels of the cervical spinal cord

There were no neurological deficits or subjective complaints related to the tumor. Since he had no complaints and didn't want any kind of surgical approach, the meningioma was followed by a control MRI. After three months the control cervical MRI revealed the regression of the meningioma (Figure 2 A,B,C).

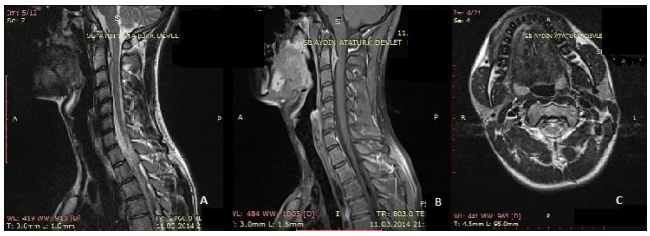

Figure 2: The control cervical MRI showing regression of meningioma in T2 weighted (A), T1 weighted sagittal (B) and axial (C) images

The length of the lesion regressed to 35 mm with a sagittal diameter of 6 mm without any signs of hemorrhage and calcification. In his third MRI after six months the lesion was completely regressed (Figure 3A,B,C).

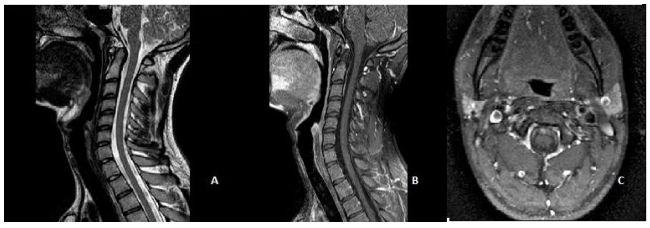

Figure 3: After six months cervical MRI showed complete regression of meningioma in T2 weighted (A), T1 weighted sagittal (B) and axial (C) images with lineer contrast enchancement without any solid lesion

There was only a linear contrast enhancement without any signs of a solid lesion. During this period he had got no kind of medical treatment.

Discussion

The typical meningioma is a dural-based, markedly enhancing extra-axial mass. It may orginate from meningothelial cells whether intracranial, spinal or ectopic. Their common intracranial localisations are parasagittal, and sphenoid regions (75%). They can be also seen in spine (12%), cerebellopontine angle (2-4%), intraventricular (2-5%), orbital (1%) and ectopic (<1%) [1]. Although the definite treatment for symptomatic meningiomas is complete surgical resection, less common locations and asymptomatic meningiomas are important for the diagnosis and the appropriate treatment. Our patient had a spinal meningioma which is less common than intracranial ones. It was asymptomatic and incidentally diagnosed. We presented our case for being a rare asymptomatic spinal meningioma showing regression within six months without anykind of treatment.

Regression can be seen in both malign and benign tumors. The underlying mechanisms are associated with mainly related to the apoptosis and activity of the immune system, and the microenvironment of the tumor. The oncogenic suppressors of DNA are also related to this process [9]. Reports of spontaneous regression of meningiomas in the literature are rare. In literature spontaneous regression of meningiomas are reported in association with intratumoral hemorrhage [4] and progestative hormonal treatment withdrawal [5-7]. It can also gradually shrink together with a decrease of edema and increase of calcification [8]. The intratumoral hemorrhage and calcification may cause necrosis of the tumor.

In literature it has been also reported that the administration of medroxyprogesterone acetate can cause the growth of multiple meningiomas. As expected, hormonal withdrawal is associated with spontaneous regression [10]. This has been also reported in association with cyproterone acetate withdrawal [5]. It is also wellknown that meningiomas may get smaller in size after pregnancy. Shimizu et al also reported a similar regression of meningioma during a 2-year period due to chlormadinone acetate (a progesterone agonist) which was used for the treatment of benign prostatic hyperplasia [6].

Meningiomas are known to cause local vascular disturbances, mainly venous congestion and enhanced circulation. The decrease in dural blood supply and venous drainage may also contribute the regression process. The hemodynamic alterations

may have contributed to vessel and tissue ischemia and, consequently, led to tumor hemorrhage and eventual necrosis [11].

All these data reinforces the link between meningioma regression and hormonal status, hemorrhage and calcification. However our patient had an incidentally diagnosed meningioma without any symptoms. He refused any kind of treatment and he hadn't been using any medication previously. He had no hemorrhage and vascular pathology either. It is difficult to explain the underlying mechanism of the resolution of meningioma since his medical history was unremarkable.

The resolution of meningiomas due to intratumoral hemorrhage may be seen within 7 years [4] or in a shorter period of time (6 months - 2 years) in some cases of drug withdrawal [6, 10]. In our patient the meningioma at C2-C3 levels of the cervical spinal cord disappeared within 6 months. The regression of the tumor could be seen in repeated MRIs. To our knowledge this is the first spinal meningioma which was regressed spontaneously without any clinical symptoms. Additionally we couldn't find any reasons to explain the regression. There had been no signs of hemorrhage or calcification. Our patient was male and had no previously treated with drugs that may cause growth or regression of a meningioma. To date, there have been no reports showing spinal meningioma regression in such a short time without any symptoms.

In conclusion, according to the present data the regression of meningioma may not be uncommon in cases of intratumoral hemorrhage, calcification or drug withdrawal such as medroxyprogesterone acetate, cyproterone acetate and chlormadinone acetate. Our case had none of these factors related to regression of meningioma. We believe that the factors related to tumoral growth and regression is still unclear and further studies would be beneficial to clarify the mechanisms and factors related to spontaneous regression.

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Case Report

Surgical Treatment of Anomalous Origin of Right Coronary Artery in a Patient with Mitral Stenosis

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Key words: Coronary anomalies; right coronary artery; bypass grafting; mitral stenosis; surgical technique.

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Abstract

BACKGROUND: An anomalous origin of the right coronary artery is rarely observed, with a reported incidence between 0.026% and 0.25%. This condition is often completely asymptomatic and is found incidentally during angiographic evaluation for other cardiac diseases. However some patients present with exertion angina or sudden death. Surgical treatment in patients with anomalous RCA is still controversial. Treatment can be conservative, angioplasty or surgery.

CASE PRESENTATION: A 59-year-old man was admitted with severe mitral stenosis. He complained exertion and rest dyspnea, NYHA III class. He had sequels of embolic stroke, results of left atrial thrombus. Echocardiography showed calcified severe mitral stenosis with mitral orifice area of 1.1 square centimeters with PSPAP 60 mmHg and normal LV function. Routine coronary angiography before surgery showed aberrant origin of RCA from the left sinus of Valsalva with 90% stenosis at his origin. Multi-slice computed tomography proved the diagnosis of anomalous RCA arising from the left sinus of Valsalva and taking an inter-arterial course between the aorta and pulmonary artery. The patient underwent mitral valve replacement with mechanical St. Jude prosthesis No 29 and saphenous vein graft to RCA. We chose by-pass grafting techniques because after aortotomy, RCA was too close to LMCA, intramural course was too short and stenosis of RCA was outside of aortic wall. The patient's perioperative course was without complications and patient was discharged on the seventh postoperative day.

CONCLUSION: Correction of anomalous of the origin of right coronary artery is mandatory in cases where patient has to be operated for other cardiac causes.

Introduction

Anomalous aortic origin of the coronary artery (AAOCA) is rarely observed, with a reported incidence between 0.026% and 0.25%. This condition is often completely asymptomatic and is found incidentally during angiographic evaluation for other cardiac diseases. However, some patients present with symptoms which may include chest pain, syncope, myocardial infarction, or sudden death [1-3]. AAOCA is the second most common cause of sudden death in young athletes, accounting for approximately 10% of such events [4, 5]. Surgical repair of AAOCA is safe and extremely successful in eliminating symptoms of myocardial ischemia [6].

We report here the first case in Albania with AAOCA associated with mitral stenosis diagnosed and treated surgically at our department.

Case presentation

A 59-year-old man was admitted with severe mitral stenosis. He complained exertion and rest dyspnea, NYHA III class. No chest pain or other ischemic symptoms were seen. He had sequels of embolic stroke, results of left atrial thrombus. ECG showed atrial fibrillation and non-specific ST changes.

Echocardiography showed calcified severe mitral stenosis with mitral orifice area of 1.1 cm^2 with PASP 60 mm Hg and a normal LV function. Routine coronary angiography before surgery showed aberrant origin of RCA from the left sinus of Valsalva with 90% stenosis at his origin as seen in Figure 1. Multi-slice computed tomography proved the diagnosis of anomalous RCA arising from the left sinus of Valsalva and taking an inter-arterial course between the aorta and pulmonary artery. RCA has 90% stenosis at his origin but outside of aortic wall.

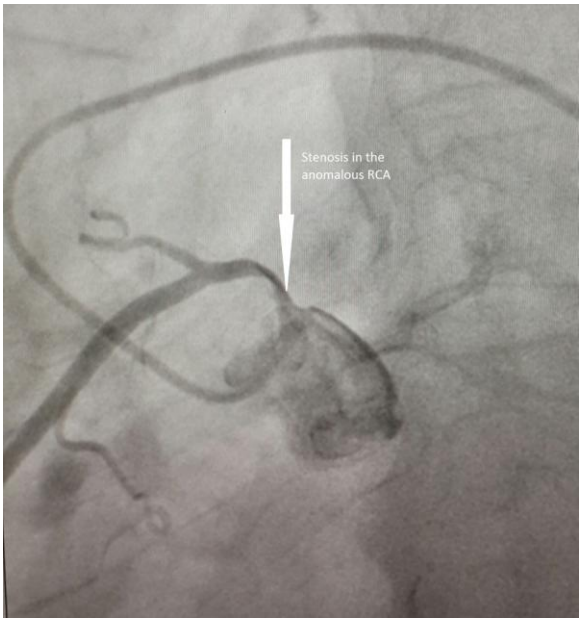

Figure 1: Coronary angiography shows anomalous origin of right coronary artery from left sinus and 75-90% stenosis at its origin

The patient underwent open heart surgery with cardiopulmonary bypass machine. After median sternotomy it was seen that right coronary artery origin was in left coronary sinus of Valsalva and the artery passed between the aorta and the pulmonary artery. After aortic clamping and mitral valve replacement with mechanical St. Jude mechanical prosthesis No 29, the aorta was divided transversally.

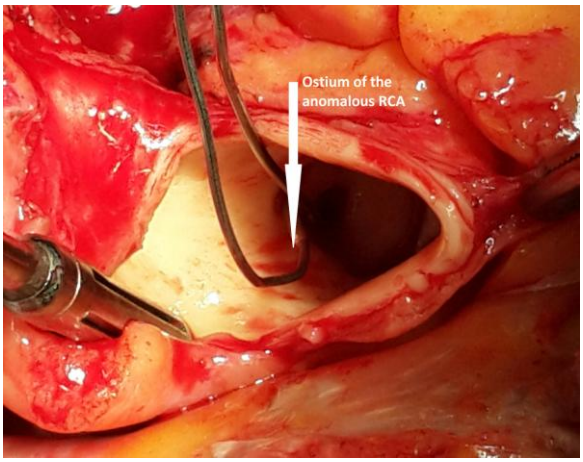

Figure 2: Intraoperative images of both ostia of the coronary arteries

It was visualized the right coronary ostium arising from left sinus of Valsalva, close to left main ostium and its inter-arterial course. We decided to perform right coronary bypass grafting with saphenous vein to RCA because the ostium of right coronary artery was small, intramural course was too short and a 90% stenosis of RCA was outside of aortic wall. We judged that it was not the case to perform unroofing techniques. The patient's perioperative course was without complications and patient was discharged on the seventh postoperative day.

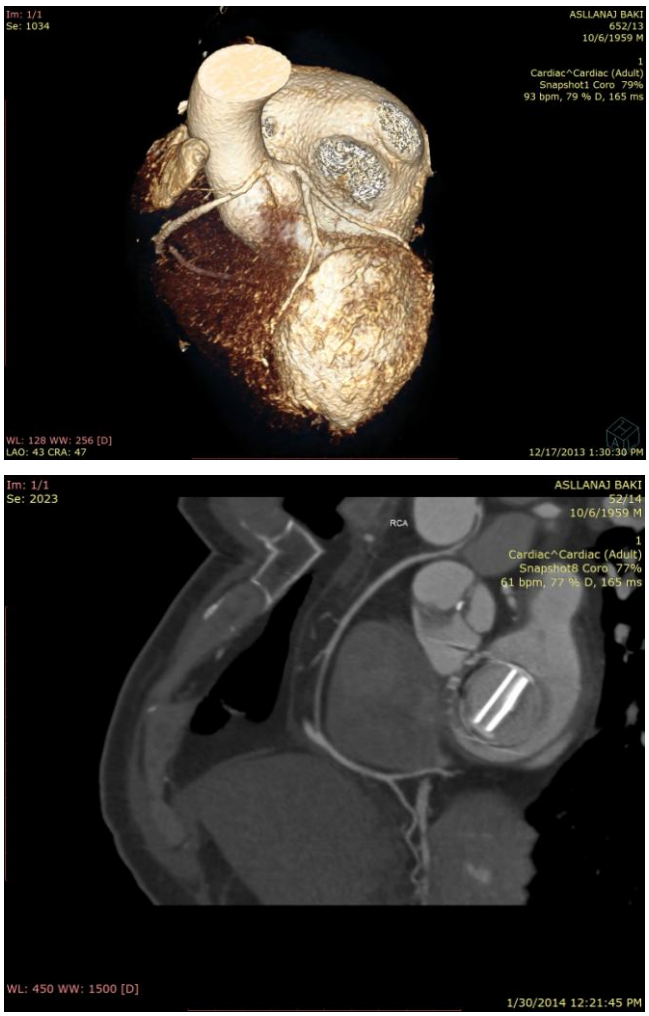

Figure 3: CT scan and 3D reconstruction of the patient before (up) and after surgery (down)

Discussion

Anomalous origin of the coronary artery from the opposite coronary sinus of Valsalva occurs with a very low incidence in the general population, but there is a high risk of sudden death due to myocardial ischemia and the resultant arrhythmias associated with them. Various mechanisms have been postulated to cause ischemia, including: origin in an acute angle

resulting in a slit-like orifice and kinking or occlusion caused by the angulation at the point of coronary artery, coronary spasm resulting from its torsion movement, mechanical compression of the coronary artery between the pulmonary and aortic trunks during physical exertion [7-9]. The majority of these complications may be exacerbated during or immediately after exercise, as exercise leads to compression of coronary arteries as well as increasing the pre-existing angulation of the proximal portion of anomalous vessel.

Patients with AAOCA are typically asymptomatic. The diagnosis is often made as an incidental finding. Symptomatic patients complain of exertional syncope, chest pain, or palpitations [10]. The physical exam, ECG and exercise stress testing are generally unremarkable. Because coronary angiography has a significant false negative rate [11], other imaging modalities are frequently employed. In particular, multi-detector computed tomography (CT) scanners and magnetic resonance angiography (MRA) now provide excellent spatial resolution allowing visualization of the coronary anatomy [10].

The choice of treatment for this coronary anomaly is controversial, medical or surgery, with most of surgeons advocating revascularization in all of cases with inter-arterial course of anomalous coronary artery [12, 13]. Most of the authors think that in symptomatic patients,without question, surgery is the best choice. For asymptomatic patients, if they have an anomalous left coronary with an intramural route, they are offered surgery. For those with a anomalous right coronary artery arising from the left sinus of Valsalva, it is a very difficult question. The level of physical activity of these patients and the dominance of their coronary artery, also play a role in decision making. A right coronary artery that has a large posterior descending artery has a large amount of myocardium at risk, and especially in young people's playing basketball even though they are asymptomatic, they are more likely to get operated on [14].

Our patient was free of ischemic symptoms, but he had to be operated for severe mitral stenosis and had a 90% stenosis of proximal RCA in the course of intramural anterior to the aorta (Fig. 1). No sign of atherosclerotic changes was seen in coronary angiography.

There are multiple surgical options for treating AAOCA. Bypass grafting was used initially but early graft failure was reported [15, 16]. Some authors explain that the early failure is due to the steal phenomenon at high levels of exertion [17] or competitive flow from patent native vessels contributing to graft thrombosis [18, 19]. For this reason bypass grafting has been used less frequently [10]. Other approaches include direct implantation of the anomalous artery [20], patch augmentation [21, 22] or pulmonary artery translocation to reduce the risk of compression of the anomalous vessel as it transverses between the aorta and the pulmonary artery [9, 23].

More recently, unroofing the anomalous vessel along its intramural segment has become the preferred management option [9, 24, 25]. This procedure, first reported by Mustafa in 1981, creates a neo-orifice at the anatomically correct sinus [26]. The advantages of unroofing are elimination the intramural segment and avoidanceof an oblique angle of take-off of the vessel.

We decided to perform CABG with saphenous graft in our patient for some reasons:

- there was a right coronary artery stenosis 90% outside of aortic wall, without signs of atherosclerosis but not spasm of coronary artery, because during the angiography procedure it was not released after nitrite injection;

- the right coronary ostium was small and too close to the ostium of left main coronary artery and intramural course was too short; and

- the right mammary artery was very small.

We conclude that correction of the anomalous origin of RCA is mandatory in cases where patient has to be operated for other cardiac causes.

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Case Report

Vesicoureteral Reflux Detected with ^{99m}Tc-DTPA Renal Scintigraphy during Evaluation of Renal Function

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Key words: VUR; renal function; ^{99m}Tc-DTPA scintigraphy; ^{99m}Tc-DMSA scintigraphy; hydronephrosis.

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Abstract

BACKGROUND: Radionuclide techniques, as direct radionuclide cystography and ^{99m}Tc-DMSA scintigraphy, have been used in evaluation of vesicoureteral reflux (VUR) and reflux nephropathy (RN) in children. Dynamic ^{99m}Tc-DTPA scintigraphy is reserved for evaluation of differential renal function and obstruction in children, where hydronephrosis is detected by ultrasonography (US) pre- or postnatally.

CASE REPORT: Six year old boy was prenatally diagnosed with bilateral hydronephrosis. Postnatal, severe bilateral VUR was detected by voiding urethrocytography. US and ^{99m}Tc-DTPA scintigraphy performed in the first month of life showed small left kidney that participated with 2% in the global renal function. Bilateral cutaneous ureterostomy has been performed in order to obtain good renal drainage and promote optimal renal growth. Twelve months later, classic antireflux procedure was done. Control ^{99m}Tc-DTPA scintigraphy, 5 ys after antireflux surgery, revealed persisting radioactivity during the diuretic phase, in the left kidney that indicated antireflux procedure failure with VUR reappearance.

CONCLUSION: ^{99m}Tc-DTPA scintigraphy is the first method of choice for long-term monitoring of individual kidney function in children with VUR and other congenital urinary tract anomalies. Additionally, it can be used as indirect radionuclide cystography when rising of radioactivity in the kidney region, during the diuretic phase can indicate presence of VUR.

Introduction

Vesicoureteral reflux (VUR) is a congenital defect of the urinary tract leading to retrograde urine flow – from the bladder towards the kidney. It occurs in 1% of the general population and is one of the main risk factors in children for renal scar development after infection of the urinary tract [1].

Several studies stress the importance of diagnosing VUR as a risk factor for repeated urinary tract infections (UTIs), which if left untreated, can lead to serious kidney damage in the future. Especially severe VUR (grades 4 and 5) has been associated with renal damage and represents an important cause of chronic renal failure in children [2].

Radionuclide techniques, as direct radionuclide cystography and cortical scintigraphy with ^{99m}Tc-dimercaptosuccinic acid (^{99m}Tc-DMSA), have been used in evaluation of VUR and reflux nephropathy in children. The dynamic renal scintigraphy with ^{99m}Tc-diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) provides important functional information of kidney function in children with VUR.

We aimed to report vesicoureteral reflux detected with ^{99m}Tc-DTPA renal scintigraphy during evaluation of renal function in a boy who was prenatally diagnosed with bilateral hydronephrosis.

Case Report

We present a case of VUR detection during evaluation of renal function by dynamic scintigraphy with ^{99m}Tc-DTPA.

A 6-year old boy was prenatally diagnosed with bilateral hydronephrosis. Postnatal imaging by voiding urethrocystography revealed severe bilateral VUR (grade IV/V on left and grade III/V on the right side). Renal ultrasonography showed bilateral hydronephrosis and reduction of the renal parenchyma of the left kidney. The renal function was evaluated by dynamic scintigraphy (year 2005). After i.v bolus injection of ^{99m}Tc-DTPA, 120 short time frames (4 s in vascular phase and 10 s in dynamic phase) within 20 minutes were taken, matrix size 64 x 64, using one headed SOPHA gamma camera. It has been shown that left kidney participated in the global renal function with only 2% (Fig. 1).

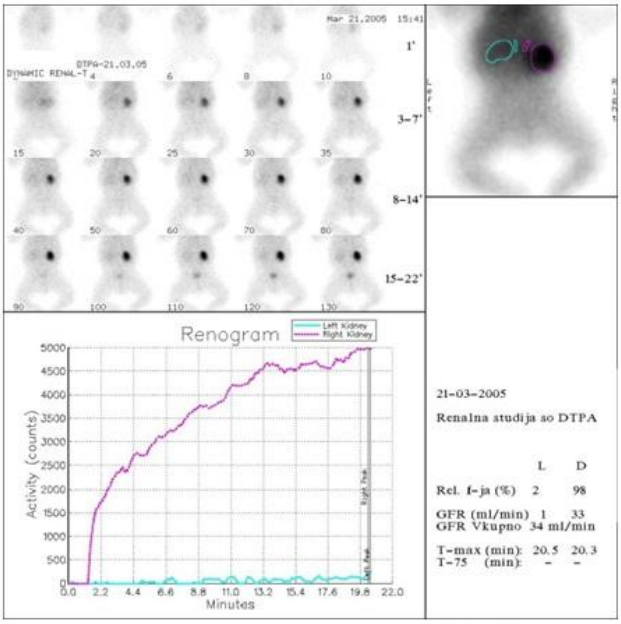

Figure 1: Dynamic renal scintigraphy with ^{99m}Tc-DTPA

Static ^{99m}Tc-DMSA scintigraphy (year 2005) was performed for evaluation of renal cortical morphology. Three hours after the injection of ^{99m}Tc-DMSA (dose of 2 MBq/kg body weight), static images in supine position were obtained (posterior-PA, anterior-AP, left oblique-LO and right oblique-RO), using a double-head MEDISO gamma camera, 256 x 256 matrix size (100-300 Kcnts for each image). The scan data showed focal cortical parenchyma defect in the mid-third of the right kidney and very small functional renal tissue on the side of the left kidney - state D according Smelly classification for reflux nephropathy (Fig. 2).

Surgical treatment was implemented, initially bilateral cutaneous ureterostomy have been created in order to obtain good renal drainage and promote optimal renal growth. Classic antireflux procedure (Leadbetter-Politano ureterocystoneostomy) was performed after 12 months.

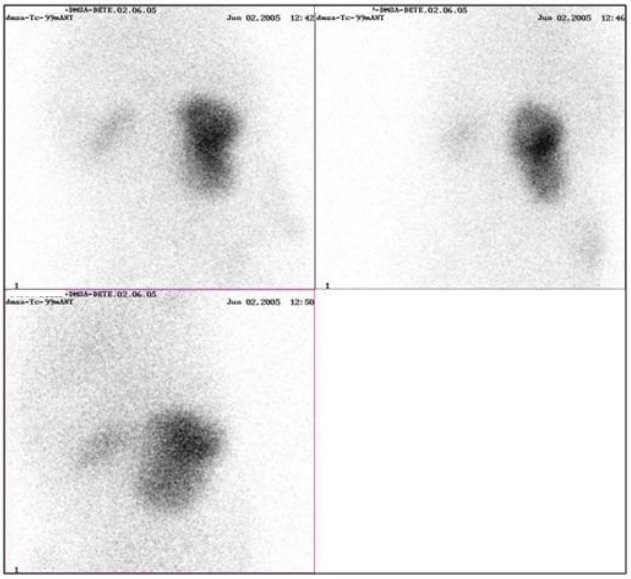

Figure 2: Renal scintigraphy with ^{99m}Tc-DMSA

During the following five years, the child has been regularly checked in outpatient pediatric Nephrology Clinic. Renal functional studies, as degradation products and clearance of endogenous creatinine, were in normal range, as well as proteinuria. The child had neither urinary infection, nor hypertension.

The follow up ^{99m}Tc-DMSA scintigraphy (year 2008), showed improvement of the findings at the side of the right kidney, without any change in the left renal function (Fig. 3).

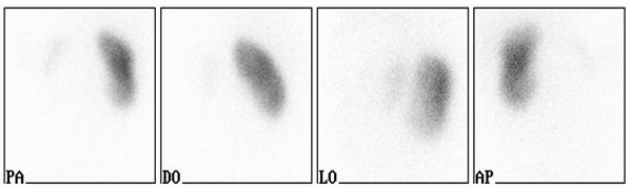

Figure 3: Renal scintigraphy with ^{99m}Tc-DMSA. Relative uptake of ^{99m}Tc-DMSA: L = 3%, D = 97%

Three years after (year 2011), control renal diuretic scintigraphy with ^{99m}Tc-DTPA was performed. Diuretic (furosemid) was given 15 minutes after the start of the dynamic renal scintigraphy. This scan data showed almost identical findings concerning the renal function in comparison with initial scan. However, during the diuretic phase an increase in the radioactivity in the region of the left kidney was noticed. This finding suggested failure of the antireflux procedure on the left side, with reappearance of VUR (Fig. 4).

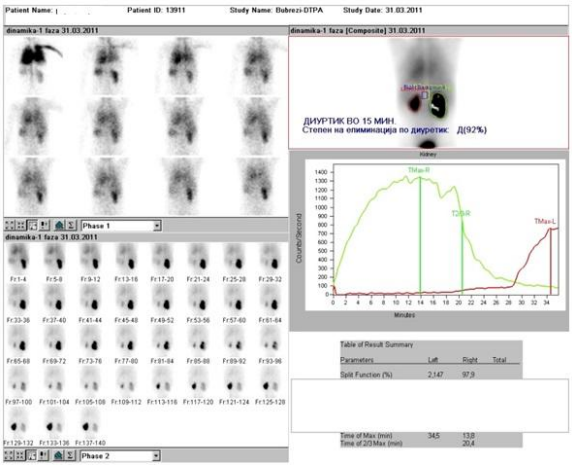

Figure 4: Dynamic renal scintigraphy with ^{99m}Tc-DTPA

Discussion

Neonatal hydronephrosis (identified if pelvic diameter is > 4mm on antenatal ultrasound) is a common abnormality that can be diagnosed ante- or postnatal, with incidence of 2-9 per 1000 infants [3]. Involving of ultrasonography as a routine test during pregnancy, allows detection of eventually intrauterine anomalies especially if they are performed during 18-20 week of gestation [4].

Among the urinary tract anomalies, hydronephrosis is the most common one. The appearance of intrauterine hydronephrosis was first described by Garett et al., in 1975 [5]. The causative factors of antenatal hydronephrosis (AH) can be categorized into those leading to obstruction (ureteropelvic junction obstruction - UPJO), those leading to reflux (vesicoureteral reflux - VUR) and a group of non-obstructing and non-refluxing “idiopathic” hydronephrosis [6]. VUR as a common cause of AH occurs in 10-15% of them [7].

Primary VUR is associated with congenital defect in the valve mechanism that prevents urine to flow backward from the bladder into the ureters. The valve does not close properly resulting in an ureterovesical junction anomaly. It is diagnosed in the early child age, as a consequence of often repeated UTIs resistant to conservative treatment. Secondary VUR, where valve mechanism is intact, is due to urinary tract malfunction, often caused by infection or increased vesicular pressures associated with obstruction. This conditions result in elevation of the bladder pressure, which distorts the ureterovesical junction [8].

In 1981, international grading system consisting of five grades was established.

The most important consequence of VUR is reflux nephropathy and renal scarring which occurs in 25% of children and younger adults with chronic renal failure. In the study of Ajdinovic et al., children with UTI and VUR (53%) had significantly higher percent of abnormal DMSA findings, than children with UTI without VUR (15%) [9].

VUR is relatively common disorder in childhood which is associated with recurrent urinary tract infection, hydronephrosis, hypertension, renal dysplasia and parenchyma damage, failure to thrive and end-stage renal disease.

Dilating reflux (grades III-V) has been shown to be significantly associated with reflux nephropathy. Prognosis is worst when RN is bilateral. Unilateral RN is compensated for the hypertrophy of the contralateral normal kidney [10].

In the study of Ali et al. the most common causes of hydronephrosis in fetuses are VUR (40.2%), UPJO (32,8%), PUVs (13.4%) and transient hydronephrosis [3]. In the study of Hamid this percent was less for the VUR (17.8), for UPJO (43.6%) and for transient hydronephrosis (20.3%). VUR was identified in 60 (29.7%) of newborns, 48 males and 12 females. The rate of VUR was 27.1% in unilateral hydronephrosis and 34.8% in bilateral.

Farhat reported 48% of neonates with AH had high grade of VUR (IV-V), while Ismail showed 36% of 43 infants with primary VUR had high grade VUR [11, 12].

Direct radionuclide cystography with ^{99m}Tc-labeled agent (sulfur colloid, DTPA, or pertechnetate) is a well-accepted alternative to fluoroscopic VCUG. It is an investigation for initial diagnosis, follow-up examination of children with VUR or for postoperative evaluation after ureteral reimplantation. This technique requires bladder catheterization and it cannot delineate anatomy of bladder and urethra. The advantages of this method include continuous monitoring and imaging, high sensitivity, and a decreased radiation dose for a voiding imaging study [13].

In the study of Thakral et al., DRCG was found to be a sensitive technique for the detection of VUR. There was a direct relationship between the grade of reflux and renal scarring.The study also reveals that there is a cause-and-effect relationship between UTI and renal scarring that is made worse by VUR [14].

The detection of VUR during dynamic renography is unusual. Conventional ^{99m}Tc-MAG3 scintigraphy can work as indirect cystography to detect reflux. Nizar et al. evaluated 5 year old boy with moderate bilateral hydronephrosis, with ureteric dilatation presented on US, but VCUG did not demonstrate any VUR and showed normal urethral caliber. Dynamic renal scan with MAG3 and post-void images with calculation of residual urine volume detected the VUR [15].

Arena et al. found VUR in 17% infants with antenatal hydronephrosis. Low grade VUR (I-III) may be missed on antenatal evaluation as there may not be dilatation of the renal pelvis [16].

In our case during control dynamic renal scintigraphy with ^{99m}Tc-DTPA, as a standard procedure of evaluation of the renal function in child with surgically treated VUR and reflux nephropathy, progressive accumulation of radioactivity was noted on the late scan images, in the region of the left non-functional kidney. This finding suggested presence of VUR and has been confirmed by subsequent conventional voiding uretrocystography. Consequently, ^{99m}Tc-DTPA dynamic renal scyntigaphy in our case served as an indirect radionuclide cystography, giving additional information and helping clinicians to optimize further clinical workout.

In conclusion, early undiagnosed VUR could be a reason for developing urinary tract infection, which can lead to serious renal damage as increasing risk of pyelonephritis and progressive renal failure. The gold standard method for diagnosing VUR is voiding cystourethrography (VCUG), but it is invasive, exposes the patient to radiation and increases the risk of reinfection.

^{99m}Tc-DMSA is a sensitive and specific method for detecting renal scars in children with persistent UTI, as a consequence of VUR.

^{99m}Tc-DTPA scintigraphy is the first method of choice for long-term monitoring of individual kidney function and structure in children with VUR and other congenital urinary tract anomalies. Additionally, it can be used as indirect radionuclide cystography, as an important independent predictor of early failure to resolve VUR.

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Case Report

Pure Motor Stroke Secondary to Cerebral Infarction of Recurrent Artery of Heubner after Mild Head Trauma: A Case Report

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Key words: childhood stroke; Heubner artery; post-traumatic stroke; head trauma.

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Abstract

BACKGROUND: The recurrent Heubner's artery is the distal part of the medial striate artery. Occlusion of the recurrent artery of Heubner, classically contralateral hemiparesis with fasciobrachiocrural predominance, is attributed to the occlusion of the recurrent artery of Heubner and is widely known as a stroke syndrome in adults. However, isolated occlusion of the deep perforating arteries following mild head trauma also occurs extremely rarely in childhood.

CASE REPORT: Here we report the case of an 11-year-old boy with pure motor stroke. The brain MRI showed an acute ischemia in the recurrent artery of Heubner supply area following mild head trauma. His fasciobrachial hemiparesis and dysarthria were thought to be secondary to the stretching of deep perforating arteries leading to occlusion of the recurrent artery of Heubner.

CONCLUSION: Post-traumatic pure motor ischemic stroke can be secondary to stretching of the deep perforating arteries especially in childhood.

Introduction

The recurrent artery of Heubner (RAH), the junction of the anterior cerebral artery and ACoA or distal A1 segment coursing backward along the A1 segment, arises as a single vessel from the portion near to the anterior communicating artery (ACoA) [1]. It supplies the head of the caudate nucleus, the anterior inferior part of the internal capsule's anterior limb, the anterior globus pallidus and putamen and the anterior thalamus, and some parts of the uninate fasciculus, olfactory region and the hypothalamus [2]. Classically, contralateral hemiparesis with fasciobrachiocrural predominance is attributed to occlusion of the RAH and is widely known as stroke syndrome in adults [3, 4]. However, isolated occlusion of the deep perforating arteries following mild head trauma occurs extremely rarely in childhood.

Here we report the case of an 11-year-old boy with a pure motor stroke secondary to an acute ischemic infarction at the RAH supply area immediately following mild head trauma.

Case presentation

An 11-year-old boy was brought to the emergency room (ER) because of difficulty in speech and weakness on his right arm, which appeared soon after he hit his head upon falling from his bicycle. He was not unconscious but 20 minutes after falling

difficulty in speech and right arm paresis was seen. In his initial neurological examination GCS was 14 and his pupils were reactive to light and isochoric. He had dysarthria and right fasciobrachial hemiparesis with muscle strength of 4/5. The brain CT revealed neither skull fracture nor hemorrhagia of the intra-/extra-axial structures. A brain MRI after 24 hours showed a hyperintense lesion at the left putamen and globus pallidus interna and externa on T2 weighted images (Figure 1A), and diffusion restriction was also seen at the aforementioned structures consistent with the RAH supply area (Figure-1B). His cerebral and carotid MR angiography revealed no pathology and his parents did not give permission for cerebral angiography, and so this could not be performed. Isolated post-traumatic occlusion of the perforating arteries is extremely rare in children. Therefore, a large scale laboratory assessment was performed to exclude coexisting systemic vasculitic diseases, emboligenic heart diseases and haematological disorders. Erythrocyte sedimentation rate, CRP, platelet count, PT, PTT, antithrombin III, homocycteine, von Willebrand factor, activated protein C resistance, and protein C and S assays were all normal. Lupus anticoagulants, anticardiolipin and antiphospholipid antibodies were negative as were antinuclear and anti-ds DNA antibody titers. He was also assessed for ophthalmological and dermatological manifestations of the vasculitic diseases, but these revealed negative results.

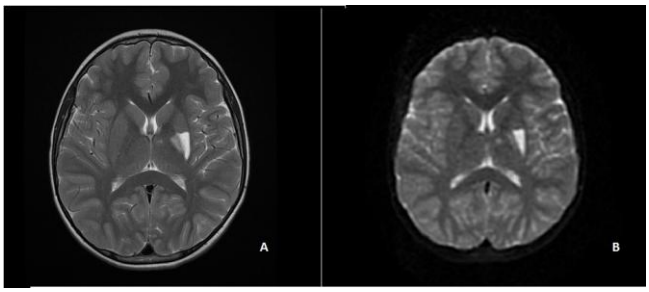

Figure 1: The cerebral MRI showing a hyperintense lesion at the left putamen and globus pallidus interna and externa on T2 (A) and diffusion weighted images (B)

During the hospitalization period he did not develop any further neurological deficits or convulsive episodes. A physical rehabilitation program was applied and he was discharged ten days later. The latest neurological examination after discharge was performed six months later and he had full muscle strength on the right side. Apart from the possibility of a mild cognitive dysfunction, his school performance was acceptable.

Discussion

Moderate to severe head injuries in children can lead to skull fractures, shearing injury or infarct/oedema [5, 6]. The majority of cerebral artery occlusions occur in the anterior and middle cerebral arteries due to vasospasm, embolus, thrombus or dissecting aneurisms. There also may be traumatic dissection of the carotid arteries or vessels of the circle of Willis as well as congenital predisposition to rupture of intracranial arteries. However, cerebral ischemic lesions are extremely rare in childhood with an incidence of 0.2-0.63/100 000 per year [3, 7]. Less than 2% of all ischemic strokes in childhood occur in the basal ganglia after mild head trauma, which is attributed to the vasospasm of the deep perforating arteries. Most pure motor ischaemic strokes are due to small arterial vessel disease in old patients with a mean (SD) age of 72.2 years [8, 9]. In 15%, they may be associated with other stroke subtypes [10].

The deep perforators of the carotid system refers to the anterior choroidal artery and posterior communicating artery, the lateral lenticulostriate arteries that originate from the middle cerebral artery and the medial lenticulostriate arteries and the RAH that originates from the anterior cerebral artery [1, 2, 7, 9, 11, 12]. Unlike the small penetrating arteries, selective occlusion of the main trunk of either the RAH or lenticulostriate arteries may result from vasospasm or thromboembolism following head injury, probably because of the anatomical characteristics of these arteries in children. The occlusion of the lenticulostriate, thalamoperforating, or choroideal arteries may result in basal ganglionic infarction after head injuries [13].

According to a recent report history, mild head trauma is one of the most frequent risk factors for ischemic stroke of basal ganglia in children [8]. Several mechanisms of arterial stroke have been proposed. Mechanical disruption of the flow in the perforating branches or intimal trauma and subsequent thrombosis can lead to occlusion as well as transient arterial spasm induced by trauma [8].

Although the affected area is relatively small, this can cause contralateral hemiparesis with fasciobrachiocrural predominance. Together with this, dysarthria, epilepsy, athetosis, and cognitive and behavioural disturbances are reported [11]. Sometimes it may be hard to attribute these clinical findings to minor head trauma without loss of consciousness. Therefore, differential diagnostic assessment is important. Assessment of common risk factors of cerebral infarctions of adults, imaging and laboratory work should be directed to determine secondary causes such as traumatic dissection of the common carotid and internal carotid arteries or vessels of the circle of Willis, congenital predisposition to rupture of the intracranial arteries, heart disease leading to embolus formation and congenital thrombophilia. Although cranial MRI showed the infarction of the RAH supply area in our patient, a cerebral angiography or CT angiography would have been informative and excluded arterial dissection if it

had been performed. Moreover, laboratory assessment to differentiate systemic diseases leading to vasculitis or prothrombotic states revealed normal test results.

We concluded that post-traumatic pure motor ischemic stroke can be secondary to stretching of the deep perforating arteries during hemispheric movement at a perpendicular angle, leading to occlusion of RAH.

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Case Report

Endothelial Nitric Oxide Synthase *T-786C* Mutation, Prothrombin Gene Mutation (*G-20210-A*) and Protein S Deficiency Could Lead to Myocardial Infarction in a Very Young Male Adult

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Key words: (eNOS) *T-786C* mutation; prothrombin gene mutation (*G-20210-A*); protein S deficiency; myocardial infarction; young male.

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Abstract

INTRODUCTION: Myocardial infarction is a rare medical event in young people. The main reasons include congenital coronary abnormalities, coronary artery spasm, and coronary thrombosis due to hypercoagulable states (hereditary and acquired).

AIM: We present a case of a young male adult with myocardial infarction caused by a combination of gene mutations and anticoagulation protein deficiency.

CASE PRESENTATION: A 19 years old young man was admitted to our hospital complaining of chest pain during the last two weeks. The patient did not have any known cardiovascular risk factors, except a positive family anamnesis. Subacute inferior nonST segment myocardial infarction was diagnosed according to the patient's history, electrocardiographic and laboratory findings. Coronary angiography revealed suboclusive thrombus in the proximal, medial and distal part of the right coronary artery (TIMI 2). Percutaneous coronary intervention was performed. Anticoagulant and antiagregant therapy (heparin, acetilsalicilic acid and clopidogrel) according to protocol was started. The hospital stay was uneventful. Homozygous endothelial nitric oxid synthase (eNOS) *T-786-C* mutation, heterozygote prothrombin gene mutation (*G-20210-A*), and protein S deficiency were verified from the thrombophilia testing. Other trombophilic tests were normal. Three months after discharge from hospital another coronary angiography was performed. It revealed normal coronary arteries. Four years after the attack, the patient is free of symptoms and another cardiovascular event.

CONCLUSION: Combination of genetic mutations and anticoagulation protein deficiency could be a reasonable cause for myocardial infarction in a very young male adult without any other cardiovascular risk factors.

Introduction

Acquired or hereditary hypercoagulability or thrombophilia may be defined as a tendency to venous or arterial thrombosis. Acquired hypercoagulability leading to arterial thrombosis is seen in every day surgical practice, while the genetic predisposition for development of arterial occlusive disease is still inconclusive. Past, as well as more recent publications suggest a correlation between the existence of particular genetic mutations and arterial thrombosis [1]. There is clear evidence that hyperhomocysteinaemia [2] and the antiphospholipid antibody syndrome [3] are a genetic predisposition to arterial thrombosis, but the role of prothrombin gene *G20210A* variant mutation and the factor V Leiden deficit is still unclear [4]. It seems that genetic mutations are particularly important for young patients and women since the data suggest that they increase the risk of myocardial infarction and ischemic stroke [5, 6].

Three to ten percent of all myocardial infarctions of young people occur due to non atherosclerotic disease such as congenital coronary artery anomalies, myocardial bridging, coronary artery dissection, septic vegetations, coronary artery aneurysms, hypercoagulable states (hereditary and

acquired) [7, 8] and drug abuse.

We present a case of a young adult with subacute myocardial infarction, without the traditional coronary artery disease risk factors, and with a combination of gene mutations and anticoagulant protein deficiency.

Case Presentation

A 19 year old young man was admitted to our hospital complaining of chest pain during the previous two weeks. He was not obese; he was an active athlete, and a nonsmoker. He does not consume alcohol regularly nor does he abuse drugs. The patient had no hypertension, hyperlipidemia or diabetes mellitus. He had no prior history of cardiovascular events. He has a positive family anamnesis for coronary artery disease. Electrocardiography showed sinus rhythm (HR 55/min), normal cardiac axis, 2 mm Q waves in D3, AVF, T wave negative in D2, D3, AVF, no ST segment elevation. Transthoracic echocardiography revealed hypokinesia of the inferior wall with preserved systolic function of the left ventricle. Laboratory analysis showed increased levels of the cardiac enzymes: creatine kinase 411 U/L (32-211 U/L), creatine kinase MB 8.8 ng/ml (0-15 ng/ml), troponin 16 ng/ml (0.78 ng/ml), and lactate dehydrogenase 947 U/l (208-378 U/l). Subacute inferior nonST segment myocardial infarction was diagnosed according to the patient's history, electrocardiographic and laboratory findings [9]. Coronary angiography was performed. It revealed subocclusive thrombus in the proximal, medial and distal part of the right coronary artery (TIMI 2) (Fig. 1).

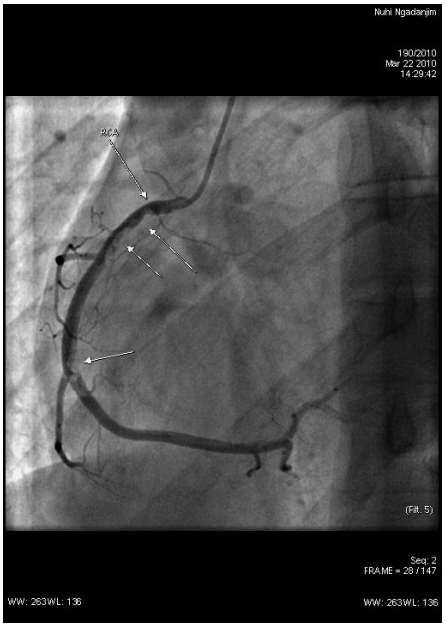

Figure 1: *Coronary angiography. Sub occlusive thrombus in the proximal, medial and distal part of the right coronary artery (TIMI 2)*

The other coronary arteries had no stenosis. Anticoagulant and antiagregant therapy (heparin, acetilsalicilic acid and clopidogrel) was started according to protocol [9]. The hospital stay was uneventful. After discharge, blood was drawn according to the guidelines for thrombophilia tests and sent for thrombophilic testing.

Homozygous endothelial nitric oxid synthase *(eNOS) T-786C* mutation, heterozygote prothrombin gene mutation (*G-20210-A*), and protein S deficiency were verified from the thrombophilia testing. Protein S deficiency activity was estimated at 10% (reference range 60-150%).

Deficit of antithrombin III, protein C and presence of antiphospholipid antibodies were excluded. There were no genetic mutations for factor V Leiden, genetic variants of the beta fibrinogen gene, gene for *PAI-1*, polymorphism in human platelet antigen (*HPA1*) (*1a/1a*), *MTHFR C677T* gene mutation, high levels of apolipoprotein B and factor XIII.

We recommended dual antiagregant therapy (acetilsalicilic acid a 100 mg plus clopidogrel a 75 mg per day) during one year after the myocardial infarction. After that, we recommended lifelong treatment with acetilsalicilic acid tablets.

Another coronary angiography was performed three months after discharge from hospital. It revealed normal coronary arteries (Fig. 2).

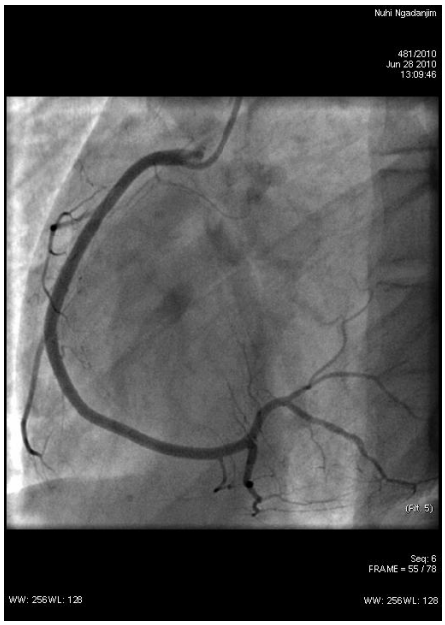

Figure 2: *Coronary angiography. Normal flow through the right coronary artery*

During 4 years follow up the patient was asymptomatic, without another acute coronary event.

Discussion

In our paper we present a case of a very young male patient with subacute myocardial infarction due to thrombosis of the right coronary artery. After excluding the conventional cardiovascular risk factors, thrombophilic tests were performed and revealed homozygous endothelial nitric oxid synthase *(eNOS) T-786C* mutation, heterozygote prothrombin gene mutation (*G-20210-A*), and protein S deficiency.

Arterial thrombosis is a multi-factorial condition, where most of the risk factors do not overlap with those for venous thrombosis. An increased risk for arterial cardiovascular diseases by heritable thrombophilia could not be established firmly. The treatment and secondary prevention should relate to the established cardiovascular risk factors. Testing for heritable thrombophilia is not recommended, although there are many studies that confirm the coexistence between genetic mutation and arterial thrombosis.

Nitric oxide is produced by endothelium and plays an important role as a smooth muscle relaxant in maintaining vascular tone as well as inhibiting activation and aggregation of platelets [10]. Endothelial nitric oxid synthase catalyses the synthesis of nitric oxide. Presence of a *T-786-C* mutation in the *eNOS* gene results in decreased synthesis of nitric oxide and confers a greater risk for coronary artery spasm [11], ulcerative lesions in internal carotid artery [12]. Homozygous endothelial nitric oxide synthase *(eNOS) T-786-C* mutation is known as an independed risk factor in combination with other known cardiovascular risk factors, such as cigarette smoking [13]. In addition to the clinical connection between *eNOS T-786* mutation and risk for arterial thrombosis and atherosclerosis, there is molecular evidence for changes in markers of oxidative stress in high risk patients [14]. The presence of the *T-786* gene mutation in our patient, without other cardiovascular risk factors, but in combination with other gene mutations, suggests that this gene mutation is a potential cause for arterial thrombosis in such a constellation.

Prothrombin is a precursor of thrombin, acting as a pro-coagulant, via platelet activation and the generation of fibrin and factors Va, VIIIa, and XIIIa, and subsequently as an anticoagulant, by activating protein C. The prothrombin gene mutation is associated with elevated prothrombin levels, which is a genetically determined trait that increases the thrombosis risk [15]. About 1% to 2% of the general Caucasian population is heterozygous for the prothrombin gene which increases the risk of developing blood clots 2 to 5 times. Homozygous people have an even greater risk. Large number of studies demonstrated an association between the prothrombin *G20210A* mutation and venous thromboembolism. Recently, one study demonstrated a significant influence of prothrombin gene polymorphisms on myocardial perfusion [15]. The prothrombin *G20210A* mutation might play role in arterial thrombosis especially in young people [16] with other known cardiovascular risk factors.

Protein S, a vitamin K dependent physiological anticoagulant, acts as a nonenzymatic cofactor to activated protein C in the proteolytic degradation of a factor Va and factor VIIIa. Protein S deficiency may be hereditary or acquired (due to hepatic disorders or a vitamin K deficiency). Hereditary protein S deficiency represents an autosomal dominant trait and manifestations of thrombosis are observed in both heterozygous and homozygous individuals. Protein S deficiency usually manifests clinically as venous thromboembolism and the absolute risk of thrombosis in patients with protein S deficiency has been estimated to be 8.5 times higher than in individuals with no defect [17]. There are studies that suggest an association between arterial thromboses (stroke, heart attack) in patients with protein S deficiency, but its role in arterial disease is still being explored [18]. In patients younger than 55 years of age protein S deficiency should be considered as atherothrombotic risk factor [19].

Venous and arterial thromboses are complex genetic traits, with various gene-gene and gene-environment interactions. A number of studies have evaluated and confirmed that the coinheritance of two or more defects increases the appearance of thrombosis [20].

In spite of the fact that opinions about protein S deficiency and prothrombin *G20210A* mutation and their role in the appearance of arterial thrombosis are divided, the combination with other genetic defects like *T-786-C* mutation in the *eNOS* gene, may enhance the probability of arterial thrombosis like myocardial infarction, especially in young individuals without any other risk factors. In these clinical cases, testing for coagulation disorders should be performed in order to recommend a long term therapy which could prevent any other thrombotic events.

In conclusion, the combination of genetic mutations and anticoagulation protein S deficiency could be a reasonable cause for myocardial infarction in a very young male adult without any other cardiovascular risk factors. This case suggests that in premature coronary artery disease thrombophilia tests are justified. In addition to its scientific value, genetic tests could be a solid basis for further therapy recommendation.

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Case Report

Long-Term Outcome after Rehabilitation of Bilateral Total Hip Arthroplasty in Renal Transplant Recipient – A Case Report

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Key words: renal transplantation; hip replacement surgery; rehabilitation; exercise; outcome.

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Abstract

INTRODUCTION: Total hip replacement is generally proposed for renal transplant patients with avascular osteonecrosis of the femoral head.

PURPOSE: The purpose of the study is to report the long-term outcome after rehabilitation of bilateral total hip arthroplasty in a patient with renal transplantation suffering from avascular osteonecrosis of the both femoral heads.

MATERIAL AND METHOD: The patient S.D, 49 years old at follow-up. Few months after renal transplantation, the patient had got avascular osteonecrosis of both femoral head. One year after transplantation the total hip arthroplasty for both hip joints were performed. Three years later repeat total hip arthroplasty surgery for left hip was performed. After any surgery intervention the patient was referred for inpatient rehabilitation. For clinical assessment the clinical findings and Harris Hip Score have been used. The rehabilitation program consisted of exercises, occupational therapy, and patient education.

RESULTS: After any rehabilitation treatment the patient had improvement of clinical findings. At follow-up assessment outcome for both hip function was good - Harris Hip Score was 81 points.

CONCLUSION: Rehabilitation is integral part of multidisciplinary treatment of renal transplant recipient after total hip arthroplasty. Regular exercise training of these patients is very important for improving of their long-term outcome.

Introduction

Avascular osteonecrosis of the femoral head is a serious osseous complication after renal transplantation. It is caused by disruption of blood flow. Although the precise mechanism is still uncertain, the administration of glucocorticoid has been considered to play an important role in the occurrence of osteonecrosis. It prevalence clearly decreased from 20% to 4% after introduction of cyclosporine and reduction of steroid doses [1]. These patients have been proposed to be treated by elimination of weight–bearing, conservative treatment, but also they have been undergone core decompression and total hip replacement (THR) [2, 3].

This is our first patient with bone complication after renal transplantation who was admitted for inpatient rehabilitation treatment.

The purpose of the study is to report the long-term outcome (six years' outcome) after rehabilitation of bilateral total hip arthroplasty in a patient with renal transplantation suffering from avascular osteonecrosis of the both femoral heads.

Material and Method

The patient S.D, male, 49 years old at follow-up assessment, single, carpenter with 17 years

working experience, with previously received disability pension due to chronic renal failure. Non smoker, he was previous smoker until six years ago. He has been undergone to renal transplantation on the right kidney because the chronic renal failure six years ago. His age at the time of the operation was 42 years. He had regular check in by nephrologists. Following surgery he has received immunosuppressive drugs, like cyclosporine and glucocorticoids to prevent rejection of the new organ.

Few months after renal transplantation, the patient had got hip pain due to avascular osteonecrosis of both femoral heads. The diagnosis was confirmed with native radiologic findings and magnetic resonance imaging (MRI) of the pelvis with both hips. In December 2008 the decompression of bilateral femoral head was performed. One year after transplantation (2009) a total hip arthroplasty (non-cemented) of the right hip and few months later a total hip arthroplasty (non- cemented) of the left hip were performed (Figure 1). Three years after first arthroplasty (2012) the patient has undergone the repeat total hip arthroplasty surgery for left hip due to prosthesis loosening. After any surgery intervention the patient was referred for inpatient rehabilitation in regional spa center and further rehabilitation in the rehabilitation hospital.

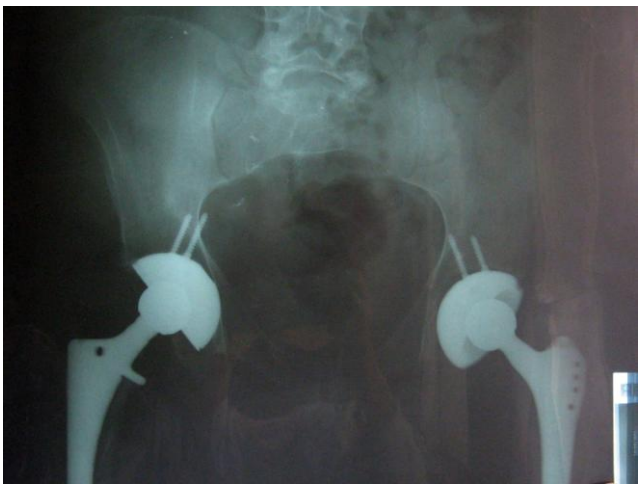

Figure 1: X-ray of pelvis with both hips after both total hip artroplasty

In January 2010 our patient has been suffering from low back pain and bilateral sciatica, and has been conservatively treated by orthopedic surgeon. The findings on computerized tomography (CT) scan of lumbar spine in the transversal plane confirmed lumbar disc herniation at L4-L5 level with compression of the cauda equina and nerve roots, with mild spinal canal stenosis. HLA typisation showed A2, A3, B18, B 27 positive findings. At the same time, dual energy x-ray absorptiometry (DXA) confirmed osteoporosis (t- score on right forearm -3.6, and L1-L4 -1.8), and bisphosphonate ibandronic acid 150 mg and vitamin D were administrated.

The patient was admitted for rehabilitation treatment six times, after total hip arthroplasty surgeries and also for exercise training and maintenance of his functional status.

At the first admission the patient also has suffered from low back pain, that reffers in left leg to the knee, pain in the left knee after long walking, limited range of motions in the both hips, weakness of the hip muscles, walking with the pair of below elbow crutches.The patient was obese, his height was 185 cm, weight was 100 kg, body mass index (BMI) 34.2. The patient's cardiovasular system was well.

For clinical assessment the clinical findings, measurement of the both hip range of motion, leg length and leg circumference, hip muscle strength (manual muscle testing- MMT), Harris Hip Score [4] and Visual Analogue Scale (VAS) score for low back pain have been used. Assessments were made at baseline and at discharge during the all admissions, and at follow up 6 years after first admission.

The clinical findings of musculoskeletal system on the first admittion after both artroplasty surgery was: lumbar spine was slight scoliotic deviation in thoracolumbar spine, without spasm of lumbar spinal muscles, with reduced range of motions in the lumbar spine, anteflexion finger-floor distance 40 cm, reduced retroflexion. The right leg was shorter for 1 cm (umbilicus-maleol). There was hipotrophy of the tight muscles and hipotrophy of the calf muscles bilaterally, mostly peroneal muscles and slight hipotony of the below knee muscles on the left leg. There are scars from operaive cut on the outer part of the both hips. Active movements in the right hip were limited (especially flexion with extended knee and abduction because the muscle weakness (illiopsoas and gluteus medius MMT 4-), and in both knees and ankles were in normal range. Active movements in the left hip were limited with weakness of the hip flexor (MMT 3-) and abductor (MMT 4-), in the left knee were in normal range. Left foot was in slight equinovarus, dorsal flexion in the ankle was limited, especially eversion, but pasive dorsal flexion was 10^0, plantar flexion was in normal range. There were muscle weakness of the dorsal flexion of the second, trird and forth finger on the left leg. There wasn't any sensitive disturbance. Patelar reflex was symetrical, reflex of Achilles tendon was diminished on the left. He walked with the aid of below-elbow crutches. VAS score for low back pain at admission was 7.

The rehabilitation program consisted of range of motion exercises, strengthening exercises for hip muscles (abductor, flexion, extensor muscles) (Figure 2), strengthening exercises for quadriceps, stationary bicycle exercises, isometric exercise for spinal, abdominal and gluteal muscles, occupational therapy and patient education about hip arthroplasty and ergonomic advices for spine biomechanics. For low back pain transcutaneous electrical nerve stimulation (TENS) and dyadinamic currents were applied.

Figure 2: Exercises for hip abductor

During the rehabilitation he continued to receive immunosuppressive drugs like mycophenolate mofetil, cyclosporine, glucocorticoids; and also antihypertensive therapy, bisphpsphonate, statine, diuretic, folic acid, and acetilsalicil; and bisphosphonate therapy and vitamin D for osteoporosis.

Results

After all inpatient rehabilitation treatment the patient had improvement in clinical findings (improved muscle strength, slightly increased range of motions in both hips, better ambulatory with one or both crutches, decreased low back pain).

At follow-up examination six years after bilateral replacement surgery and repeat hip arthroplasty of the left leg functional status of both hips was good with Harris Hip Score 81 points. The patient was obese and had mostly sedentary life-style without any physical activities. He hasn't performed exercises at home regularly, he didn't lose the weight. He drove his car. Clinically there was some muscle weakness of the hip muscles more on the left leg, and limited range of motions in the both hips (Table 1).

He has suffered periodically from slight low back pain. VAS score for low back pain was 2. His gait was with a pair of below elbow crutches. The follow-up x-ray of the pelvis with hips presents situation after total arthroplasty on both hips.

Table 1: Range of motions (in degrees) in both hips during the first, second, the last admission on rehabilitation treatment and at follow-up 6 years later

Active range of motion of both hips (in degrees) at admission (a) and at discharge(d)	Left leg I admission a/d	Left leg II admission a/d	Left leg Last dmission a/d	Right leg I admission a/d	Right leg II admission a/d	Right leg Last admission a/d	Follow-up Six years later
Flexion with extended knee	$0^0/10^0$	$50^0/55^0$	$50^0/80^0$	$65^0/65^0$	$20^0/60^0$	$80^0/80^0$	10^0 left 70^0 right
Flexion with flexed knee	$0^0/30^0$	$70^0/75^0$	70^0	90^0	$90^0/95^0$	95^0/N	20^0 left 85^0 right
Extension	$-5^0/0^0$	$-5^0/-5^0$	$-10^0/-5^0$	$0^0/5^0$	$0^0/5^0$	$-10^0/-5^0$	-10^0 left -5^0 right
Abduction	$0^0/10^0$	$15^0/20^0$	$25^0/35^0$	$5^0/10^0$	$20^0/25^0$	$40^0/40^0$	30^0 left 35^0 right

Discussion

Bone diseases in kidney transplant recipients have been previously reported. This is our first patient with bone complication after renal transplantation who was admitted for inpatient rehabilitation treatment. Following renal transplantation he has permanently received immunosuppressive therapy which includes corticosteroids. Avascular osteonecrosis of the both femoral heads was developed during the first year after renal transplantation.

Avascular necrosis under immunosuppressive therapy is a well known sequel following solid organ transplantation. Most cases affect hip, knees or shoulders in more than one location and occur in conection with the use of high-dose steroids. In a case report was presented that despite the steroids had been completely withdrawn after avascular necrosis of the femoral head, 2 years after renal transplantation, MRI revealed bilateral tibial and tarsal bone necrosis [5]. Shibatani M. et al. investigated the development of avascular osteonecrosis after renal transplantation using MRI in 150 patients (96 males). They confirmed that the total dose of steroids given within the first 2 months after renal transplantation had a great influence on the incidence of avascular osteonecrosis [6].

In the retrospective study of 758 kidney transplant patients, for post-operative immunosupression, 374 patients had received high-dose corticosteroids (average 12.5 g during the first year post-operatively), and 376 patients had received low-dose corticosteroids (average 6.5 g during the first year post-operatively) and cyclosporine A. In high-dose steroid group 11.2% developed femoral head

necrosis, and in the low-dose steroid group only 5.1% patients developed this complication at an average of 26.2 months and 20.5 months respectively post-transplantation [7].

In the retrospective study, among 305 renal transplant recipients 14 patients (4.5%) developed osteonecrosis of the femoral head, which was bilateral in 12 patients and unilateral in two. Thus, total number of hips with osteonecrosis was 26. The mean interval between transplantation and magnetic resonance imaging was 8.9 months. Extensive necrosis was found in most cases at the first evaluation (> 25% in 15 cases and > 50% in eight). Eleven patients were treated by elimination of weight-bearing and conservative treatment and 15 underwent core decompression. Mean follow-up was 33 months since transplantation. A poor functional outcome (Lequesne's index > 7 or total hip arthroplasty) was seen in 61.5% of cases [2].

In the historical cohort study consisted of 42,096 renal transplant recipients enrolled in the United States Renal Data System between 1 July 1994 and 30 June 1998, renal transplant recipients had a cumulative incidence of total hip arthroplasty of 5.1 episodes/1000 person-years, which is 5-8 times higher than reported in the general population. Avascular necrosis of the hip was the most frequent primary diagnosis associated with total hip arthroplasty in this population (72% of cases). Repeat surgeries were performed in 27% of the patients with avascular necrosis, vs.15% with other diagnosis. Total hip arthroplasty is well tolerated and is not associated with increased mortality in this population [8].

One year after kidney transplantation in our patient the total hip arthroplasty for both hip joints were performed due to femoral head osteonecrosis. Three years later repeat total hip arthroplasty surgery for left hip due to prosthesis loosening was performed. After any surgery intervention the patient was referred for inpatient rehabilitation.

A review of the literature reveals that cemented hip arthroplasty provides good to excellent functional outcomes for renal transplant patients. Recent studies have found that despite decreased bone stock in these patients, porous-coated prostheses are not contraindicated [9].

During the inpatient rehabilitation treatment our patient performed strengthening exercises for hip muscles (abductor, flexion, and extensor muscles), quadriceps exercise, range of motion exercises, stationary bicycle exercises, isometric exercise for spinal, abdominal and gluteal muscles, exercises in open and closed chain, occupational therapy and education.

Total hip arthroplasty has improved the care of patients with end-stage joint disease, leading to pain relief, functional recovery, and substantial improvement in quality of life. However, long-term studies indicate persistence of impairment and functional limitation after total hip arthroplasty, and the optimal rehabilitation protocols are largely unknown. In the systematic review the convincing evidence for the effectiveness of single interventions in addition to usual exercise programs exists for each of the three following options: treadmill training with partial body-weight support, unilateral resistance training of the quadriceps muscle (operated site), and arm-interval exercises with an arm ergometer. In the late postoperative phase (operation interval > 8 weeks) exercise programs consistently improve both impairment and ability to function. Weight-bearing exercises with hip-abductor eccentric strengthening may be the crucial component of the late-phase protocols [10].

Despite a successful surgical procedure, deficits in muscle strength and physical function are documented 1-2 years after total hip replacement. There is a lack of evidence concerning which rehabilitation strategy is most effective after THR. In the single-blinded, cluster randomized controlled trial with consecutive sample of 46 patients undergoing primary THR surgery for osteoarthritis, 44 patients completed the trial. An intervention group received 12 weeks of intensified exercises (e.g. rubber band resistance) and a control group received standard rehabilitation exercises without external resistance. Hip abduction strength was significantly weaker in the leg operated compared with the leg not operated on after the intervention in both groups. The authors concluded that the majority of THR patients tolerated early-initiated intensified exercises without additional pain and with high patient satisfaction, but some of the patients need supervision to perform intensified exercises [11].

In the other clinical trial with 26 patients who had had a total hip arthroplasty the effect of home versus in-hospital exercise under supervision programs on hip strength, gait speed and cadence were evaluated. The best improvement in maximum isometric abduction torque was showed in the group which exercised under physiotherapy supervision in hospital [12].

Our patient made DXA examination two years after renal transplantation that demonstrated signs of osteoporosis, so bisphosponate medication and vitamin D were administrated.

Osteoporosis, osteopenia, and osteonecrosis are common in renal transplant recipients. In the clinical study of 85 renal transplant recipients with mean age of 36.25 ± 10.5 years and mean duration of posttransplantation follow-up of 9.82 ± 2.72 months; t scores of forearm and lumbar vertebrae were normal in 29.4% and 21.2%; osteopenia in 56.5% and 49.4%; and osteoporosis in 12.1% and 29.4% of patients, respectively.They concluded that bone disease including osteopenia and osteoporosis was observed among 70%. During the follow-up period, BMD

increased significantly from baseline at 9.82 ± 2.72 months. VitD therapy caused more prominent improvements in BMD [13].

In the review of 24 RC trials (1.299 patients) was presented that fracture risk for a kidney transplant recipient is four times that of the general population and higher than for a patient on dialysis. Bisphosphonates (any route), vitamin D sterol, and calcitonin all had a beneficial effect on the bone mineral density at the lumbar spine. Bisphosphonates and vitamin D sterol also had a beneficial effect on the bone mineral density at the femoral neck. Bisphosphonates had greater efficacy for preventing bone mineral density loss when compared head-to-head with vitamin D sterols. The authors concluded that treatment with bisphosphonates, vitamin D sterol or calcitonin after kidney transplantation may protect against immunosuppression-induced reductions in bone mineral density and prevent fracture. Adequately powered trials are required to determine whether bisphosphonates are better than vitamin D sterols for fracture prevention in this population. The optimal route, timing, and duration of administration of these interventions remain unknown [14].

In the other systhematic review of RCTs conducted to assess the evidence avalible to guide targeted treatment to reduce bone disease in transplant recipients, the authors concluded that bisphosphonates and vitamin D have a beneficial effect on BMD at the lumbar spine and femoral neck [15]. After renal transplantation, both bisphosphonates and vitamin D metabolities, variably associated with calcium supplementation, have been demonstrated to have beneficial effect on bone loss, at least in the first year after renal transplantation. However, there are no data about the possible efficiacy of these treatments on fracture rate [16].

At follow-up assessment our patient has good postoperative outcome with Harris Hip Score of 81 points. Our opinion is that he should have better outcome if he had reduced his weight and if he performed the exercises regularly.

In conclusion, renal transplant recipient may develop avascular femoral head necrosis and osteopenia/osteoporosis due to corticosteroid therapy. Some of these patients need total hip arthroplasty. Rehabilitation is a integral part of multidisciplinary treatment of renal transplant recipient after total hip arthroplasty. Regular exercise training of these patients is very important for improving of their long-term outcome.

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Stomatology

Clinical Therapeutic Effects of the Application of Doxycycline in the Treatment of Periodontal Disease

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Key words: periodontal disease; doxycycline; sub-dose; gingival inflammation; gingival bleeding.

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Abstract

OBJECTIVE: To compare the therapeutic effects of the application of doxycycline-full dose (100 mg) and sub-dose (20 mg) in the treatment of periodontal disease.

MATERIAL AND METHODS: A total of 60 patients with periodontal disease were examined. Patients are divided into two groups: A) treated with antimicrobial dose of 100 mg doxycycline once daily for 30 days, and B) treated with 2 x 20 mg/day. doxycycline, during 75 days. Among all patients a conservative treatment was carried out and ordinated the proper dose doxycycline in total dose during treatment from 3 gr. Index of dental plaque by Löe-Sillness, index of gingival inflammation and gingival bleeding by Cowell were followed.

RESULTS: Values of dental plaque in relation first examination, 10th, 20th day, 1 month and 2.5 months, showed that after 2.5 months, average value (x = 0.83) of dental plaque in second group is slightly less than the value (x = 0.93) of dental plaque in the first group. The average value (x = 0.17) of gingival inflammation in second group is significantly less than the value (x = 0.50) of gingival inflammation in the first group. The average value (x = 0.97) of gingival bleeding in patients from the first group was significantly higher than value(x = 0.37) of gingival bleeding in the second group.

CONCLUSION: Patients whose therapy was helped by a sub-dose doxycycline demonstrated positive therapeutic effects on gingival inflammation and bleeding.

Introduction

Treatment of periodontal disease includes removing of dental plaque and other irritating factors (conservative treatment) or performing surgical methods, which sometimes include the use of antibiotic therapy administered systemically or locally [1, 2]. The administration of local and systemic antibiotics, anti-inflammatory drugs, or sub-antimicrobial small doses of doxycycline applied as adjuvants have the opportunity to provide additional positive therapeutic effects [3, 4]. Sub-antimicrobial dose of doxycycline is 20 mg dose of the antibiotic applied twice daily in the treatment of periodontal disease. It is believed that a dose of 20 mg provides quite effective inhibition of enzymes, cytokines and osteoclasts than any antibiotic that would be applied in full or normal dose. On the other hand research studies have not spotted noticeable changes in the oral flora or bacterial flora in other organs in the individual's body because of which they identified as extremely recommended dosage which is a serious clinical utility when applied only in addition to periodontal pockets debridement [3]. For the clinician, this is particularly favorable moment, which enables broad applicability.

Well known fact is that periodontal disease is

a disease known as infection of the periodontal tissue complex. The primary initiation belongs to the bacteria which alter local immune response and result in appropriate tissue destruction. Due to the infectious nature of the disease the main aim of treatment should be suppression of the effective bacterial populations responsible for the dissolution of the periodontal tissues [5, 6]. Recent findings show that the local causal therapy conducted in the affected periodontium is not always sufficient to eliminate bacteria, so under certain conditions in the overall therapeutic treatment, systemic treatment with antibiotics may be included [6-8].

Bacterial plaque is a major factor for the initiation of chronic periodontitis (CP), but characteristic clinical signs (periodontal pocket depth, epithelial apical migration and alveolar bone destruction) primarily are a result of disrupted and insufficient immune and defense mechanisms of the host [9]. The success of the results varies between individuals and depends on the production and the level of proinflammatory mediators, enzymes and cytokines in all development stages of the disease [10].

The key between those two extremes are matrix metalloproteinasis (MMPs), a family of proteolytic enzymes released by macrophages, tissue fibroblasts, epithelial cells and neutrophils (PMNs). Damage of the collagen, the major structural protein in periodontium has his part in the destruction of the epithelial attachment and bone resorption [10]. LDH which helps the destructive and resorptive processes in periodontal tissue complex joins on these pathogenetic mechanisms of chronic periodontitis.

Considering these facts we have set the goal of this paper: to compare the therapeutic effects of the application of doxycycline - from the full dose (100 mg) and sub-dose (20 mg) in the treatment of periodontal disease.

Material and Methods

The choice of patients that are part of the study is made by chance, and is based on a personal position on each of them. Of those who voluntarily showed a wish to be part of the study, is provided written consent for joining in the investigation. In the selection of patients are taken into consideration the criteria for inclusion and exclusion of the study, according to the protocol approved by the Ethic Commission of Faculty of Dentistry at UKIM in Skopje.

The study was performed of 60 patients in age from 30-70 years with clinically manifested mild form of chronic periodontitis where depth of periodontal pockets on certain representative group of teeth was estimated at 3-5 mm. The diagnosis of each individual of the study group was set according collected anamnestic data which also includes dental and medical history of the patients, clinical examinations and analysis the X-ray image.

The selection of patients is made at the Clinic of oral pathology and periodonthology, at the University of Dental Clinical Centre in Skopje. As additional criterias are taken into account were the criteria for inclusion and exclusion of the study:

Criteria for inclusion: patients with periodontal disease which manifest the periodontal pockets depth of 3-5 mm; patients who in the last two months are not receiving drugs from any group of antibiotics; patients with at least three teeth in one side of the jaw; and patients who have at least 12 teeth in the mouth.

Criteria for exclusion: patients receiving systemic antimicrobial drugs; patients receiving non-steroidal anti-inflammatory drugs; patients with diagnosed any cardiac, renal or acute infectious illness; patients in whom not conducted curettage in periodontal pockets in the last 3 months; smokers, alcoholics, pregnant women, patients with brest cancers; patients allergic to antibiotics; patients with poor oral hygiene; patients who are using any herbal drugs and extracts.

Patients are divided into two groups (total n = 60). Group A is treated with antimicrobial dose of 100 mg doxycicline capsule (30 patients) once daily in progress in 30 days, and in group B with the same number of subjects where the ordinated dose were 2 x 20 mg doxycicline capsule daily for 75 days.

In all subjects, after the diagnostics and determining the condition, there were conducted conservative treatments which mean removal of dental calculus, soft deposites, and dental plaque and other iritative factors, scaling and root planing. After that, there was administrated doxycycline in the appropriate dose (depending on the group to which it is intended).

The total dose that the patients received in the course of therapeutic treatment without distinction of dosage and dynamics of applications was 3 grams. The first group has the systemic administration of doxycycline 100 mg. once daily, and total planned dose received within 30 days. The second group of patients who received doxycycline twice by 20 mg daily (in the morning and evening) ended this therapy after 75 days, also in total dose of 3 g.

Patients were conducted on following clinical research: Index of dental plaque, according to Löe-Sillness; Index on gingival inflammation and gingival bleeding according Cowell.

The index of dental plaque, according to Löe-Sillness was registered with visual method through coloring with methylene blue, according to which was the numbered index which belongs to.

The index of gingival inflammation and gingival bleeding index according Cowell were measured separately.

The inflammation was determined with inspection, and for gingival bleeding was applied the method of sonding, too.

The statistical data processing was performed in a statistical program STATISTICA 7.1. The differences in the values of the dental plaque, gingival inflammation, gingival bleeding, periodontal pockets depth, clinical attachment level, overview on 10th day, 20th day, 1 month and after 75 days, were tested with Friedman ANOVA test (ANOVA Chi Sqr.)

The differences in the values of the dental plaque, gingival inflammation and gingival bleeding between the two subgroups of patients (patients who received doxycicline 2 x 20 mg daily 2.5 months and patients who received doxycicline 100 mg daily for 30 days) were tested by Mann-Whitney U test (Z).

Results

Differences in values of dental plaque in the relation: first examination, 10th day, 20th day, 1 month and 2.5 months, as a result of the applied therapy in both groups are shown on Table 1. At the first examination for Z = 0.00 and p < 0.05 (p = 0.99) there was no significant difference in the average value of the dental plaque in both groups.

After 10 days from the applied therapy the average value of dental plaque (x = 0.97) in patients from the second group (doxycycline 2 x 20 mg/2.5 months) for Z = -3.15 and p < 0.01 (p = 0.002) was significantly higher than the average value of the in dental plaque (x = 0.37) in patients from the first group (doxycycline100 mg/30 days).

After 20 days from the applied therapy the average value (x = 0.43) on dental plaque in patients from the second group (doxycycline 2 x 20 mg/2.5 months) for Z = 0.22 and p> 0.05 (p = 0.82) is only slightly lower than the average value (x = 0.47) of dental plaque in patients from the first group (100 mg doxycycline/30 days).

After 1 month of the applied therapy the average value (x = 0.33) of dental plaque in patients from the second group (doxycycline 2 x 20 mg/2.5 months) for Z = -0.44 and p > 0.05 (p = 0.66) was only slightly higher than the average value of the (x = 0.27) in dental plaque in patients from the first group (doxycycline100 mg/30 days).

After 2.5 months, the average value (x = 0.83) of dental plaque in patients of the second group (doxycycline 2 x 20 mg/2.5 months) for Z = 0.63 and p < 0.05 (p = 0.53) unsignificantly is less than the average value (x = 0.93) of dental plaque in patients from the group A (doxycycline 100 mg/30 days) (Table 1).

Table 1: The difference between the investigated groups according to the received value of dental plaque in all investigated time intervals

Dental plaque	Rank Sum 1 x 100 mg	Rank Sum 2 x 20 mg	U	Z	p-level	Valid N 1 x 100 mg	Valid N 2 x 20 mg
First examin	914.00	915.00	450.00	0.00	0.99	30	30
10th day	702.00	1128.00	237.00	-3.15	**0.002**	30	30
20 th day	930.00	900.00	435.00	0.22	0.82	30	30
1 month	885.00	945.00	420.00	-0.44	0.66	30	30
2.5 months	957.50	872.50	407.50	0.63	0.53	30	30

Mean differences between index of gingival inflammation in relation with first check up, 10th day, 20th day, one month and 2.5 month later, as a result of applied therapy in both examined groups, are shown in Table 2.

On the first check up mean value (x = 1.57) of gingival inflammation was slightly greater than the average value in patient from second group x = 1.47), Z = 0.41 and p > 0.05 (p = 0.68).

Ten days after applied therapy average values of gingival inflammation in patient from the first group (x = 0.83) was significantly greater than the average values in second group (x = 0.40), Z = 2.62 and p < 0.01 (p = 0.009).

Twenty days after applied therapy there are not significantly differences in the average values of index of gingival inflammation between examined groups, Z = 0.00 and p > 0.05 (p = 0.99).

One month after applied therapy there are not significantly differences in the average values of index of gingival inflammation between examined groups, Z = 0.00 and p > 0.05 (p = 0.99).

On the check up two and a half months after applied therapy average values conducted in patients from second group were significantly smaller than the consequently values in patient from the first group, Z = 2.22 and p > 0.05 (p = 0.03) (Table 2).

Table 2: Difference between examined groups according to the obtained values of index of gingival inflamation between all tested intervals

Gingival inflamation	Rank Sum 100 mg	Rank Sum 2 x 20 mg	U	Z	p-level	Valid N 100 mg	Valid N 2 x 20 mg
First examin	942.50	887.50	422.50	0.41	0.68	30	30
10th day	1092.00	738.00	273.00	2.62	**0.009**	30	30
20th day	915.00	914.00	450.00	0.00	0.99	30	30
1 month	914.00	915.00	450.00	0.00	0.99	30	30
2.5 months	1065.00	765.00	300.00	2.22	**0.06**	30	30

Mean differences between index of bleeding of probing BOP in relation with first check up, 10th day, 20th day, one month and 2.5 month later, as a result of applied therapy in both examined groups, are shown in Table 3.

On the first check up there are not any

significant differences between two examined groups, Z = 0.00 and p > 0.05 (p = 0.99).

Then days after applied therapy average values on BOP index was slightly greater in patients which consisted second group, but without significantly differences between both examined groups, Z = 1.12 and p > 0.05 (p = 0.26).

Twenty days and one month after applied therapy there are not any significant differences between two examined groups, Z = 0.00 and p > 0.05 (p = 0.99).

On the check up two and a half months after applied therapy average values conducted in patients from second group were significantly smaller than the consequently values in patient from the first group, Z = -3.49 and p < 0.001 (p = 0.000) (Table 3).

Table 3: Difference between examined groups according to the obtained values of BOP in all tested intervals

Gingival bleeding	Rank Sum 2 x 20 mg	Rank Sum 1 x 100 mg	U	Z	p-level	Valid N 100 mg	Valid N 2 x 20 mg
First examin	914.00	915.00	450.00	0.00	0.99	30	30
10th day	991.00	839.00	374.00	1.12	0.26	30	30
20th day	915.00	914.00	450.00	0.00	0.99	30	30
1 month	914.00	915.00	450.00	0.00	0.99	30	30
2.5 months	679.00	1151.00	214.00	-3.49	**0.000**	30	30

Discussion

Today there are a lot of antibiotics which may be ordained, locally or systemically [11]. Tetracyclines are one of the antibiotics that have a broad spectrum of activity and are able to inhibit the anaerobic microorganisms. The literature shows that it is possible to happen in a very short period, usually 5 days [12]. It has been proven that doxycycline is the most common antibiotic that belongs to the group of antimicrobials that are active against most periopatogens due to low minimum inhibitory concentration (MIC) [13].

In our study are compared clinical effects in patients with chronic periodontitis treated with doxycycline 100 mg. once a day for one month and 20 mg doxycycline twice daily in period of 2.5 months.

It is evident from the study, that despite the first clinical examination in patients from both groups, all index values recorded improved clinical findings whether it is a dose of 100 mg or 40 mg daily.

Descriptive statistics of index of dental plaque according to Löe-Sillness at patients undergoing systemic application of doxycycline 100 mg once daily for 30 days, showed variation and systematic reduction from the first examination to period of 75 days. The differences between the values of dental plaque in relation to first examination, 10th, 20th day, 1 month and 2.5 months for ANOVA Chi Sqr. = 81.00 and p < 0.001 (p = 0.000) there is significant difference between the values of dental plaque in analyzed relation. We get almost identical findings with gingival inflammation and gingival bleeding.

The average value of gingival bleeding (x = 0.93) after 2.5 months Z = 3.30 and p <0.001 (n = 0.000) is significantly lower than the average value of gingival bleeding (x = 1.53) at the first examination.

From the obtained findings it is quite certain that improved clinical effects were obtained after applying doxycycline in dose 2 x 20 mg. Differences in values of dental plaque in relation first examination, 10th, 20th day, 1 month and 2.5 months, as a result of the applied therapy in both groups shows that after 2.5 months average value of dental plaque (x = 0.83) in patients in the second group (2 x 20 mg doxycycline/2.5 months) for Z = 0.63 and p > 0.05 (p = 0.53) is slightly lower than the average value (x = 0.93) of dental plaque of patients in the first group (doxycycline 100 mg/30 days). Evaluation of all remaining clinical parameters showed definite advantages in the group treated with sub-dose 2 x 20 mg doxycycline. After 2.5 months average value (x = 0.17) of gingival inflammation in patients in the second group (2 x 20 mg.doxycycline/2.5 months) for Z = 2.22 and p > 0.05 (p = 0.03) was significantly lower than the average value (x = 0.50) of gingival inflammation in patients from the first group (doxycycline 100 mg/30 days).

In terms of index of gingival bleeding, after 2.5 months, average value of gingival bleeding (x = 0.97) at patients in the first group (doxycycline 1 x 100 mg daily/2.5 months) and Z = -3.49, p < 0.001 (n = 0.000) was significantly greater than the average value of gingival bleeding (x = 0.37) at patients in the second group (doxycycline 2 x 20 mg/30 days). The rationale for choosing the application of doxycycline in addition to conventionally applied method is supported by its ability to achieve higher concentrations in gingival cervical fluid, and the possibility of modulating the clinical effect on the host. Doxycycline inhibits matrix metalloproteinase's, which mediates in reduced opportunities for periodontal breakdown, which first manifests itself in reduced gingival inflammation and reduced gingival bleeding [14]. Some studies have failed to find a positive therapeutic effect of doxycycline doxycycline [15-17].

Although in the literature exists quite conflicting results, tetracycline and its related drugs are known to participate in the activation of latent pro-MMP, MMP de-escalation, and neutralizing oxidative mechanisms responsible for periodontal tissue destruction]18, 19]. This evidence provided the basis for a new therapeutic approach to control periodontal disease not only in healthy people, but also in those with compromised medical health, especially among patients with diabetes mellitus for the use of tetracycline and their derivatives in the treatment of periodontal disease [20[. Our findings are consistent with the findings of many authors [4, 21-25].

In this regard, we support the notion that systemic antibiotics administered as adjunctive therapy in sub- antimicrobial dose, may offer an additional advantage over the conducted conventional therapy. In this context primarily refers to the clinical loss of attachment and periodontal pocket depth. However, differences in study methodology and lack of certain facts can be a serious handicap for comprehensive analysis. It was difficult to extract definitive conclusions from the study, although patients with deep pockets, progressive or "active form" of the disease or specific microbial profile, can be beneficial after this additional treatment [26]. In contrast to these findings [27] concluded that the evidence for the additional benefit of adjuvant therapy with antibiotics in patients with chronic periodontitis is insufficient and unconvincing. Based on the obtained findings of the survey we can conclude that in patients whose therapy was supported by a sub-dose doxycycline 2 x 20 mg demonstrated positive clinical therapeutic effects on gingival inflammation and gingival bleeding.

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Stomatology

The Effects of NBF Gingival Gel Application in the Treatment of the Erosive Lichen Planus: Case Report

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Abstract

The therapy of erosive lichen planus (ELP) has been particular problem in the treatment of oral lesions. This case of ELP in male patient 29 years old was treated with topic application of the NBF gingival gel, three times a day after meal, previously rinsed with Clorhexidine gluconate 0.12%. After 5 days of treatment, initial improvements were recorded, and after two weeks of application of the NBF gingival gel we observed significant improvement. Clinical monitoring after the fifth day showed mild epithelialization of the eroded mucosa, yet still present erythematous base of the lesion. After the second week the erythema area was significantly reduced and the eroded surfaces of the mucosa were minimal, measured less than 0.5 mm. After the third week there were no erosions to detect on the oral mucosa, yet still present vague redness, which completely pulled after the fourth week. Treatment ended after the fifth week when the topical application of the NBF gingival gel was terminated, and therapy was done, and clinically achieved effects remained stable even after the third month of the treatment. Topic application of the NBF gingival gel with ELP patients showed positive clinical effects in relatively short time period.

Introduction

Oral lichen planus (OLP) is a chronic disease, with unknown etiology, where the autoimmune mechanisms take a particular place. It is believed that the increased production on free radicals (ROS), may play an important role in the pathogenesis of various skin diseases, such as atopic dermatitis, psoriasis, vitiligo [1], and lichen planus, where the presence of oxidative stress is recently been proved [2]. Actually, the oxidative stress is caused between theimbalance in production of reactive free radicals (ROS) and the organism's ability to neutralize reactive intermediaries, which are the consequence of the caused impairment [3]. Created lesions with potentially-present comparative DNA, result of the oxidative stress, can contribute to the development of the malignancy in the oropharyngeal area [4, 5]. It happens at lichen planus atrophic, bullous and erosive type, but usually, not at reticular type.

If we are called on the present oxidative stress, it is completely logical, that the disease will be treated with topical antioxidants, which on some level are our therapeutic experience.

Although the findings suggest that corticosteroids are the number one cure of ELP even when they are applied topically, though it is likely that certain patients can not apply them because of the

numerous long-term effects. From this follows the need for new preparations to treat ELP.

In the last years, in the treatment of oral lesions advantages belong to the natural products containing propolys. This bee product is a rigid resin containing wax and herbal extracts used in the prevention of oral disease and has no side effects [6, 7]. Besides numerous anti-inflammatory properties [8] also it has antioxidants [9].

Antioxidants control oxidative stress in wound healing and there is a belief that it can accelerate the process of healing. Antioxidant therapy is in its beginning and is the most modern approach in the treatment of oral lesions. Propolys can be a component of tooth paste, water for washing, and gels and it can be easily applied to the oral cavity [10].

In dentistry it is widely used, and the findings are the subject of several studies. It is used for reducing dentinal hypersensitivity [11], prevents caries [12], affects the improvement of oral mucositis [13], and it was found that it could be useful for oral cancer [14].

Products based on nanotechnology, which contain antioxidants, may be excellent adjuvants in the healing of lesions. The only such product in the world is NBF gingival gel, which contains propolys, vitamin C and vitamin E in form of nanoemulsion.

This paper presents the case of oral erosive lichen planus localized in the buccal mucosa in a young male patient, which is topically applied NBF gingival gel and monitored for three months.

Case Report

Male patient, 29 year old, was accepted to the University Dental Clinic Center ,,St. Pantelejmon" in Skopje, at the Department for oral medicine and periodontology, with basically complaints of discomfort, a sense of burning pain that intensifies during eating. Patient complains on serious problems in maintaining everyday oral hygiene.

Clinical investigating of buccal mucosa was presented with erosions superficial ulceration, erythematous background, Wickham stretch marks and solitary papules. Changes were localized unilaterally in retro molar region easily extended to occlusal line with dimensions of 1 mm in width and 1.5 mm in length on buccal mucosa on the right side (Fig. 1).

Clinical findings and history of the disease in addition of lichen planus were suspicious, so we applied additional criteria. For the definition of the diagnosis were implemented following procedures: biopsy for histopathological verification and direct immunofluorescence. Histopathological researches of buccal mucosa confirmed: canceled epithelium, degenerative changes in basal layer, and the individual basal cells atrophic-term changes.

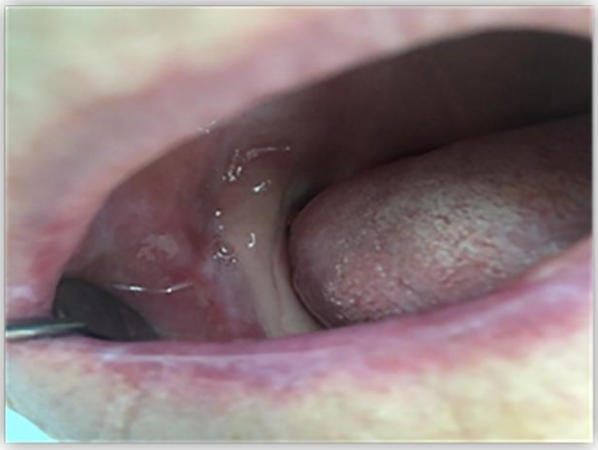

Figure 1: Erosive lichen planus in the retro molar right buccal mucosa

The surface of the epithelium was canceled with signs of erosion or deeper ulcers, around which was presented expressed cellular infiltration. In papilar layer was recorded intense lympho-histiocytes infiltration, which extend in strips. The present infiltrate has touched the epithelium and destroy the relation among the epithelium and corium (Fig. 2).

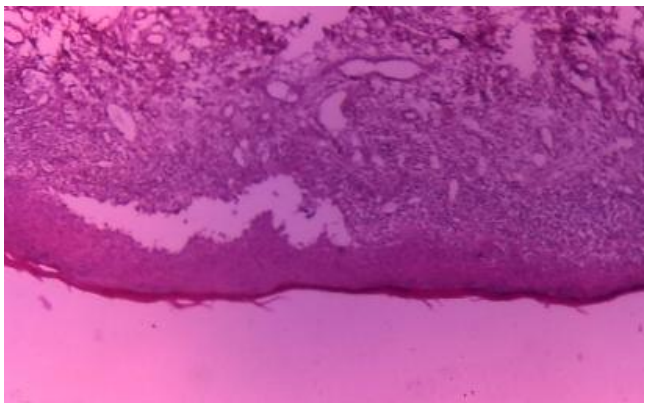

Figure 2: Histopathological findings at erosive lichen planus

The direct immunofluorescence displayed an easy deposit of IgG and C3 component of complement at length on epithelial basement membrane (Figure 3 and Figure 4).

After 3 months of tissue biopsy, buccal mucosa resulted with completely epithelialization. After 2 months, and fully satisfactory findings, patient came again with the same subjective complaints as 5 month earlier and clinical findings presented with erosion which lie on the erythema surface of right retromollar area of buccal mucosa. Patient suggests to begin with topical use of the NBF gingival gel three

times a day after eating (after they get a check, manually check dinners), with previous rinsing with Clorhexidine gluconate 0.12 %.

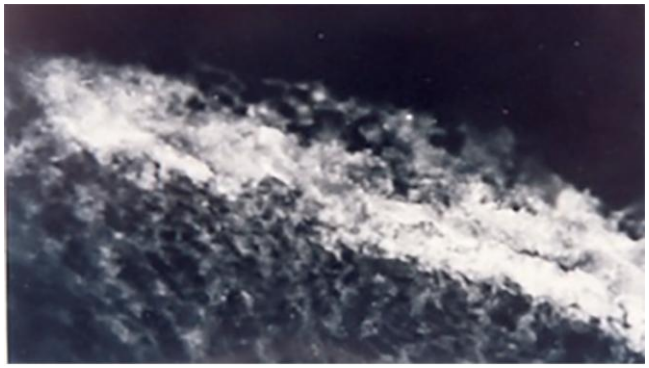

Figure 3: Deposits of immunoglobulin G by the length of the epithelial basement membrane and superficial layer of the lamina propria at oral lichen planus

The patient was suggested not to receive food or drinks in the next 20 minutes, and gel to be protected by sterile gauze. Also we administered Nystatin, 4 times per 25 drops orally, due to prevention of candidiasis.

Figure 4: Deposits of immunoglobulin C3 by the length of the epithelial basement membrane and superficial layer of the lamina propria at oral lichen planus

Systemic application of corticosteroids at this moment was avoided (Figure 5).

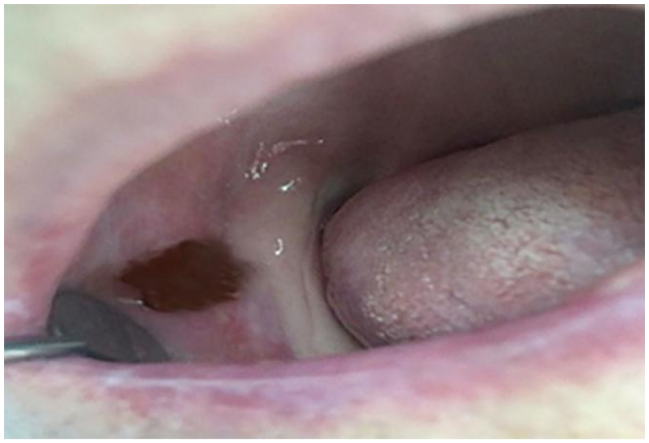

Figure 5: Application of the NBF gingival gel at the site of the buccal mucosae lesion

The patient was followed continuously from the beginning until the end of the treatment.

The first three weeks controls were conducted three times a week, and after the fourth week, once a week in the following three months.

After 5 days of treatment, initial improvements were recorded, and after two weeks of application of the NBF gingival gel we observed significant improvement. Clinical monitoring after the fifth day showed mild epithelialization of the eroded mucosa, yet still present erythematous base of the lesion.

After the second week the erythema area was significantly reduced and the eroded surfaces of the mucosa were minimal, measured less than 0.5 mm.

After the third week there were no lesions to detect on the oral mucosa, yet still present slight redness, which completely pulled after the fourth week. Treatment ended after the fifth week when the topical application of the NBF gingival gel was terminated, and therapy was done (Figure 6).

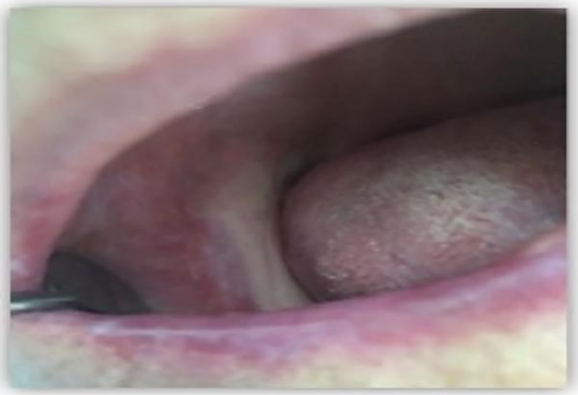

Figure 6: Healed mucosal lesion after third week of therapy with NLB gingival gel

The results remained unchanged after the third month. Patients had no symptoms, and the clinical findings were satisfactory with no longer presence of erosions or erythema. Only present were Wickham stretch marks as whitish strings in stripe layout.

Discussion

The main cause in the therapy of ELP is complete withdrawal of patients' painful symptoms, epithelialization of the eroded mucosal lesions and oral carcinoma prevention. There are various therapeutic procedures described and implemented in practice till this day including topical medications, steroid ointments, ultraviolet rays (PUVA) [15] and laser [16]. However, the era of new medications

increasingly conquers the world, and is also applied in the treatment of the oral mucosal lesions, including ELP. Various drugs are used either as topically applied or systemically applied medications [17].

In this field in the last 10 years there has been considerable development in the nanotechnology. Nanotechnology brought mankind in completely new period of its development where we have new therapeutic options in which central spot belongs to the nano materials. They contain particles that can be resorbed quickly and transported to the eroded tissues, where they stimulate healing, which is result of numerous biochemical and cellular interactions [18]. In the recovery process priority belongs to the antioxidants. With their use, the oxidative stress in the wound healing process is effectively controlled and probably due to them the whole healing can be accelerated. For now, the use of antioxidant therapy in the treatment of ELP is still in its infancy and is the newest and most modern therapeutic approach.

The products which are based on nanotechnology and contain antioxidants can be used as excellent adjuvants in the healing of oral mucosal lesions. Such product in the world is NBF gingival gel, containing vitamin C, vitamin E and propolis in form of nano emulsion.

In this case report where recovery process has been followed there had been significant solid therapeutic effects after topical application of the NBF gingival gel. Reduced painful symptomatology, facilitated mastication and complete epithelialization in period of four weeks are accounted as quite satisfactory findings. Easily accessible lesions can be treated by applying adherent pastes. They allow long-term retention of the paste on the site of the lesion, easy and precise control of the therapeutic effects of its application [19].

The main advantages of the topical application is that it provides reduced side effects, efficient delivery of small amounts of the drug in the affected area, without the possibility the drug to be lost in vain, distributed throughout the whole organism, simultaneously with maximizing in the affected area [20].

Essentially, pathological diagnosis of all parts of the body including those of the oral mucosa is the consequence of the oxidative stress which is an imbalance between the oxidants and antioxidants. This imbalance results in increased production of free radicals, including the reactive free radicals (ROS). They easily damage the oral cells, because the mucous membrane allows rapid absorption of the substances through their surfaces.

ROS and antioxidant imbalance can play leading role in the development of ELP [21, 22]. In fact, antioxidant defense mechanisms are very complex, and its debatable point is in the estimate amount and effects of the various antioxidants in vivo in assessment in the overall antioxidant status [21, 23].

Relevant data indicate that the role of the nutrients, especially of those so-called antioxidants as vitamin A, vitamin C and vitamin E is great significance in the pathogenesis of the ELP [24].

In the study of Abdolsamadi [25] level of anti-oxidants A, C and E in the saliva were significant lower in patients with OLP than healthy control group. The possible protective effects of vitamins in reducing the risk of OLP may be related to anti-oxidant role in eliminating free radical damage and single oxygen. The fact that antioxidant vitamins are effective against oxidative stress may explain the significant difference in oral mucosa [26, 27].

NBF gingival gel is high functional nano-bio fusion gel, created for the first time, with a new technology named nano-bio fusion. The gel contains three bio-compatible nano-emulsion contents (Vitamin C, Vitamin E, and Propolis extract) which have antibacterial, ant inflammatory and anti-oxidative effect. Vitamin C and Vitamin E have power anti-oxidant effect and Propolis which content flavonoids.

According to well-known effect of anti-oxidants, in this case report we used nano emulsion in treatment of ELP at different periods of time. The obtained results were satisfied.

The first improvement was evident after the fifth day with less epithelization. After two weeks, it was evident more epithelization with erithema reduction. Re-epithelization process reaches a maximum after three weeks, without erithema. We believe that all effects are due to Vitamin C, Vitamin E, and Propolis.

Vitamin C is known to activate phagocytes, and have the primary role in organism defense [28].

It‘s a co-factor for about eight enzymatic reaction in the synthesis of collagen. Vitamin C is shown to accelerate gingival wound healing in experimental animals, preventing the bleeding [29, 30]. The molecule of nano Vitamin C that is present in the gel, is 2 times smaller, and 110 times more potent in the synthesis of collagen, than vitamin C on its own. So, its elimination can be realized faster, easier, and more efficient.

Anti-oxidant property of propolis was evaluated by Krol [31], biocompatibility by Bankova [32], and antibacterial, antifungal, ant oxidative and anti-inflammatory properties by Sforcin [33].

One of the important components which contribute to the achieved result following topical application of NBF gingival gel is adhesiveness. Its constitutive component – propolis ensures very good adhesion, while the nanoemulsion itself has a much smaller surface tension due to the small dimensions of the vitamin molecules, being the reason for the

instantaneously rapid absorption through the mucose in the target cells. Furthermore, the main component of the propolis - flavonoids adds to the antibacterial, antifungal, antiviral, anti-oxidative and anti-inflammatory properties.

Without limitations, and within the findings of this case report, it can be concluded that topic application of NBF gingival gel at ELP, showed positive clinical effects in a relatively short period of time, thus avoiding application of systematic or topical steroids due to their numerous adverse effects.

NBF gingival gel, with its own therapeutic modalities,allows us to recommended in the treatment of OLP.

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Stomatology

3D Finite Element Study on: Bar Splinted Implants Supporting Partial Denture in the Reconstructed Mandible

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Key words: Finite element method; Stress Distribution; Bar splinted implants; Reconstructed Mandible; Partial Denture.

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Abstract

AIM: This study aimed to estimate the stress patterns induced by the masticatory loads on a removable prosthesis supported and retained by bar splinted implants placed in the reconstructed mandible with two different clip materials and without clip, in the fibula-jaw bone and prosthesis using finite element analysis.

METHODS: Two 3D finite element models were constructed, that models components were modeled on commercial CAD/CAM software then assembled into finite element package. Vertical loads were applied simulating the masticatory forces unilaterally in the resected site and bilaterally in the central fossa of the lower first molar as 100N (tension and compression). Analysis was based on the assumption full osseointegration between different types of bones, and between implants and fibula while fixing the top surface of the TMJ in place.

RESULTS: The metallic bar connecting the three implants is insensitive to the clips material. Its supporting implants showed typical behavior with maximum stress values at the neck region. Fibula and jaw bone showed stresses within physiologic, while clips material effect seems to be very small due to its relatively small size.

CONCLUSION: Switching loading force direction from tensile to compression did-not change the stresses and deformations distribution, but reversed their sign from positive to negative.

Introduction

Mandibular defect is the defect affecting the mandibular integrity following surgical removal of oral neoplasm or trauma. It is debilitating and causes a significant impact on the patient's quality of life. This is because segmental resection of the mandible leads to significant patient morbidity [1, 2]. It is in the form of loss of mandibular support to the teeth, tongue and lip. In turn, this leads to dysfunctional mastication, swallowing, speech, impaired airway protection and oral incompetence [3, 4].

Patients also suffer from disfigurement following segmental mandibulectomy because the mandible is an aesthetic landmark. The degree to which dysfunction and disfigurement occur depends on both the location of mandibular segment removed and the amount of soft tissue excised. Therefore, the overall goal of mandibular reconstruction is to restore the patient's aesthetic deformity and functional loss that occur with this defect [5, 6].

Mandibular reconstruction and oral rehabilitation pose many challenges to the surgeon to restore function and esthetics. Many orofacial reconstruction strategies have been developed to provide an ideal bony reconstruction for later dental rehabilitation aiming to restore function and appearance to resemble the normal condition as closely as possible [5]. Wide variety of techniques and materials has been used to repair defects resulting from mandibular resection. In 1990, the use of bone grafting for mandibular defect repair was first described; where, all of these techniques had some

degree of success but none was reliable enough to be routinely used [7].

Advances in microvascular surgical reconstruction techniques and the dental use of vascularized free bone grafts have broadened the possibilities for oral restoration for these patients, offering the ability to restore the hard and soft tissue orofacial defects and providing the suitable foundation for osseointegrated implant placement [8].

The fibula graft is reported to be preferred over other vascularized grafts as it is a stable one, due to its high cortical content which has as well high percentage of bone morphogenic proteins that osteoinductively promote the bone healing process [9, 10]. Fibula graft can be used to bridge gaps up to 25cm in length, as it is a long bone and has high mechanical resistance to pressure and torsion. Moreover, the fibula flap can be easily harvested with convenient sized blood vessels for anastomoses allowing rapid healing of vital flap. Implants inserted into the fibula grafts were studied and there had been no significant reduction of the success rate when compared to implants inserted into healthy mandibular bone [11].

On the other hand, the fibula free flap is limited in vertical height that is challenging for the final oral rehabilitation; either creating a significant step at the graft–mandible junction challenging placement of dental implants posing more stresses and unfavourable bending moment delivered to such implants or the fibula is aligned at the level of the alveolar crest thus not restoring the contour of the lower border of the mandible jeoparadizing aesthetic result [12]. The "double barrel" technique was described by Bahr in 1998 to overcome such problem in which a long fibular flap is halved and folded onto itself to increase the height of the "neomandible" [13].

Implant tooth supported partial overdenture are preferred over fixed options since the denture flange helps to improve the facial appearance, more posterior placement of the artificial teeth and better tongue management of food. Moreover, overdentures provide daily access for hygiene maintenance of the implant abutments to minimize periimplant soft tissue problems [14]. Dental implants used with implant supported partial overdentures improve retention, and stability where, masticatory performance is restored to presurgical levels, compared to conventional tissue borne ones particularly on the defect side [15, 16]. Compromise between retention, the need for stress distribution and maintenance of bone around the implants is a major factor affecting attachment selection [17].

Different attachment designs in maxillofacial prosthesis as splinted implants using resilient bar with clips, ERA, O-ring and OSO attachments are available. Although the bar with O-ring attachments resulted in more favourable stress distribution than either the bar-clip or the bar-ERA deign, the O-ring design was not as retentive as the other attachment systems used [17]. Bar-splinted dental implants supporting the overlay prosthesis allow better stress distribution and less prosthetic maintenance in comparison to non splinted implants. The incorporation of a clip with such prosthesis will improve its retentive quality. Clip attachments may be plastic or metallic preferably gold. Plastic clips take up more space, more prone to be dislodged, suffer more wear and tear and offer less retention in comparison to gold ones [18].

Recently, the finite element method (FEM) has been widely applied to prosthetic dentistry to predict stress and strain distribution at periimplant region, investigating the influences of implant and prosthesis designs, the magnitude and direction of load, bone mechanical properties as well as modeling different clinical scenarios [19-21].

As the stress distribution is an important factor for bone resorption during rehabilitation, the attachment system should present an adequate stress transfer to avoid bone resorption and improve treatment prognosis. Finite element analysis (FEA) was used to study the effect of different types of attachment systems on stress distribution; one study revealed that overdenture retained by unsplinted implants displayed stress concentration at both mesial and distal sides of the implants while for splinted implants stress concentration was observed at the distal side of the implant [22].

This study aimed to estimate the stress patterns induced by the splinted implants with bars prostheses and splinted implants with bars retained with plastic or metal (Gold) clips placed in the reconstructed mandible using FEA.

Material and Methods

A partially edentulous female patient having her mandible reconstructed with vascularized free fibula graft in the right segment of the mandible with the last standing tooth being the lower right canine was selected and a Cone Beam CT scanning of the patient was used to obtain an accurate geometric 3D model of the reconstructed mandible, Figure (1).

The construction of the printed reconstructed mandible model was divided into three steps; image acquisition of the 3D Cone Beam Computed Tomography scans-Imaging protocol of patient having mandible reconstructed with vascularized free fibula graft, construction of the 3D model using the Mimics software (Materialize Software Solutions, Leuven, Belgium. Version 10.01) through importing, threshold, mask creation and 3D reconstruction, and finally production of the printed model by the help of multi-jet

modeling (thermal material application with UV curing) rapid prototyping machine (Invision Si_2, 3D systems, Rock Hill, SC, USA, Present in the Central Metallurgical Research and Development Institute, Helwan, Egypt).

(a) (b)

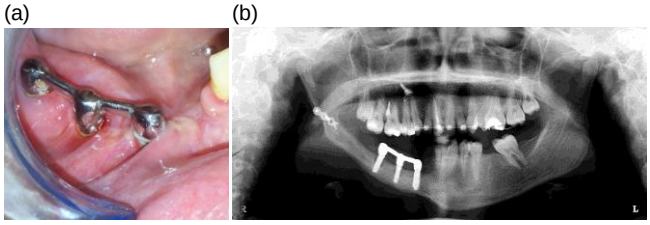

Figure 1: (a) Showing intraoral view of three implants splinted with bar inserted in the right side of partially edentulous reconstructed mandible (b) radiographic view

Three conventional implants (Osseo-Link, Global Implant Solutions, LLC, MA, USA) were inserted into the fibula on the reconstructed mandible in the premolar molar area; anterior and middle implants were 5mm in diameter and 11 mm in length while the posterior implant was 3.5 mm in diameter and 11 mm in length. The length of the edentulous area was measured and 1.5 mm was left from the last tooth. The implants were placed in the resultant space equidistant from each other, thus the implant neck coincides with the crest of the ridge as in Figure 2. Three abutments were secured to the implants and then shortened that the future occlusal plane of the artificial teeth coincides with that of the natural ones. The plastic bar and wax pattern for dome shaped copings covering the abutments were cast and secured in place.

(a) (b)

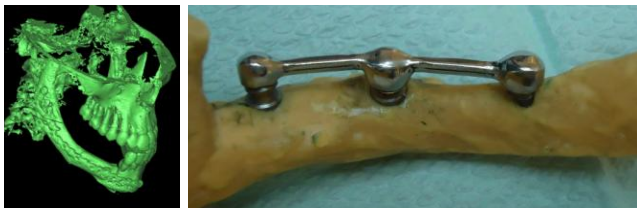

Figure 2: The printed model of the reconstructed mandible (a) bone masking, (b) final prototype

Rest seats for the double Aker clasp were prepared on the occlusal surface of the lower left first and second molars. Undercuts below the bar were blocked out using rubber base material. Mandibular impression was made in a stock tray using rubber base impression material for the fabrication of the partial overdenture which was then drawn. Clips were placed over the bar and relief of the fitting surface of the denture base was made for pick up of the clip.

In this study, two 3D FE models were constructed to simulate implant resection prosthesis placed in the reconstructed mandible supported and retained by bar splinted implants with and without clips. The printed model of the reconstructed mandible, three implants, bar, clips and mandibular partial overdenture were modeled in 3D as separate components (parts as; jaw bones, fibula, implant complex, bar, clips and overdenture).

Table 1: Number of Nodes and Elements

	Nodes	Elements
Fibula Cancellous Bone	23,742	110,487
Fibula Cortical Bone	4,611	14,339
3 x Implant-abutment Complex	41,108	212,828
Bar	5,123	20,475
2 x Clips	1,189	3,960
Overdenture	14,450	53,096
Mandible	14,219	65,187

Model components were modeled in 3D on commercial general purpose CAD/CAM software AutoDesk Inventor (Autodesk Inc., San Rafael, CA, USA, version 8.0). That each component was exported as SAT file before importing them into ANSYS (ANSYS Inc., Canonsburg, PA, USA).

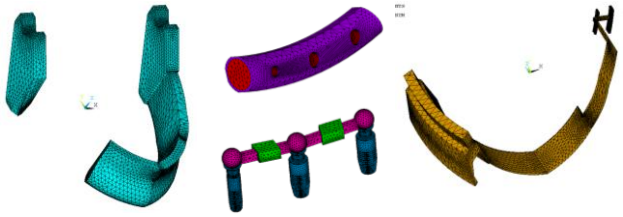

Figure 3: Screen shots of ANSYS screens showing all parts of the model separately

Set of Boolean operations were carried out to assemble all the model components before meshing. The meshing software was ANSYS version 12 and the used element in meshing all three-dimensional models is 10-node tetrahedral structural solid element (SOLID187), which has three degrees of freedom (translations in the global directions) [23]. Mesh density compromise is an important parameter that, affects the results accuracy. The final mesh density of all modeled components is tabulated in Table 1.

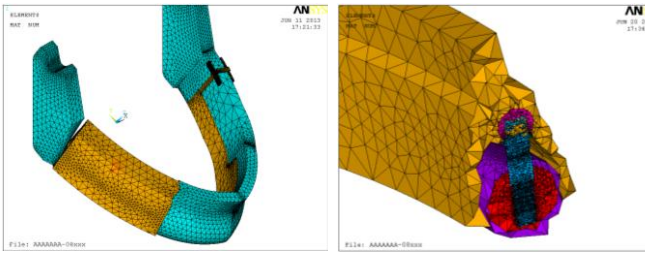

Figure 4: Assembled model and sectional cut showing fibula, supporting system, and overdenture. Overdenture; Coping; Implant; Fibula cortical; Fibula cancellous

Figures 3 and 4 show the meshed components of the model, where colors indicate different material properties. Where, all materials used in this model including alveolar bone, fibula, implants, bar, clips and overdenture were assumed to be isotropic, homogenous and linearly elastic. Modulus of elasticity and Poisson's ratio of each model component were fed to FEA package (listed in Table

2).

Table 2: Material properties

Material	Young's modulus [MPa]	Poisson's ratio
Fibula Cancellous Bone	700	0.20
Fibula Compact Bone	14,000	0.40
3 x Implant / Abutment complex (titanium)	110,000	0.33
Bar (chrome-cobalt alloy)	218,000	0.33
2 x Clips (plastic)	3,000	0.28
2 x Clips (gold)	97,000	0.33
Overdenture (Acrylic resin)	3,000	0.35
Mandible (weighted average of alveolar cancellous and compact bones)	4,450	0.30

Frictional contact between prosthesis, clips, and bar was defined by the elements CONTACT 174 and TARGET 170 as surface to surface contact [23]. In addition, the model was fixed in place at TMJ top surface as a boundary condition, while loads were applied in a vertical direction (tissue ward and tissue away) perpendicular to the occlusal surfaces, at the central fossa of the lower first molar for each model as 100N [24]. The solid modeling and linear static analysis FEA were performed on a personal computer Intel Pentium Core 2 Duo, processor 3.0 GHz, 4.0GB RAM.

Four runs were performed on the model two for compressive and tensile load with using two plastic clips. While in the third run a different clips material (Gold clip) was evaluated under tensile loading, and the last one was performed without clips under tensile loading.

Results

Finite element analysis resulted in a huge number of graphical illustrations of stresses, strains, and deformation distributions on each component in the studied model. Such results can be presented on the whole model and/or each component, showing color variation from dark blue (represent minimum value) to dark red (represent maximum value).

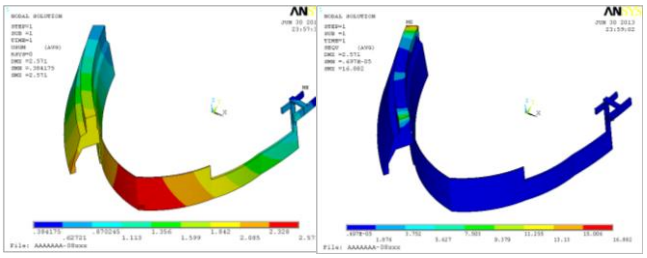

Figure 5: Sample of overdenture results, showing (left) total deformation and (right) Von Mises stress distribution under compressive loading of 100N

As illustrated in Figure 5, total deformation and Von Mises stress distribution on the overdenture under compressive loading of 100N. Maximum value of Von Mises stress appeared under the applied load, while the maximum value of deformation was found at lingual side (downward or upward) according to the applied load direction (compression or tensile respectively).

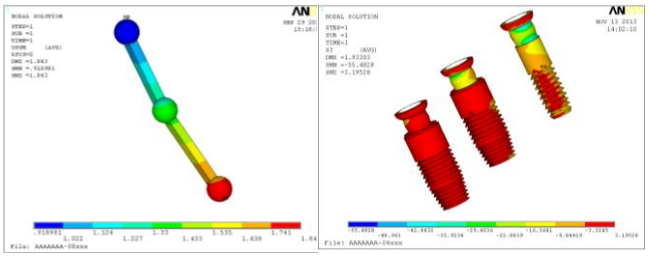

Figure 6: Using gold clips under tensile loading showed (left) Bar total deformation distributions (right) compressive stress distribution on the three implants

Harder clips material (gold) receives higher level of stresses than the softer one (plastic). While, the metallic bar connecting the three implants is insensitive to the clips material. In this study, typical implant complex behavior was obtained, that it showed maximum stresses at implant neck at the connection with cortical bone as in Figure 6-b. The first and second mesial implants away from TMJ showed higher stresses and deformations than the third distal implant.

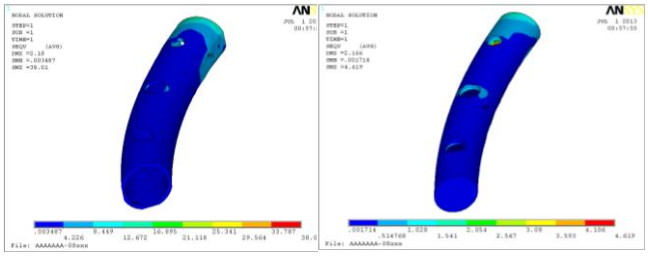

Figure 7: Fibula (left) Compact (right) cancellous bone Von Mises stress distribution under tensile loading

Fibula compact and cancellous bones behavior are presented as Von Mises stress distributions in Figure 7. Similar distribution can be obtained in compressive loading, but generally both showed Von Mises stress values within physiologic limits.

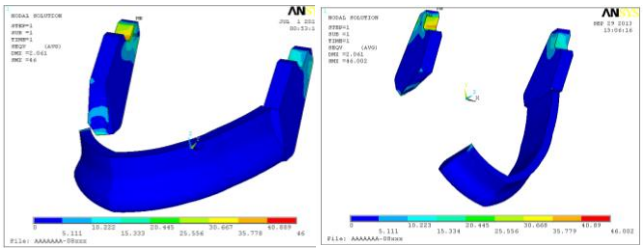

Figure 8: Jaw bone behavior under tensile loading with two clips materials (left) plastic as soft material and (right) gold as hard material

Table 3: Prosthesis behavior summary

	3 Implants		Bar		Clips		Over Denture		Fibula Cancellous		Fibula Compact	
Run	U_{sum}	S_{von}	U_{sum}	S_{von}	U_{sum}	S_{von}	U_{sum}	S_{von}	U_{sum}	S_{von}	U_{sum}	S_{von}
With plastic clip Compression	1.93	44.24	1.84	76.13	1.65	2.55	2.57	16.88	2.16	4.61	2.18	38.01
With plastic clip Tension	1.93	44.24	1.84	76.13	1.65	2.55	2.57	16.88	2.16	4.61	2.18	38.01
Without clip Tension	1.93	44.24	1.84	76.13			2.57	16.88	2.16	4.61	2.18	38.01
With gold clip Tension	1.93	44.21	1.84	76.56	1.66	2.70	2.57	16.89	2.17	4.62	2.18	37.99

Where: U_{sum}: total deformation in mm
S_{von}: Von Mises stress in MPa

Finally, the mandible is insensitive to clips material as in Figure 8 and showed Von Mises stress values within physiologic limits in all case studies included within this research. Finally summary of most important results obtained in this study are tabulated in Table 3.

Discussion

FEA was used for studying different designs for prosthetic rehabilitation of reconstructed mandibles. That, the stress level on the bone graft can be determined and quantified in every portion of the reconstructed mandible in addition to evaluation of prosthetic components deformations by the help of such studies [24-27]**.**

In this study, the fibula free flap was utilized as bone graft due to long edentulous span that offers extensive periosteal blood supply and multiple osteotomies, to be made helping to precisely adapt the bone flap to the recipient site and to replicate the contour of the resected mandible [28]. Furthermore, the success rate of implants inserted into the fibula grafts shows no significant difference from those placed into healthy mandibular bone [29, 30].

Three implants were used for better stress distribution, where the anterior implant was placed 1.5mm from the lower right canine to provide support near that terminal tooth thus preventing excessive stresses to be delivered to it. Furthermore, this anterior implant acts as terminal rest for the lingual plate major connector instead of placing a terminal canine rest on the lower right canine.

The remaining space of the edentulous span after the anterior implant was divided for equidistant placement of the implants helping with more favourable anteroposterior distribution of force. Bar helps with wide anteroposterior distribution of forces, provides even support over a great surface area, thus helping to reduce the load on the soft tissues. Bar/Clip attachment helps with abutments splinting, offers high retention capacity and minimizes prosthesis movement during function. Two clips were placed one over each segment of the bar.

Overdenture overall volume and stiffness are negligibly affected by removing small volume to place the clips inside. Clips are usually made from plastic material which is too close to the overdenture resin material. That is why using clips or not is insignificant. Similarly, using harder clips material as gold has a negligible effect on overdenture behavior. Although there is a considerable difference in friction coefficient between, the plastic clips and gold clips, and the metallic bar the clips design do not depend on friction in fixing the overdenture on the metallic bar. Therefore gold clips are not recommended due to the high cost without obtaining effective difference.

On the other hand, plastic attachments housed in the prosthesis wear rapidly rendering such attachments becoming ineffective and leading to bone loss around the implant adjacent to the defect. Thus frequent replacement for such attachments is needed a factor that contributes to increased cost. Furthermore, Plastic clips take up more space, more prone to be dislodged, suffer more wear and tear and offer less retention in comparison to gold ones [31-33].

Fibula cancellous and compact bones, in addition to jaw bone are safe under the expected loads bilaterally in the central fossa of the first molar as 100N (tension and compression).

Unilateral load of 100 N was applied in tissueward and tissueaway direction to evaluate the stress pattern induced in the reconstructed mandible in the two models. The load was applied at the central fossa of the lower first molar in vertical direction mimicking the effect of load in centric occlusion [27].

If the assumption of full osseointegration between different types of bones, and between implants and fibula existing, the levels of generated stresses on bones will be far enough from endurance limit. Thus such system for mandible reconstruction is optimal, and it behaves well under the expected loads [18-21].

Within the limitations of the present study,

there was a stress concentration in the region of the grafted bone/mandible interface and at the region of the sigmoid notch, coronoid process and condylar neck on the reconstructed side as proven by a previous study [19]. This can be explained in the view of size discrepancy between grafted bone and native mandible. Thus, to obtain better fixation and healing after bone grafting and to reduce the influence of stresses, osteosynthesis plates or supplementary fixation in the inferior border of reconstructed mandible should be applied [19, 34]. Due to discontinuity of the stress line in the mandible reconstructed with the fibula, the stresses on the healthy side were less than on the defective side [19].

The principal stresses obtained were the same on comparing the absence and presence of the plastic clip attachment. This may be attributed to the similarity of clips/overdenture material in addition to the splinted implants that were assumed to be 100% osseointegrated with the surrounding bone; that these implants can withstand all the stresses within its physiologic limit regardless the use of the clip/bar attachment.

When the results were analyzed regarding the stress distribution in all loading situations, the highest stresses were concentrated in the bar followed by the cortical bone around the implant neck as proven in other studies [19, 20]. Similar conclusions of previous studies regarding the location of the maximum stresses on the cortical layer is closely related to the material properties assigned to bone; as the cancellous core has modulus of elasticity less than that of the cortical layer, the implants were only supported by cortical bone which would absorb most of the stresses, while the reaction forces of the cancellous bone upon the loaded implant would be underestimated [35, 36].

In the view of the mechanical principle stating that when two materials of different moduli are placed together with no intervening material and one is loaded, a stress contour will be observed where the two materials come into contact. These stress contours are of the greatest magnitude near the point of the first contact [37].

Regarding this principle, the importance of using the bar to splint the implants is observed, that the bar will bear the greatest stresses as demonstrated in the results. This prevents the greatest stresses to be developed around the implant neck and subsequent bone resorption; thus implant success rate is improved especially with the irregular bending patterns that arise during mastication due to asymmetric nature of reconstructed mandibles [37].

The forces resulting from occlusal loading is damaging to the implants, thus it is preferable to splint the implants with a rigid bar to direct most of the occlusal forces along the long axis of the implants. Additionally, as the modulus of elasticity of the plastic clip is similar to that of acrylic resin, the values of the stresses delivered to the reconstructed mandible did not change in the analysis of the two models with/without plastic clips.

Despite the great stresses developed in the reconstructed part and at its interface with the native mandible, it is worth to be mentioned that constant recurrence of increased stresses during the ossification process results in thickening of the reconstructed parts according to Wolff's law; which says that bone remodels itself to shapes that are suited to bear external stresses [38-41].

Within the limitations of this study, the following conclusions can be drawn:

1. Using clips or not has a negligible effect on all other parts of the studied model. In addition clips material rigidity did not influence the jaw bones, implant complex, and metallic bar.

2. Switching loading force from tensile to compressive did not change stresses and deformations distribution, but it reverses their sign from positive to negative and vice versa.

3. Fibula and jaw bones showed safe behavior with the assumption of full osseointegration between different types of bones, and between implants and fibula.

Ethical approval

This research was approved by Research Ethics Committee (REC) of Medical Faculty, Ain Shams University (FMASU R 33/2015, September 13, 2015).

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Public Health

Misuse of Psychologically Active Substances of Convicts being in Prisons and their Treatment

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Abstract

Due to the data of the Ministry of Justice of the Republic of Serbia, over 70% of persons being in prisons and serving their sentences are drug addicts, and 50% of them are drug users. In the European prisons, the percentage of persons using drugs in the entire prison population is 20-70%, and in the USA 70-80%, in the Australian prisons between 50 and 80% of the convicts in prisons are addicts of psychologically active substances. The results of our survey are pursuant to official statistics data for Serbia, the European countries, USA and Australia, since 80% of our convicts in prisons have misused psychologically active substances during the period of 30 days, the previous period before coming to these institutions. More than a half of our examinees (60%), misuses narcotics and alcohol occasionally or permanently, the alcohol users only 12.7%, and only narcotics 7.3% of the ones, meaning that a treatment of addiction disease should have a significant role in prevention of recidivism.

Introduction

Dissemination of addict disease in the world, and us, has reached such rations that we can call it an epidemic, reasonably. Statistical data of different organisations emphasise epidemiological features of drug addition, being alarming ones. The World Health Organisation conducted a research on exposure of psychologically active substances and alcohol between 2001 and 2006 (NIDA, 2007 [1]). The exposure means that a person has tried, used or still uses psychologically active substances. The research included 54,069 examinees in 17 countries, from all continents. This research had shown that a degree of usage of some substances is different. Alcohol usage over exceeds 90% in eight of seventeen countries included in. Alcohol drinking is more presented in America, Europe, Japan and New Zealand than in Africa, China, and Middle East, and the highest usage is in Ukraine (97%). The rates of drug usage is higher in the USA (cannabis 41.9%, and cocaine 16.2%), in New Zealand (cannabis 41.9%, and cocaine 4.3%) than in other countries. The rates of tobacco usage are mostly similar, being between 45-74%, except in Nigeria (16.8%) and South Africa (31.9%). There is reduction of tobacco usage in some countries.

Pursuant to the data of the *European Monitoning Centre for Drugs and Drug Addiction* (2004) [2], the percentage of drug addicts in the European prisons is from 22% to 86%. In the USA, in the period from 1984 until 1999, it was recorded an increase of drug addicts in prisons, being 3% annually (Scalia, 1999) [3]. The report from 2002 stated that in the American prisons were placed 440670 convicts, having 112447 drug addicts, 48823 for possession of drug amounts, and 56574 for possession and selling of drugs (Karberg, James, 2005 [4]). The American *Bureau of Justice Statistics* (2006) [5] stated that in 1997, 70% of convicts in the state prisons and 57% in federal prisons used drug and alcohol before being arrested. In the surveys conducted in 2002, 52% of women and 44% of men satisfy criteria to be drug addicts. In testing of juvenile convicts, it was determined that 56% of boys and 40% of girls were positive to drugs during their arresting in 2000, while in 2006, 60% of boys and 50% of girls, and this means that the number of juveniles increases, the

ones who had problems with the law, but were also positive to drugs. The data from other countries also reveal a significant number of drug addicts among criminal persons. In England and Wales, 63% of male prisoners being sentenced are alcohol addicts, and 43% are drug addicts (Singleton et al., 1998 [6]). In Australian prisons, between 50% and 80% of convicts being in prisons are addicts of psychologically active substances (Australian Bureau of Criminal Intelligence, 2000 [7]).

By increasing of criminal acts related to psychologically active substances, it has been increased the number of addicts in prisons. The number of registered drug addicts being arrested in 2010 was 6211 from the entire number of 11 211, and this means 55.40%. From the entire number of convicted persons 7167, who are being served their prison sentence, 3 286 or 85% are drug addicts, while from the entire number of convicted ones 3332, 2115 or 63.48% misuse psychologically active substances (Ministry of Justice, Republic of Serbia, 2011). In the criminal structure of convicted ones in 2011, from the entire number of 7933 persons who are being served their sentence, the most frequent ones were larceny, theft, concealing and similar (1 984 convicts), drug misuse (1 688), robbery (800), body injuries (313), gun possession (244), family violence (392), raping (109), murder and murder attempts (268) and other criminal acts (1554) of convicts (Ministry of Justice, Republic of Serbia, 2012 [8]).

The situation is similar in the region. Pursuant to the Ministry of Justice of Republic of Croatia, Prison Administration Office, among 7 572 convicts who served their prison sentence in 2010, there was 22.36% of drug addicts [9]. From the entire number of convicts who were drug addicts in 2010, 55.4% made convicts who served their prison sentence in this period. During 2010, there were 1034 new persons who were received to serve their prison sentence, also drug addicts. Two years behind, in Croatia, there was an extremely high increase of criminal acts among recidivists of convicts. In 2010, even 88.59% of drug addicts who were previously sentenced, and it was 27.21% more compared to 2009, i.e. 39.63% more than in 2008 (Ministry of Justice, Republic of Croatia, Prison Administration Office, 2011 [9]).

The aim of this study was to investigate the misuse of psychologically active substances of convicts being in prisons and their treatment in the District Prison in Novi Pazar, Serbia during October and November in 2013.

Material and Methods

The research was realised in the District Prison in Novi Pazar, Serbia during October and November in 2013. In the stated facility, male adults are being served their prison sentences, age 20 to 35. The duration of the sentences is up to12 months in prison, but usually several minor offences are joined, and thus the convicts are kept for a longer inhere. The District Prison in Novi Pazar can accept up to 80 convicts, and usually 50 of the ones serve their sentences. There is a Behavioural Service, Security Service and General Administration Service within this prison. Training Service and Health Service do not exist here, but a doctor comes on certain weekdays.

A sample makes 55 male convicts being in the prison of the District Prison in Novi Pazar at the time, speak Serbian and have agreed to participate in the research. The age of the examinees in the sample is from 20 to 60 years (AC = 29.98; CD = 8.01). The majority of the examinees live in the city (85.5%), and a smaller number in a village (14.5%). Pursuant to their nationality, the examinees can be divided into three groups: Bosniacs (76.4%), Serbs (20.0%) and Roma (3.6%). The total of 7.3% of the examinees do not have primary school finished, 54.5% have finished primary school, 12.7% has started high school, 23.6% has finished high school, and only one of the convicts has started higher education. Before their arrival to the prison, 58.2% of the examinees have been employed and 41.8% have not. The sample structure pursuant to their marriage status is: 56.4% unmarried, 7.3% are divorced or live separately, 32.7% married for the first time and 3.6% married for the second time. In Table 1, the sample structure has been presented. In Table 1, the sample structure has been presented.

Table 1: The Sample Structure

Variables	Categories	No.	%
Place of residence	Village	8	14.5
	City	47	85.5
Nationality	Bosniac	42	76.4
	Serb	11	20.0
	Roma	2	3.6
Schooling	Unfinished primary school	4	7.3
	Finished primary school	30	54.5
	High school started	7	12.7
	High school finished	13	23.6
	Higher education started	1	1.8
Employment	Employed	32	58.2
	Unemployed	23	41.8
Marital status	Married once	18	32.7
	Married twice	2	3.6
	Separated	1	1.8
	Divorced	3	5.5
	Not married	31	56.4

Results

Prevalence and the Patterns of Misuse of Psychologically Active Substances

The results show that 80% of the convicts being in the prison misuse psychologically active substances, and 20% do not. These results are pursuant to the official statistical data for Serbia. Pursuant of the data of the Sanction Performance Administration Office, the number of addicts of

psychologically active substances among persons being in prisons since 2012 was 4741 drug addicts, alcoholics 1723 or 16.85% (N10226), 2011-the number of drug addicts was 4929 or 44.43%, alcoholics 1880 or 16.95% (N11094), 2010-the number of drug addicts was 6211 or 81,08%, alcoholics were 2090 or 27.28% (N7660), in 2009-the number of drug addicts was 4495 or 41.64%, alcoholics 1674 or 15.51% (N10795), in 2008-the number of drug addicts was 6063 or 62.50%, alcoholics 1695 or 17.47% (N9701), in 2007-the entire number of addicts of psychologically active substances was 73.35% (N = 6580), 53.07% (N = 4189) in 2006, 31.67% (N = 2559) in 2005 (Ministry of Justice of the Republic of Serbia, 2013 [8]).

The data on prevalence of misuse of psychologically active substances among the examinees from the sample has been presented in Table 2.

Table 2: Prevalence of Misuse of Psychologically Active Substances (PAS)

Misuse of PAS	No.	%
Exists	44	80.0
Does not exist	11	20.0
Total	55	100.0

We can notice that a number of convicts being serving their sentences Serbian in prisons misuse psychologically active substances. Prevalence of misuse in prison population in Serbia started to be over 50% in 2006, and their number grows constantly. In 2008, the number of drug addicts was 6063 or 62.50%, alcoholics 1695 or 17.47% to have its culmination in 2010, when it had the scope of epidemic, where from the entire number of convicts (7660), there were 6211 drug addicts or 81.8%, alcoholics 2090 or 27.28%, and its number was constant in 2011-drug addicts 4741, alcoholics 1723 and in 2012-drug addicts were 4929, alcoholics-1880. As we have already mentioned, the similar situation of increase of prevalence of misuse in prison population is similar in the region, either. In Croatia, the number of addicts of psychologically active substances being in their prisons was 349 in 2002, while in 2011-it was 3303 (Ministry of Justice of the Republic of Croatia, 2013), and this means it has increased nine times [9].

Each psychologically substance affects in a specific way, and it means it causes a specific type of addiction (Henslin, 1996 [10]). Table 3 contains data on prevalence of misuse of certain kinds of psychologically active substances. The examinees have been classified depending of whether they just use narcotics, only alcohol or a combination of narcotics and alcohol or they do not misuse psychologically active substances.

More than a half of the examinees (60%) misuse a combination of drugs and alcohol either temporarily or permanently, but only 12.7% use just alcohol and 7.3% only narcotics of all examinees. The drug addicts using any of drugs in great amounts (high-frequency users) probably use several types of drugs in combination with alcohol (Elliot et al., 1989 [11]).

Table 3: Prevalence of Misuse of Certain Types of Psychologically Active Substances

Type of PAS	No.	Percentages
Narcotics	4	7.3
Alcohol	7	12.7
Narcotics and alcohol	33	60.0
Do not misuse PAS	11	20.0
Total	55	100.0

Since a majority of the examinees combine different psychologically active substances, the use of certain psychologically active substances has been identified by a survey in the time period prior their arrival to serve sentences (30 days and 6 months). The data is presented in the Table 4. In the last 30 days prior their arrival to this facility, the examinees mostly used alcohol (41.8%), cannabis (38.2%), heroin (34.5%), hypnotics (29.1%) and cocaine e (29.1%). In the same period, the examinees have used other psychologically active substances: methadone (5.5%), analgetics (25.5%), barbiturates (5.5%), amphetamine (7.3%), and LSD have been used by only one examinee (1.8%). In the period of six months prior their arrival to serve their sentences, the examinees have mostly used alcohol (69.1%), heroin (63.6%), cannabis (60%), cocaine (45.5%), analgetics (32.7%) and hypnotics (30.9%). A lower frequency of usage of the following psychologically active substances has been discovered: methadone (12.7%), barbiturates (10.9%), amphetamine (14.5%), LSD (5.5%) and inhalers (5.5%). The majority of the examinees mostly used alcohol, heroin, cannabis and hypnotics in both observed periods. Numerous researches have shown that within a number of recidivists we can find over drinking or alcohol addiction (Maruna, Immarigeon, LeBel, 2004 [12]). The Sanction Performance Administration Office has warned onto a high number of alcoholics among the prison convicts. The number of registered alcoholics among persons being in prisons for serving their sentences was 697 persons in 2005, while in 2010-this number was 2090, and in 2012-the number was 1723 (Ministry of Justice of the Republic of Serbia, 2013 [8]).

Our data is also the same as the official statistics of indicators of the Ministry of Justice Republic of Serbia, on what are the most frequently used substances. Marihuana uses in average 40% and 80% (2008-65%, 2009-89%, 2010-60%, 2011-40%) (Ministry of Justice of the Republic of Serbia, 2013 [8]).

Pursuant to a report of the Treatment Service, Ministry of Justice, Prison Administration Office of the Republic of Croatia [9], the structure of the examinees for their primary psychologically active means N = 209 in 2007 was as following: the most often used

psychologically active substance was heroin 82% (N = 172), cannabis 11% (N = 22), amphetamine 4% (N = 8), cocaine 3% (N = 7), 2009-heroin made 40%, cannabis-14%. For the year 2010-active substances were 44.2%, cannabis-14.3%; in 2011-active substances were 28.8% and in 2012-active substances were 39.14%, cannabis-18% (Ministry of Justice of the Republic of Croatia, 2013 [9]), and all these results point onto primary distribution of heroin as a far more often used psychologically active substance in our areas and the ones nearby.

Table 4: Distribution of the Examinees Pursuant to the Last Time Period of Usage of Psychologically Active Substances

The time of the last PAS usage	Yes		No		Total	
	No.	%	No.	%	No.	%
Getting drunk in the last 30 days	23	41.8	32	58.2	55	100
Getting drunk by alcohol in the last six months	38	69.1	17	30.9	55	100
Heroin usage in the last 30 days	19	34.5	36	65.5	55	100
Heroin usage in the last 6 months	35	63.6	20	36.4	55	100
Methadone usage in the last 30 days	3	5.5	52	94.5	55	100
Methadone usage in the last 6 months	7	12.7	48	87.3	55	100
Analgetic usage in the last 30 days	14	25.5	41	74.5	55	100
Analgetic usage in the last six months	18	32.7	37	67.3	55	100
Barbiturate usage in the last 30 days	3	5.5	52	94.5	55	100
Barbiturate usage in the last 6 months	6	10.9	49	89.1	55	100
Hypnotics usage in the last 30 days	16	29.1	39	70.9	55	100
Hypnotics usage in the last 6 months	17	30.9	38	69.1	55	100
Cocaine usage in the last 30 days	16	29.1	39	70.9	55	100
Cocaine usage in the last 6 months	25	45.5	30	54.5	55	100
Amphetamine usage in the last 30 days	4	7.3	51	92.7	55	100
Amphetamine usage in the last 6 months	8	14.5	47	85.5	55	100
Cannabis usage in the last 30 days	21	38.2	34	62.8	55	100
Cannabis usage in the last 6 months	33	60	22	40.0	55	100
LSD usage in the last 30 days	1	1.8	54	98.2	55	100
LSD usage in the last 6 months	3	5.5	52	94.5	55	100
Inhalants usage in the last 30 days	1	1.8	54	98.2	55	100
Inhalants usage in the last 6 months	3	5.5	52	94.5	55	100

A high frequency of heroin usage among convicts matches with the researches that heroin stimulates aggression, and in this way, criminal behaviour of addicts of psychologically active substances. With heroine users, there is a high rate of criminal, and with other drug adducts, the intensity of crminal behaviour varies with the intensity of drug usage (Anglin, Speckart, 1986 [13]). Adult criminals, multiple offenders that have used heroin before being 16 years old, also participated in different robberies and thefts (Chaiken, Chaiken, 1982 [14]). A previous beginning of heroin and cocaine usage gives a higher possibility for these addicts to become serious offenders as adults (Colins, Bailey, 1987 [15]). Regular heroin users can sustain of criminal actions and continue to use it as a part of everyday life activates, though it stimulates criminal behavior and brings energy within its users (Hanson et al., 1985 [16]). Cocaine has stimulation effects and causes skin, hearing and visual hallucinations, delirium ideas, a condition of hyper sensitivity of psycho-motor sphere and increased physical and psychological powers (Williams, 1989 [17]).

Methadone (heptanon) is misused by addicts not only as a substitution for heroin, but as a primary substance or a combination with the heroin. Methadone is also a psychologically active substance, used by the examinee addicts being in prisons for serving their sentences mostly as a treatment and quitting of heroin. Methadone substitution is being applied within addicts where there is estimation that the possibility of abstinence is very low. Some researches have shown that addicts who receive optimal doses of methadone can be more able to work, as people who do not take any medication (Munjiza, 2009 [18]). In our research, only one of the examinees has been to a methadone treatment, while the others quitted under an excuse that the one has the same addiction effects as the others. In Croatia, methadone has been used in surgeries as a substitute for heroin addiction treatment, and a medicine *subutex* has been introduced, being used more in the prison system. The main critique to methadone therapy is that the one drug is replaced by another. During 2008, 521 of convicts in prisons used this medicine (Ministry of Justice of the Republic of Croatia, 2013 [9]).

By studying patterns of drug misuse, there is knowledge on most people use illegal drugs and they are limited to a temporary usage of marihuana, while relatively small number start using other forbidden drugs such as barbiturates, amphetamine, cocaine and heroin (O'Malley, Bachman, Johnston 1988 [19]). A general characteristic of all drugs is to cause psychological addiction and have a special effect onto perception, feelings and moods (Regan, 2001 [20]).

The accomplished data on misuse of other psychologically active substances is pursuant to the official data of Ministry of Justice of the Republic of Serbia, except for cocaine. Amphetamine has been misused pursuant to the official data of Ministry-in 2008-17%, 2009-20%, 2010-40%, 2011-50%; cocaine -in 2008-5%, 2009-10%, 2010-10%, 2011-50%; ecstasy- in 2008-17%, 2009-20%, 2010-40%, 2011-50% (Ministry of Justice of the Republic of Serbia, 2013 [8]).

Pursuant to the data from Croatia, the frequency of misuse of sedatives, hypnotics and psychologically simulative drugs is 6%, and the combination with other psychologically active substances is 37.87%; 3.38% is cocaine addicts; 3.77% is stimulation means without cocaine; 11.39% is cannabis addicts; less than 0.1% uses inhalants (Prison Administration Office of the Republic of Croatia, 2007 [9]). During 2012, except all stated active substances use of 39.14% and cannabis 18%, they have used polytoxics – 30.34%, sedatives-hypnotics-7.03%, cocaine - 3.49% and stimulation means - 1.72 % (Ministry of Justice of the Republic of Croatia, 2013 [9]).

The Treatment Need with Persons Serving Their Prison Sentences

For determination of treatment needs with convicts who do not misuse and who misuse psychologically active substances, an instrument has been used under a title *Addiction Severity Index-ASI.* It is the instrument being applied for decades in

evaluation of existence and nature of this issue within criminal offenders. ASI is a relatively short, semi-structured questionnaire, designed to offer significant information on different life aspects of persons being in prisons for serving their sentences. All the gained data shall be confidential and it has been presented to all examinees in the beginning, and the data shall be only used for the scientific purposes. In the beginning of each ASI survey, there is a detailed instruction chapter for each examinee on the way of answering and to be as honest as possible, since in that way, the survey shall justify its primary purpose. The ASI test gives information on the basic aspects of life that can be connected to misuse of psychologically active substances. Life areas covered by the survey are: health status, educational and employment statues, legal status, family background, family relations and psychological status. The results of the survey shall be presented pursuant to life areas. Based on gained answers on existence, duration and intensity of the issue, it is being given a mark for each issue status: 1 – no problem, a treatment has not been indicated; 2 – smaller problems, a treatment probably has not been indicated; 3 – mild problems, a type of treatment is needed; 4 – significant problems, a treatment needed; 5 – very significant problems, a necessary treatment.

Health Status

We have not included health issues being directly connected with misuse of psychologically active substances in evaluation in expression of certain degree of health issue status. If an examinee has a chronically disease, receives a certain therapy, and the ones does not need additional treatment, then marking is lower, since there is no need for additional treatment. A higher mark has been given if there are more frequent of long-lasting health issues, serious health problems in the last 30 days, and if the examinee wants to have a treatment. In Table 5, the number and percentage of the examinees within a group who misuse and do not misuse PAS have been presented, the ones who express a certain degree of problems in the health life area.

Table 5: Distribution of the Examinees who Misuse PAS and Do Not Pursuant to their Health Status

Health status	Misuse PAS		Do not misuse PAS		Total	
	No.	%	No.	%	No.	%
No problems	8	18.2	3	27.3	11	20.0
Smaller problems	14	31.8	3	27.3	17	30.9
Mild problems	12	27.3	2	18.2	14	25.5
Significant problems	9	20.5	2	18.2	11	20.0
Very significant problems	1	2.3	1	9.1	2	3.6
Total	44	100.0	11	100.0	55	100.0

Generally considered, it has been determined the existence of smaller problems within the majority of the examinees (30.9%), i.e. the absence of the problems (20%). This means that within half of the examinees, there is no need to apply treatment being directed towards the improvement of the health status, and within the other half, there is the need. There are a higher number of the examinees who do not have problems and those with smaller ones (54.6%), than of the examinees who misuse psychologically active substances (50%). Mild problems have been discovered within 27.3% examinees who misuse psychologically active substances and 18.2% of those who do not misuse psychologically active substances. Significant problems have existed within 20.5% of the examinees who misuse psychologically active substances and 18.2% of those who do not misuse them. Very significant health problems have had one examinee who misuses psychologically active substances and two who do not use the ones.

Pursuant to the data of the Ministry of Justice of the Republic of Serbia from 2012, the number of those being sick in prisons is the following: 4353 of the musculoskeletal diseases, 8461 of respiratory ones, 3825 of digestive disease, 6720 of cardiovascular diseases, and 3445 of a nervous system, 18 858 of mental disease, and the entire number is 45 662. The type and number of infection diseases among the persons being in prisons for serving their sentences in 2012 was: 20 of HIV, 2 of hepatitis A, 1569 of hepatitis B and hepatitis C, and 52 of TBC. The number of those being sick of hepatitis C within the persons being in prisons in Serbia grows rapidly. From 2005 until 2007, the number of the sick ones was 528 in 2005, while in 2006, it increased to almost three times 1431, and in 2007, the number of them was 1784, while it has been constant for five years period, and in 2012 it was 1569 (Ministry of Justice of the Republic of Serbia, 2013 [8]).

Educational and Employment Status

By evaluating schooling of the examinees, whether the one has a profession, trade or a special skill, a permanent job in the last year, whether someone helps them socially, the source of their incomes and supporting of other ones, we have gained the following results on their educational and employment status. Juvenile criminals continue to perform criminal acts as adults, if they had problems at school as very young (irregular school attendance or leaving it), (Blumstein, Farrington, Moitra, 1985 [21]). In Table 6, the data has been shown on the issues in educational and business domain of examinees' life domain of the ones who misuse and do not misuse PAS.

Table 6: Distribution of the Examinees who Misuse PAS and Do Not Pursuant to their Educational and Employment Status

Educational and employment status	Misuse PAS		Do not misuse PAS		Total	
	No.	%	No.	%	No.	%
No problems	0	0	0	0	0	0
Smaller problems	5	11.4	2	18.2	7	12.7
Mild problems	21	47.7	8	72.7	29	52.7
Significant problems	15	34.1	1	9.1	16	29.1
Very significant problems	3	6.8	0	0	3	5.5
Total	44	100.0	11	100.0	55	100.0

The highest is the number of the examinees

with mild problems (52.7%) and significant problems (29.1%), making more than 2/3 of the total number of the examinees. It emphases a low level of educational and business status of the examinees and their need for education treatment and business education, but also a classification of the ones. Mild and significant problems (more than 80%) have had the examinees that misuse and do not misuse psychologically active substances. The number of 18.2% examinees has smaller problems and they do not misuse psychologically active substances, and 11.4% use the ones. Mild problems have 47.7% of the ones using psychologically active substances, and 72.7% of the examinees do not use the ones. Significant problems have had 34.1% of the examinees who misuse psychologically active substances, and 9.1% of the ones who do not use them. Significant problems have not had the ones who do not misuse psychologically active substances, while 6.8% had been the ones who misuse psychologically active substances with very significant problems.

Legal Status

The evaluation of the legal status has been performed based on the current and previous verdicts of the examinees, and we have also determined the type of criminal acts and their recidivism. Per a legal model, violation of law can be considered deeds related to drug, such as usage, possession and production (Goldstein, 1985 [22]). There are criminals who committed numerous criminal acts and they do not use drug (Innes, 1988 [23]). The data describing legal status of the examinees has been shown in Table 7.

Table 7: Distribution of the Examinees who Misuse PAS and Do Not Misuse PAS pursuant to their Legal Status

Legal status	Misuse of PAS		Do not misuse PAS		Total	
	No.	%	No.	%	No.	%
No problems	0	0	0	0	0	0
Smaller problems	3	6.8	2	18.2	5	9.1
Mild problems	14	31.8	6	54.5	20	36.4
Significant problems	24	54.5	3	27.3	27	49.1
Very significant problems	3	6.8	0	0	3	5.4
Total	44	100.0	11	100.0	55	100.0

It has been determined that smaller problems with the law have 49.1%, and mild problems have 36.4% of all examinees. It tells us that within the majority of the examinees, there is need for treatment, directed to the legal remedies and different programmes being applied with the aim not to do the same criminal acts and other ones, i.e. not to have recidivism. Mild problems with the law have 54.5% of the examinees who do not misuse psychologically active substances, while the ones who do misuse these substances are 31.8% of the examinees. Significant problems with the law has 54.5% of the examinees who misuse psychologically active substances, and 27.3% has the same problems but they do not misuse them. A smaller and very significant problem has 6.8% per each group of the ones who misuse psychologically active substances, while the marks have not been given for the prisoners who do not misuse the ones.

Family Background

In Table 8, there has been presented data on family background of the examinees, i.e. whether some of the closer siblings and family members have had problems with alcohol usage or significant psychological problems, and have been sent to treatment and hospitalisation. In case any of the examinees' siblings do not use psychologically active substances, either has some mental disorder, it has been given a mark "no problems". The mark "very significant problems" has been given in the cases where several siblings and family members have serious problems with the misuse of psychologically active substances or chronically mental disorders, and thus had been treated in the hospital in the past.

Table 8: Distribution of the Examinees who Misuse Pas and Do Not Misuse PAS pursuant to Family Background

Family background	Misuse PAS		Do not misuse PAS		Total	
	No.	%	No.	%	No.	%
No problems	16	36.4	6	54.5	22	40.0
Smaller problems	11	25.0	2	18.2	13	23.6
Mild problems	15	34.1	1	9.1	16	29.1
Significant problems	2	4.5	2	18.2	4	7.3
Very significant problems	0	0	0	0	0	0
Total	44	100.0	11	100.0	55	100.0

If we reconsider a family background of the examinees, we can determine that a high percentage of them do not have problems (40.0%), smaller have problems (23.6%), while mild problems has 29.1% of all examinees. In the categories with smaller and mild problems, there are a higher number of the ones who misuse psychologically active substances (59.1%), than those who do not misuse psychologically actives substances (27.3%). Significant problems have 18.2% of the examinees who do not misuse psychologically active substances, and 4.5% of those who misuse the ones. Very significant problems do not have any of the examinee categories.

Identification with social, deviant and criminal features of family members makes a base for a future behaviour of the person. A family member has a chance to learn on such behaviour from the early years of the childhood, and thus, the one has the impression of a hereditary feature. Family pathology and difficulties in its functioning show onto the background of such behaviour of an individual. Juvenile convicts continue to perform hard criminal acts if they originate from poor families, if they have someone in a closer family with a criminal behaviour, if they have a low IQ and inadequate parents' care (Blumstein, Farrington, Moitra, 1985 [24]). The gained data in the research of the family background of the examinees are pursuant to the data in nearby countries. In a survey conducted in the territory of Bosnia and Herzegovina, in the criminal facilities in

Banjaluka, Tuzla, Foca and Zenica, from a hundred of the examinees who misuse psychologically active substances, three parents of the ones also use them, while twenty-seven parents do not misuse psychologically active substances. Within 68 examinees addicted to psychologically active substances, there have been 38 parents being alcohol addicts, and 30 who has not been (Korac, 2008 [25]).

Family and Social Relations

During the evaluation of interpersonal relations, we have tried to evaluate whether family issues would exist if there were no misuse of psychologically active substances within the families of the examinees. Based on the data presented in Table 9, the issues on the area of family relations of the examinees can be reconsidered.

Table 9: Distribution of Examinees who Misuse PAS and Do Not Misuse PAS regarding their Family and Social Relations

Family and social relations	Misuse PAS		Do not misuse PAS		Total	
	No.	%	No.	%	No.	%
No problems	1	2.3	0	0	1	1.8
Smaller problems	6	13.6	4	36.4	10	18.2
Mild problems	19	43.2	5	45.5	24	43.6
Significant problems	18	40.9	2	18.2	20	36.4
Very significant problems	0	0	0	0	0	0
Total	44	100.0	11	100.0	55	100.0

Within the highest number of the examinees, there have been mild problems (43.6%) or smaller ones (36.4%), while insignificant problems have had 18.2% of the examinees. This data speak on the need for treatment application in the aim of improvement of interpersonal relations and overcoming of the problems existing due to it. There have been a higher percentage of significant problems within the examinees who misuse psychologically active substances (40.9%) than to those who do not misuse psychologically active substances (18.2%). There has been almost the same presence with mild problems within both groups of the examinees (43.2% and 45.5%). The absence of problems in interpersonal relations has been recorded only with one examinee who misuses psychologically active substances. The gained data has been similar to the data in the surveys made in our nearby countries, and they shall be reconsidered in the parts on differences of the examinees regarding their socio-demographic and criminal-penology features.

Mental Status

In the evaluation of psychiatrically status and need for treatment, we have reconsidered only the symptoms that have not been directly connected with misuse of psychologically active substances. In Table 10, the data on mental (psychiatrically) status has been presented.

Table 10: Distribution of examinees who misuse PAS and do not misuse PAS regarding their mental status

Mental status	Misuse PAS		Do not misuse PAS		Total	
	No.	%	No.	%	No.	%
No problems	4	9.1	3	27.3	7	12.7
Smaller problems	8	18.2	2	18.2	10	18.2
Mild problems	18	40.9	3	27.3	21	38.2
Significant problems	8	18.2	3	27.3	11	20.0
Very significant problems	6	13.6	0	0	6	10.9
Total	44	100.0	11	100.0	55	100.0

Mild problems have had 38.2%, and significant ones 20% of all examinees. Smaller problems have been discovered within 18.2% of the examinees, and lack of problems with 12.7% of the examinees. Very significant problems have had 10.9% of the total number of the examinees, and all of them misuse psychologically active substances. None of the examinees who do not misuse psychologically active substances had any significant problems of this type. Within the group of the examinees who misuse psychologically active substances, mild problems have had 40.9%, significant problems and smaller ones 18.2% per each group, 13.6% has had significant problems, and 9.1% has not had any problems. Within the examinees who do not misuse psychologically active substances, 27.3% has had mild and significant problems each, or have been without problems, and 18.2% has had smaller problems.

The gained results of the research tells that within a high number of the examinees, there have been needs for application of treatment directed towards improvement of psychological and emotional condition. The official data of the Ministry of Justice of the Republic of Serbia in all prisons for serving sentences show on the presence of similar problems. According to this source, a total number of sick ones from mental disorders in the facilities for serving prison sentences was 3893 in 2007, and 29386 of special psychiatrically check ups were performed, while in 2011, there were 27202, and in 2012-18858 (Ministry of Justice of the Republic of Serbia, 2013 [8]), and this is the highest number of check ups being performed with convicts.

Concluding Thoughts

Misuse of psychologically active substances in the population of adult convicts in Serbian prisons has reached alarming size. Prevalence of misuse of psychologically active substances in prison population grows significantly and increases 20% per year. Pursuant to the data of Prison Administration Office among persons in prisons and addicts of psychologically active substances were 31% in 2005, 53% in 2006, and even 73% in 2007; while in 2010 of the total number of 7660 those 6211 or 81.08% were drug addicts, 2009 were alcoholics or 27.28%. It is a similar situation in the entire region. In Croatia, the number of convicts being addicted to psychologically

active substances was 31% in 2005, 41% in 2006, being 41% of the prison population, and this percentage is approximately constant in this level, in 2010-it was 44.2%; and in 2012-it was 39.14%. In the European prisons, the percentage of convicts who misuse psychologically active substances is up to 80%, and similar data is valid for the USA and Australia. The relation of a high frequency misuse and high frequency of criminal actions is intensive and a long-term (Nurko, 1988 [26]). Most people who use drugs change during time and have a higher participation in criminal activities (Newcomb, Bentler, 1988 [27]).

The research results emphasise onto a necessity of introduction of wide programmes of prevalence and treatment of addiction disease, primary since a high number of addicts serving their prison sentences, but also for the other convicts who are surrounded by misuse and trade of psychologically active substances. Having the Strategy for Struggle against Drugs in Prisons, the country of Serbia and its management structures in prison system have chosen clearly for taking measures and programmes for prevention of trade and misuse of psychologically active substances in prison-treatment facilities. Unfortunately, numerous progressive ideas being in this document have not been realised until present days. It is obvious that Serbian punishment system has stayed closed to many innovations and positive experiences from the countries that marked the last decades of 20th century.

The very character of these institutions for performance of criminal sanctions is being negatively reflected onto persons who are in prisons (Plojović, S. 2013 [28]).The sense of being jeopardised is one of the most often feelings within prisoners (Johnson, 2002 [29]). An alternative position, developed mainly by criminologists, is that imprisonment is not simply a “cost” but also a social experience that deepens illegal involvement (Cullen et al., 2011 [30]). The improvement of practice in Serbian penalty institutions must start urgently to be applied, in the domain prison facilities allow it, with consultations with relevant scientific institutions dealing with this issue. The priority should be given to prevention of misuse of psychologically active substances in prisons and treatment of addicts. In the contemporary expert and scientific circles, there is an opinion that prison sentence offers ideal conditions for intervention, so as to reduce availability of psychologically active substances, and convince a convict to have an appropriate treatment. Some treatment models can look as mechanisms of social pressure onto prisoners and mechanisms of social control in a broadest sense (Garland, 2001 [31]). Having addicts at a treatment who have had their sentence verdict, they could be either voluntarily or forcedly, depending on circumstances. In the line with the practice of other countries, it can be recommended to introduce varied treatment programmes: an oral treatment programme (methadone therapy), a religious treatment programmes with a different religious influence, individual and group psycho-therapy programme, and also a family treatment programme. Based on the conducted research, two general conclusions can be derived:

- The convicts who misuse psychologically active substances represent a special group of offenders, regarding their characteristics and needs for treatment, and they all differ due to their frequency and pattern of misuse of psychologically active substances compared to a normal population. From this, the necessity is of the following and it is that
- There is a need for application of special programmes or ways when this group is considered, and there are differences in treatment needs among the examinees that misuse and do not misuse psychologically active substances.

A special significance is the work on prevention of drug addiction disease in prisons. Since the connection of criminal and drug addition disease, all convicts regardless of their history on misuse of psychologically active substances, should be treated as a high risk population. The last researches emphasise that adult criminals often continue to use psychologically active substances and do criminal acts since there is a lack of efficient treatment and supervision (Anglin, Piper, Speckart, 1987 [32]). The misuse of psychologically active substances represents a significant factor of reflecting a criminal behaviour, and thus, the treatment of addiction disease has a significant role in prevalence of recidivism (Plojović, Maksimović, 2013 [33]). Prevention programmes being applied in the world are mostly of informative character, with the aim to introduce convict population with the causes, development flow and consequences of drug addition disease. Such programmes are not demanding, since the experts employed at prisons can realise them. The efficiency of treatment is mostly reflected in improving of psychological interventions (Rohsenow, 2004 [34]). A combined treatment is possible in prisons and some authors evaluate that a treatment of addiction disease in combination with methadone therapy, counseling and treatment reduces the use of psychologically active substances within convicts that can go under such treatment, different from those who did not have any treatment (Shewan, Dalgarno, 2005 [35]).The measures of reduction of illegal drugs in prisons must be stricter. All prisons represent the most profitable market for psychologically active substance transfer, and it increases violence risk and other misuse being visible in the illegal distribution. Since all of these, a special attention must be devoted to have certain measures to reduce illegal transfer of these substances in prisons. The evaluation on the percentage of hard drugs being used by convicts emphasise onto an urgent and efficient intervention, since if there is no intervention, the usage of

psychologically active substances in Serbian prisons can be to an epidemic level.

In the end, it should be emphasised that introduction of contemporary treatment programmes of misuse of psychologically active substances demands high human and material resources, and unfortunately, some of the punishment institutions in Serbia, do not have them. Further on, a lot of programmes demand separation of the convicts being in them to have special premises, special equipment and materials necessary for the realisation of programme activities, and it is impossible to provide since Serbian prisons have too many convicts. If adequate conditions are not secured for the implementation of these programmes, it is difficult to expect to live, even those programmes being evaluated by the state as strategically significant for the struggle against drug usage in prisons.

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Review Article

Genomic Imprinting

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Abstract

BACKGROUND: Genomic imprinting is the inheritance out of Mendelian borders. Many of inherited diseases and human development violates Mendelian law of inheritance, this way of inheriting is studied by epigenetics.

AIM: The aim of this review is to analyze current opinions and options regarding to this way of inheriting.

RESULTS: Epigenetics shows that gene expression undergoes changes more complex than modifications in the DNA sequence; it includes the environmental influence on the gametes before conception. Humans inherit two alleles from mother and father, both are functional for the majority of the genes, but sometimes one is turned off or "stamped" and doesn't show in offspring, that gene is imprinted. Imprinting means that that gene is silenced, and gene from other parent is expressed. The mechanisms for imprinting are still incompletely defined, but they involve epigenetic modifications that are erased and then reset during the creation of eggs and sperm. Genomic imprinting is a process of silencing genes through DNA methylation. The repressed allele is methylated, while the active allele is unmethylated. The most well-known conditions include Prader-Willi syndrome, and Angelman syndrome. Both of these syndromes can be caused by imprinting or other errors involving genes on the long arm of chromosome 15.

CONCLUSIONS: Genomic imprinting and other epigenetic mechanisms such as environment is shown that plays role in offspring neurodevelopment and autism spectrum disorder.

Introduction

Genomic imprinting is the inheritance out of Mendelian borders. Many of inherited diseases and human development violates Mendelian law of inheritance, this way of inheriting is studied by epigenetics. Epigenetics shows that gene expression undergoes changes more complex than modifications in the DNA sequence; it includes the environmental influence on the gametes before conception.

When epigenetic changes occur in sperm or egg cells that lead to fertilization, epigenetic changes are inherited by the offspring [1].

Genomic imprinting is a process of silencing genes through DNA methylation. The repressed allele is methylated, while the active allele is unmethylated.

Some questions still await conclusive answers, particularly those concerning why mammals alone among vertebrates use imprinted genes to regulate embryonic and neonatal growth [2].

The aim of this review is to analyze current opinions and options regarding to this way of inheriting.

Results and Discussion

The classical definition of epigenetics refers to the mitotically and/or meiotically heritable changes in gene activity that does not involve alterations in DNA sequence [3]. Genomic imprinting occurs when two alleles at a locus are not functionally equivalent and is considered the primary epigenetic phenomenon that can lead to the manifestation of parent-of-origin

effects [4]. Genomic imprinting affects both male and female offspring and is therefore a consequence of parental inheritance, not of sex [2]. Epigenetic changes can be induced by environmental factors at different times in life. Epigenetic control operates on three major levels, on DNA, histones, and nucleosomes [3]. Epigenetic mechanisms encode information above and beyond DNA sequence and play a critical role in brain development and the long-lived effects of environmental cues on the pre- and postnatal brain [5] and [6].

When epigenetic changes occur in sperm or egg cells that lead to fertilization, epigenetic changes are inherited by the offspring [1].

Genomic imprinting is a process of silencing genes through DNA methylation. The repressed allele is methylated, while the active allele is unmethylated. This stamping process, called methylation, is a chemical reaction that attaches small molecules called methyl groups to certain segments of DNA [3]. DNA methylation is a biochemical process crucial for normal development in higher organisms, and it is the most thoroughly studied epigenetic mark. Methylation entails the covalent attachment of a methyl (CH_3) group to the C5 position of a cytosine residue, forming 5-methylcytosine (5 mC) [3]. DNA methylation is mediated by the cellular DNA methylation machinery, comprising Dnmt1, Dnmt3a, Dnmt3b and Dnmt3L. DNA methylation is a dynamic process during early embryonic development and undergoes parent and lineage dependent genome-wide changes [3] and [7].

There are now more than 25 identified imprinted genes, and estimates based on mouse models indicate that as many as 100 to 200 may exist [8]. The first endogenous imprinted gene identified was mouse insulin-like growth factor 2 (Igf2), which encodes for a critical fetal-specific growth factor [8] and [9].

Many theories have attempted to explain the evolution of genomic imprinting, but the most prominent are the kinship theory [10] and the sex-specific selection theory [11]. The kinship theory relies on asymmetries in relatedness between individuals' maternally and paternally derived alleles [12]. The kinship theory predicts that genes increasing an offspring's share of maternal resources, such as growth enhancers that act in development, will be expressed from the paternally derived allele and repressed on the maternally derived allele [13]. For X-linked loci, inheritance is asymmetric with respect to parental origin, and imprinting allows expression from such loci to be sexually dimorphic [10]. Under weak selection, quantitative genetic models of X-linked loci suggest that when selection is stronger against one sex, expression in the offspring of alleles derived from the other sex should be higher [10].

Although the exact molecular mechanisms involved in establishing and maintaining genomic imprints remain undetermined, much is known about the basic details [14]. Imprinted genes often occur in clusters that contain one or more imprinting control regions (ICRs). ICRs often exhibit different patterns of DNA methylation depending on whether the allele is paternally or maternally inherited [15]. The parental allele-specific epigenetic marks are heritable to the daughter cells, but must be reset in each successive generation to establish parental specific imprints. In mammals, two major genome-wide epigenetic reprogramming events take place during gametogenesis and early embryogenesis [15].

How does transcription lead to DNA methylation in oocytes? Oocyte availability is a challenge to molecular studies, but Kelsey and Feil [16] have speculated that the act of transcription results in a constellation of chromatin modifications that are conducive to interaction of DNMT3A and DNMTL, whereas other transcribed regions might be protected from methylation by CXXC-domain proteins.

Genomic imprints template their own replication, are heritable, can be identified by molecular analysis, and serve as markers of the parental origin of genomic regions. Beyond merely labeling homologous genetic alleles as descendent from father or mother, genomic imprints have the significant functional consequence of stifling gene expression from one of the parental alleles, resulting in unbalanced gene expression between homologous alleles.

The life cycle of imprints

Genomic imprints change in characteristic ways during the life cycle of the organism [17] and [18]. Imprints are ‘established’ during the development of germ cells into sperm or eggs. After fertilization, they are ‘maintained’ as chromosomes duplicate and segregate in the developing organism. In the germ cells of the new organism, imprints are ‘erased’ at an early stage [17]. This is followed by establishment again at a later stage of germ-cell development, thus completing the imprinting cycle. In somatic cells, imprints are maintained and are modified during development [17]. The imprints that are introduced in the parental germlines, maintained in the early embryo and fully matured during differentiation, they need to be read. Reading means the conversion of methylation or chromatin imprints into differential gene expression [17] and [18]. As a result of imprinting, there is biased allelic expression that favors expression from one parental locus over the other.

The dispersed patterns of CpG dyads in the early-cleavage embryo suggest a continuous partial (and to a low extent active) loss of methylation apparently compensated for by selective de novo methylation [18] and [19]. A combination of passive and active demethylation events counteracted by de novo methylation are involved in the distinct

reprogramming dynamics of DNA methylomes in the zygote, the early embryo, and PGCs [19].

Imprinted genes code for what?

A majority of the known imprinted genes code for proteins, others code for untranslated RNA transcripts.

Another category of parental genomic imprint, to be contrasted with well characterized examples of monoallelically expressed genes, are those methylation parental imprints scattered throughout the genome which are not demonstrated to be functional or associated with specific genes [18].

Clusters of imprinted genes are often controlled by an imprinting center that is necessary for allele-specific gene expression and to reprogram parent-of-origin information between generations. An imprinted domain at 15q11–q13 is responsible for both Angelman syndrome and Prader–Willi syndrome, two clinically distinct neurodevelopmental disorders [20].

The imprinted gene cluster on 15q11–q13 contains a number of paternally and maternally expressed transcripts and is reasonably well conserved, in terms of both gene content and imprinting status, between mammals [21] and [22]. The cluster has been studied intensely as loss of expression, through genetic and epigenetic mutation, leads to two distinct neurodevelopmental disorders, namely Prader- Willi Syndrome, which results as a consequence of loss of paternal gene expression, and Angelman Syndrome, which arises as a consequence of loss of maternal gene expression [22] and [23].

Prader-Willi syndrome is characterized by abnormal feeding and appetite, and learning disability, individuals with PWS may also develop a severe affective psychotic illness which is similar to bipolar disorder. This includes loss of antisense transcripts which represses the expression of UBE3A, which encodes E6-AP (E6-associated protein) ubiquitin ligase from the paternal chromosome. As a consequence, the paternal copy of this gene, which is only normally expressed from the maternal chromosome, becomes reactivated leading to increased dosage [22].

AS is a neurodevelopmental disorder characterized by severe cognitive disability, motor dysfunction, speech impairment, hyperactivity, and frequent seizures. AS is caused by disruption of the maternally expressed and paternally imprinted UBE3A, which encodes an E3 ubiquitin ligase.

In addition to AS and PWS, the 15q11–q13 imprinting region has also been linked to a number of non-syndromic neuropsychiatric illnesses. For instance, maternal duplication of this interval is associated with the incidence of autism [24].

Several studies have reported differential expression of imprinted genes between control and IUGR placental samples [24]. In other words, some may act to reduce fetal growth, resulting in IUGR (negative effectors), while others may act to enhance fetal growth in a compensatory manner to save a pathogenically growth restricted fetus (positive effectors) [25].

Some questions still await conclusive answers, particularly those concerning why mammals alone among vertebrates use imprinted genes to regulate embryonic and neonatal growth [2]. At this stage, it is clear that genomic imprinting uses the cell's normal epigenetic machinery to regulate parental-specific expression, and that everything is set in motion by restricting this machinery in the gamete to just one parental allele [2]. An improved understanding of genomic imprinting will undoubtedly continue to provide an important model to discover how the mammalian genome uses epigenetic mechanisms to regulate gene expression [2].

In conclusoon, genomic imprinting is important process of inheritance that plays important role in future genetic studies. It is a complex process that is based on DNA metylation in alleles of chromosomes. Numerous external cues influence DNA methylation, which may determine disease onset or progression. Genomic imprinting is a fairly rare phenomenon in humans, most genes are not imprinted, and most of studies are done in mice or plants, so we have a lot to do in this field. Although we do not yet know the precise mechanisms underlying epigenetic gene regulation in the pathogenesis of several diseases, there are finding that the progression of such diseases can be altered by modulating epigenetic programs.

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Book Review

Elizabeth Wager. GETTING RESEARCH PUBLISHED – An A to Z of Publication Strategy, Third Edition. Boca Raton, New York, London: CRC Press, Taylor & Francis Group, LLC, 2015. 188 pages; ISBN-13:978-1-78523-138-4 (Paperback) - CAT# K28669

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Key words: Scientific publishing, publication strategy, publication ethics.

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Abstract

PURPOSE: This book provides step-by-step guidance on developing a sound publication strategy for how to prepare and get research papers published. The book is a user-friendly guide, a route map for publishing that covers many topics, ranging from abstracts and blogs, tables and trial registration to ethical principles and conventions for writing scientific papers. Publishing the results of scientific research in the form of a scientific paper is the ultimate goal and the final stage of the research of each scientist. To write and publish papers is never going to be an easy task. With this book as their guide, researchers will be better informed and therefore should have an easier and altogether more pleasant path to publication with clear direction on how to choose the right journal, avoid publication delays, and resolve authorship disputes and many other problems associated with scientific publishing.

CONTENTS: The 188 pages of the book are distributed in 5 chapters in Part I and 249 entries ordered by the letters of Alphabet in Part II creating an A to Z of publication strategy. In the Appendices there are four sections covering further reading, organizations, guidelines and principles of good publication practice for company-sponsored medical research. The book also contains key references and useful websites within many entries where it seemed helpful. The last ten pages of the book present an index to help users to find the information of interest in the book.

CONCLUSION: The book is intended to help all authors, young and old, novice and experienced, to plan their research and publications effectively and prepare manuscripts for journals and other publications, increasing the likelihood that their work will be published. Providing essential information on publishing strategy and process, the book should be extremely useful to everyone who wants to publish research results.

Field of medicine: Scientific publishing in biomedicine

Audience: This lively and intelligent guide is primarily directed towards scientific or health-care professionals, students at all levels, medical and other professionals in biomedicine interested in publishing scientific results and observations from their professional work and experience. In particular, it is written for those who intend to carry out scientific research, especially young doctors and healthcare professionals carrying on specialization and students at all levels, from undergraduate to postgraduate. It is applicable to professionals in various clinical disciplines and public health who are involved in writing a research proposal, selecting a proper research strategy, conducting the research itself and submitting a final report. The book can also be useful as a guideline for all medical and other professionals in biomedicine in conducting and promoting their professional and research work by publishing papers in scientific journals.

Purpose: This book presents an original effort to summarize the relevant data and information about scientific publishing, obtained from authentic and highly regarded sources. It provides step-by-step guidance on developing a sound publication strategy and on the art and science of getting research papers published. The book is user-friendly and an easy-to-read-and-use guide, a route map for publishing that covers many topics, ranging from abstracts and blogs, tables and trial registration to ethical principles for writing scientific papers, conventions and often

unwritten rules of publishing in peer-reviewed journals and proceedings at conferences.

In the professional and academic environment it is necessary to continuously publish new findings and results of professional, research and academic work in order to achieve status and career advancement. Proper communication of scientific knowledge, ideas and new scientific discoveries, and knowing how to communicate in this technology-driven world for publishing papers in peer review journals is critically important. Publishing the results of scientific research in the form of a report (i.e. a scientific paper) is the ultimate goal and the final stage of the research of each scientist. The published scientific paper should be a reliable reference and lasting legacy, although it should always remain subject to review and criticism.

To write and publish papers is never going to be an easy task. It requires from scientists a sincere desire for knowledge, imagination and creativity, perseverance in the hard work and writing skills to prepare a scientific paper in accordance with internationally accepted principles and criteria for scientific communication. To write for journals and for other publications is mainly a learned activity and much more science than art. Good writing and producing a well-written article is learned through reading, imitation, repetition, and instruction. With this book as their guide, researchers will be better informed and therefore should have an easier and altogether more pleasant path to publication with clear direction on how to choose the right journal, avoid publication delays, and resolve authorship disputes and many other problems associated with scientific publishing.

Content: The 188 pages of the book are distributed in 5 chapters in Part I and 249 entries ordered by the letters of Alphabet in Part II creating an A to Z of publication strategy. In the Appendices there are four sections covering further reading, organizations, guidelines and principles of good publication practice for company-sponsored medical research (from GPP3). The book also contains key references and useful websites within many entries where it seemed helpful. The last ten pages of the book present an index to help users to find the information of interest in the book.

Highlights: The A to Z glossary format used to describe publication strategy is conceptualized upon contemporary knowledge, methods and methodological approaches in science and publishing. This format makes the relevant information easy to look for and easily accessible to readers with varying levels of publication experience and from different backgrounds. The book is a useful tool for individual authors or groups of 2-3 collaborators to understand the publication process in writing up their own small research projects and to avoid common pitfalls that often delay or prevent publication. It is also a helpful guide for authors of large-scale multicentre trials and explains the fundamentals of writing and publishing articles following the IMRAD structure. Furthermore, for undergraduate and postgraduate students and healthcare professionals with little or no experience of publishing, and for those who don't know what they don't know, the author has included an overview at the beginning of the book which contains a few longer sections to set the scene.

The publication reflects the rich scientific knowledge of the author as an active researcher, writer, teacher and advocate, with more than 20 years experience in publishing, teaching, editing and coordinating publications for drug companies and individual researchers. Her recommendations are evidence based where possible, and qualified accordingly when evidence is lacking.

Conclusion: The book should help all authors, young and old, novice and experienced, and even those who don't know what they don't know, to plan their research and publications effectively and prepare manuscripts for journals and other publications, increasing the likelihood that their work will be published. Providing essential information on publishing strategy and process, the book should be extremely useful, and I warmly and thoroughly recommend it to everyone who wants to publish research results. The book is reasonably priced and can be ordered from the publisher (CRC Press Taylor & Francis Group) at:

https://www.crcpress.com/Getting-Research-Published-An-A-Z-of-Publication-Strategy-Third-Edition/Wager/9781785231384?promo=LBP20