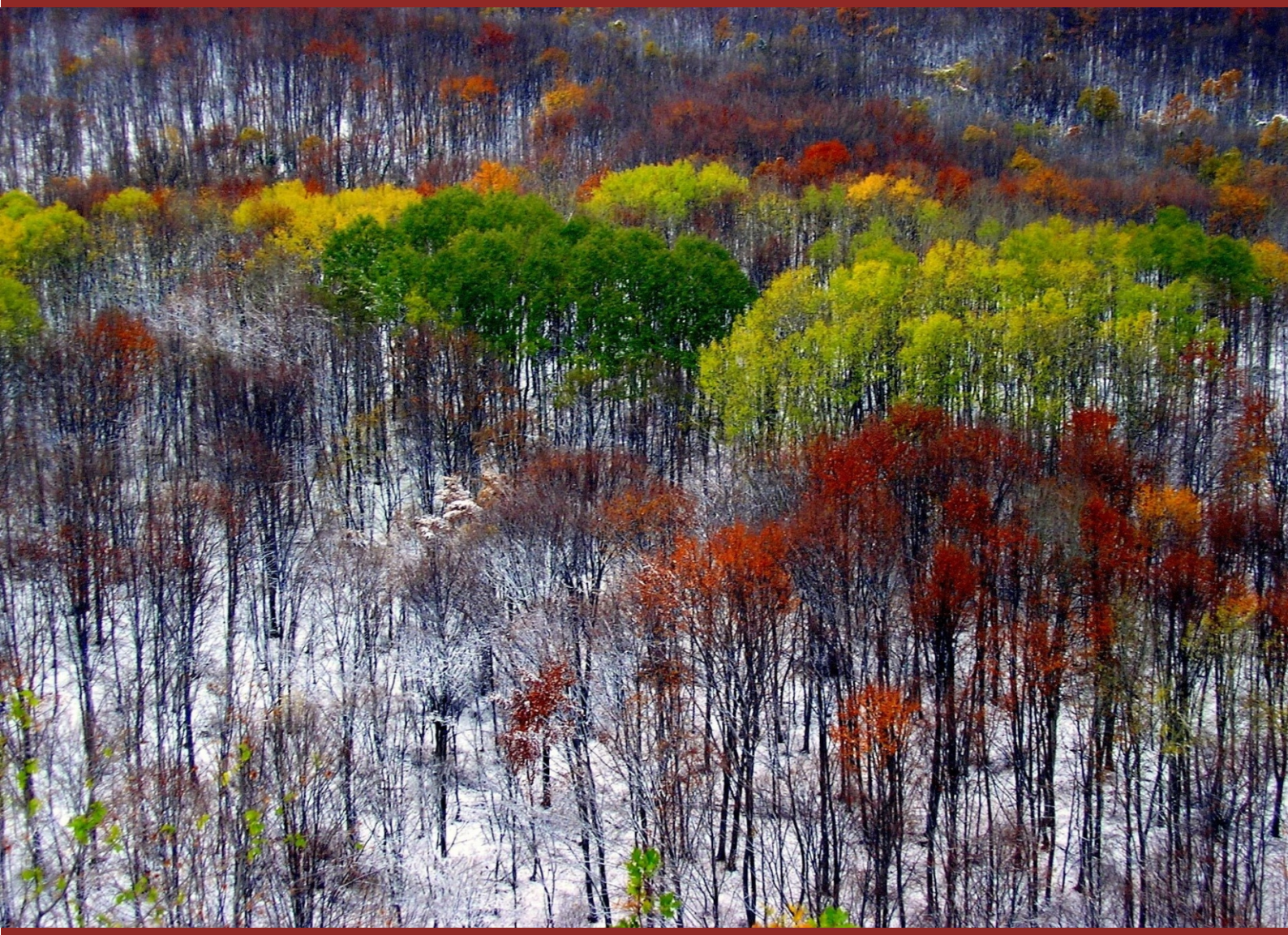
Vol 57, No 4, December, 2018
UDK 61
ISSN 0365-4478 (Printed)
ISSN 1821-2794 (Online)
www.medfak.ni.ac.rs/amm

ACTA MEDICA MEDIANAE

Naučni časopis
Medicinskog fakulteta Univerziteta u Nišu i
Podružnice Srpskog lekarskog društva u Nišu



AMM

Scientific Journal of the University of Nis Faculty of Medicine
and the Department of the Serbian Medical Society in Niš

Naučni časopis Medicinskog fakulteta Univerziteta u Nišu i Podružnice Srpskog lekarskog društva u Nišu

Scientific journal of the University of Niš Faculty of Medicine and the Department of the Serbian Medical Society in Niš

Acta Medica Medianae
Vol 57, No 4, December 2018
UDK 61 ISSN 0365-4478 (Printed version)
ISSN 1821-2794 (Online)
http://www.medfak.ni.ac.rs/amm

Acta Medica Medianae (UDK 61; ISSN 0365-4478 štampana verzija; ISSN 1821-2794 elektronska verzija) je zvanični časopis Medicinskog fakulteta Univerziteta u Nišu i Podružnice Srpskog lekarskog društva u Nišu pod pokroviteljstvom Ministarstva za nauku i tehnološki razvoj Republike Srbije. Časopis izlazi četiri puta godišnje od 1962 godine. Izdavač je Medicinski fakultet Univerziteta u Nišu, Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija. Sadržaj i celokupan tekst časopisa dostupan je na sajtu Medicinskog fakulteta http://www.medfak.ni.ac.rs/amm. Godišnja pretplata: za inostranstvo 60 USA dolara, za ustanove 2500 dinara i za pojedince 1500 dinara. Sredstva uplatiti na žiro račun Medicinskog fakulteta u Nišu br. 840-1681666-03, sa naznakom za *Acta Medica Medianae*. Uputstvo autorima se objavljuje u svakom broju, pri čemu je autor dužan da se pridržava navedenih uputstava prilikom predaje rukopisa. Radovi se mogu slati u elektronskom formatu na adresu: acta@medfak.ni.ac.rs. Naknada za štampanje rada iznosi 1000 dinara za autora, a 500 dinara za koautore, za svaki prihvaćeni rad. *Acta Medica Medianae* zadržava pravo dalje distribucije i štampanja radova.
Kontakt adresa: Časopis Acta Medica Medianae, Medicinski fakultet, Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: acta@medfak.ni.ac.rs
Tel+381-18-4533001 lok. 122 fax. +381-18-4534336
Tiraž 200 primeraka. Štampa: "Galaksijanis", Lukovo, Svrljig, Srbija.

Acta Medica Medianae je trenutno indeksirana na *Index Copernicus-u, Srpskom citatnom indeksu, DOAJ i EBSCO*

Acta Medica Medianae (UDK 61; ISSN 0365-4478 printed version; ISSN 1821-2794 online) is the official Journal of the University of Niš Faculty of Medicine and the Department of the Serbian Medical Society in Niš published with the help of the Ministry of Science and Technological Development of the Republic of Serbia. The Journal has been published four times a year since 1962. The publisher is the University of Niš Faculty of Medicine, Institutional address: dr Zoran Đinđić 81, 18000 Niš, Serbia. Table of contents and full texts of articles are available on the Institutional Home Page at http://www.medfak.ni.ac.rs/amm. Prices are subject to change. All subscriptions start with the first issue of the current year. For payment details contact the Secreteriat at acta@medfak.ni.ac.rs. Instructions for authors appear in every issue. Manuscripts accepted for publication are not returned to the author(s). *Acta Medica Medianae* retains the right for further distribution and printing of the articles.
Editorial correspodence: Journal Acta Medica Medianae, Faculty of Medicine, Dr Zoran Đinđić 81, 18000 Niš, Serbia.
Electronic submission of the papers: acta@medfak.ni.ac.rs
Phone: +381-18-4533001 lok. 113 fax. +381-18-4534336
Printed on acid-free paper; 200 issues. Press: "Galaksijanis", Lukovo, Svrljig, Serbia

Acta Medica Medianae is currently indexed in Index Copernicus, Serbian Citation Index, DOAJ and EBSCO

Naučni časopis Medicinskog fakulteta Univerziteta u Nišu i Podružnice Srpskog lekarskog društva u Nišu

Scientific journal of the University of Niš Faculty of Medicine and the Department of the Serbian Medical Society in Niš

Acta Medica Medianae
Vol 57, No 4, December 2018
UDK 61 ISSN 0365-4478 (Printed version)
ISSN 1821-2794 (Online)
http://www.medfak.ni.ac.rs/amm

Autor slike na prednjoj stranici: Vukan Milojević

ACTA MEDICA MEDIANAE

Vol 57, No 4, December, 2018

www.medfak.ni.ac.rs/amm

ACTA MEDICA MEDIANAE

Vol 57, No 4, December, 2018

Secretariat

www.medfak.ni.ac.rs/amm

ACTA MEDICA MEDIANAE

Vol 57, No 4, Decembar, 2018

www.medfak.ni.ac.rs/amm

ACTA MEDICA MEDIANAE

Vol 57, No 4, Decembar, 2018

Uredništvo

www.medfak.ni.ac.rs/amm

Original article **UDC: 616.8-009.1:[577.334:542.943`78**
doi:10.5633/amm.2018.0401

SERUM STATUS OF ENDOGENOUS ANTIOXIDANT MARKERS: BILIRUBIN, ALBUMINS, TOTAL PROTEINS AND CREATININE IN MYASTHENIA GRAVIS PATIENTS

Aleksandar Stojanov[1], Gordana Djordjević[1,2], Srdjan Ljubisavljević[1,2], Jelena Stojanov[3]

There is a lot of evidence pertaining to the ethiopathogenetic importance of oxidative stress in a number of autoimmune diseases, including some immune-mediated neurological diseases such as multiple sclerosis. However, the role of oxidative stress and oxidative status in patients with myasthenia gravis is still an under-researched area.

The aim of our research was to compare serum total and direct bilirubin, albumin, total proteins and creatinine levels in myasthenia gravis (MG) patients with healthy controls, and patients with multiple sclerosis (MS).

The subjects were divided into three groups (92 MG patients, 68 healthy controls and 74 MS patients). All MG patients were newly diagnosed, classified with MGFA Clinical Classification, and divided into two groups regarding onset age (early < 50 years , late ≥50 years), sex (male, female), thymus pathology (present, absent).

Serum antioxidant status was significantly lower in MG and MS group compared to the healthy controls ($p < 0.05$). There was no significant difference in serum antioxidant status between patients with MG and those with MS. Regarding MGFA Classification we have not found any correlation with serum levels of measured parameters.

Our findings suggested that there was a potential role of oxidative process in MG pathology. Among the analyzed parameters, direct bilirubin showed significantly lower value in women, the elderly and in the group of MG patients with pathologically altered thymus gland.

Acta Medica Medianae 2018;57(4):05-13.

Key words: *myasthenia gravis, serum antioxidants, oxidative stress*

[1]Clinic of Neurology, Clinical Center Niš, Serbia
[2]University in Niš, Faculty of Medicine, Niš, Serbia
[3]Special Psychiatric Hospital "Gornja Toponica", Niš, Serbia

Contact: Aleksandar Stojanov
32 Ozrenska St., 18000 Niš, Serbia
E-mail: astojanov1986@gmail.com

Introduction

Oxidative stress (OS) is a disorder which appears as the result of prooxidant and antioxidant balance disturbance. When the free radical production overrides antioxidant capacity there is damage to cellular metabolism and cell structures (proteins, lipids, nucleic acids), which consequently results in cell death by necrosis and apoptosis. Antioxidants are substances that delay or prevent the oxidation of a substrate, thereby removing the chain reaction that creates the reactive oxidative species (ROS) (1). The previous research in the field of autoimmune and inflammatory diseases, suggested an important role of oxidative stress in the etiopathogenesis of these diseases, e.g. multiple sclerosis (MS) (2,3).

Chronic acquired myasthenia gravis (MG) is an antigen-specific autoimmune disease of postsynaptic neuromuscular membrane, which is characterized by the impairment of neuromuscular transmission, usually mediated by antibodies against the nicotinic acetylcholine receptor (4). Myasthenia gravis is etiopathogenically a well defined autoimmune disease, but correlation between MG and OS is still insufficiently explored area. Sporadic results suggested that excessive production of free radicals can cause the inactivation of nicotinic acetylcholine receptors and damage to the neuromuscular junction (5, 6). There is also evidence of oxidative stress in other neuromuscular disorders (7).

The results indicate a significant role of bilirubin, creatinine and albumin in the antioxidant protection system. There are studies that show pronounced antioxidant activity of bilirubin (8). Furthermore, this molecule plays an important immunomodulatory role (9). Albumin is a major antioxidant in plasma, which is mainly based on its role in the binding of different molecules (10), and values of total proteins are signi-

ficantly lower in the plasma that has been treated with ROS sources (11). Creatinine is normally found as an energy depot in skeletal muscle, and has a role in the overall antioxidant status in serum and in reducing global oxidative stress (12).

The aim

The aim of our research was to evaluate serum levels of total and direct bilirubin, albumins, total proteins and creatinine as antioxidative status parameters in myasthenia gravis patients and to compare these values with those in healthy control group. Also, our objective was to liken values of these parameters in patients with another autoimmune neurological disease, such as multiple sclerosis, and finally, to perceive the obtained values of tested parameters in relation of different clinical and paraclinical patients characteristics (sex, age of onset, thymus pathology, disease classification).

Material and methods

The study included patients older than 18 years, suffering from acquired autoimmune myasthenia gravis, those hospitalized in the Clinic of Neurology, Clinical Center Niš, from January 2012 to December 2016, with a previous history of complaints for a period not longer than one year. The diagnosis of MG was based on the patients' history, physical examination, a prostigmine test, repetitive nerve stimulation test results, and the exclusion of other possible causes of symptoms, according to currently used criteria. We divided patients with MG into groups according to sex, age at the time of the disease onset (< 50 years, ≥ 50 years) and the presence of pathologically altered thymic gland. Severity of the disorder was expressed according to the classification of the Myasthenia gravis foundation of America (MGFA Classification) at the time of serum sampling.

All subjects with previous history, clinical or laboratory findings which indicate renal or liver failure, diabetes mellitus, gout or some neurodegenerative disorder, were excluded. One control group consisted of healthy subjects, nonsmokers, with no evidence of the existence of autoimmune diseases or other diseases with proved pathogenic role of oxidative stress (e.g. diabetes mellitus, gout). The second control group consisted of newly diagnosed patients suffering from multiple sclerosis, in which disease diagnosis was made on the basis of the revised McDonald criteria of 2010, and at the time the study was performed disease was clinically presented as exacerbated.

Serum samples were taken in all subjects in the early morning, after 12 hours of fasting. Immediately after sampling and sample-labeling, they were sent to a laboratory for further testing. Serum total bilirubin (normal range 5.0-21.0 μmol/L), direct bilirubin (normal range 0-3.4 μmol/L), albumin (normal range 35-53 g/L), total proteins (62-81 g/L) and creatinine (normal range 53-115μmol/L) were measured using an AU680 Chemistry Analyzer (Beckman and Coulter, Switzerland). At the same time, aspartate transaminase (AST), alanine transaminase (ALT), blood urea nitrogen and blood sugar concentrations were also measured, to exclude patients with hepatic failure, renal failure and diabetes mellitus.

All data were statistically processed by IBM SPSS statistical software (version 21) for Windows operative system. Statistically significant were considered results with p values less than 0.05. Numerical data are presented as mean ± SD. All statistical calculations were performed using appropriated parametric or nonparametric tests after verification of values distribution in each group. The comparison between subgroups was performed using the Kruskal-Wallis test.

Results

The serum samples were collected from 92 patients with MG, 68 healthy control individuals, and 72 patients with MS whose demographic characteristics (sex, age of onset) are displayed in Table 1. The patients with MG were divided in two groups, according to MSCT findings of the thymus gland (present or absent pathologic findings - thymoma and lymphoid thymus hyperplasia) as presented in Table 2. Based on the clinical assessment and data from the patients' history, all patients were classified according MGFA classification (Table 3).

The serum levels of total bilirubin, direct bilirubin, albumin, total proteins and creatinine were significantly lower in MG and MS group compared to the healthy control group ($p < 0.05$). There was no significant difference of the investigated parameters concentrations between patients with MG and those with MS ($p > 0.05$) (Table 4; Graph 1, 2, 3).

Regarding the age of onset, we found significantly lower values of creatinine in the early onset group ($p < 0.05$), and lower values of direct bilirubin, albumines and total proteins in patients with the late onset of disease ($p < 0.05$). The serum levels of total bilirubin, albumin and total proteins showed no difference ($p > 0.05$) between the patients of different sexes. The values of creatinine and direct bilirubin were significantly lower in the female group ($p < 0.05$).

Regarding thymus pathology (absent / present), we have not found any correlation to the serum levels of the measured parameters ($p > 0.05$), with an exception of direct bilirubin which levels were significantly lower in the group with present thymus pathology ($p <0 .05$). There was no statistical significance for measured parameters between groups of patients on the basis of MGFA Classification, ($p > 0.05$) (Tables 5 and 6).

Table 1. The number of examinees based on sex and age at the beginning of the disease

	N	Female	Male	<50	≥ 50
Myasthenia gravis	92	56	36	50	42
Healthy control	68	40	28	40	28
Multiple sclerosis	74	43	31	54	20

Table 2. The division of Myasthenia gravis patients in relation to the thymus pathology

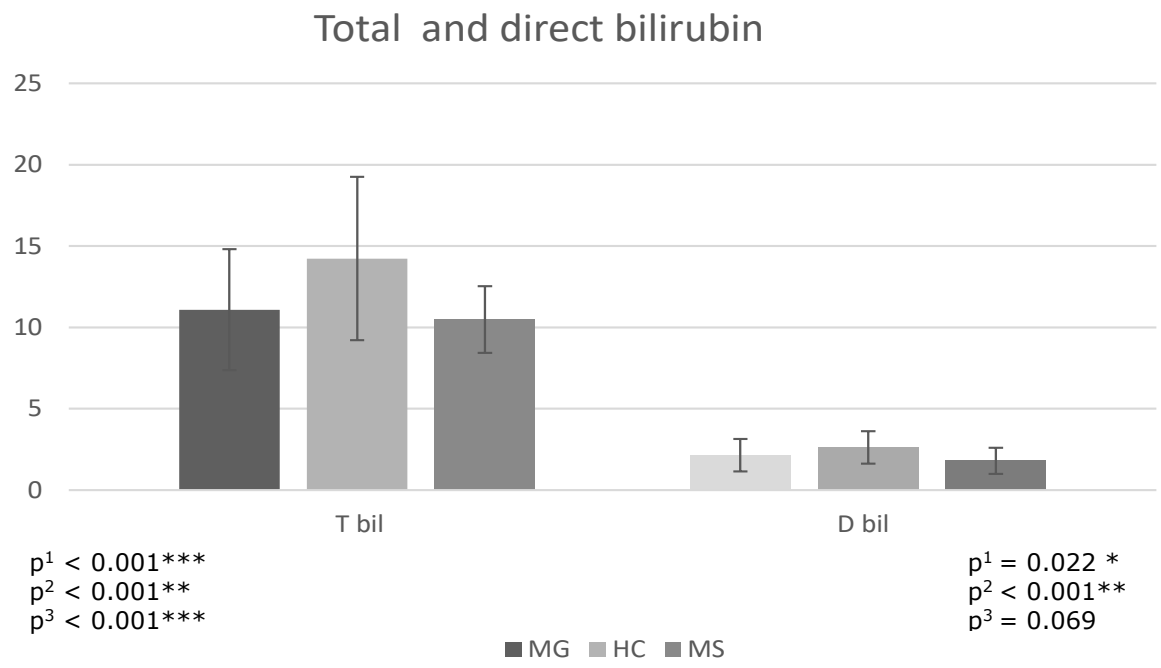
		N	Female	Male
Thymus pathology	Absent	24	14	10
	Present	68	42	26

Table 3. Number of patients according to the MGFA classification

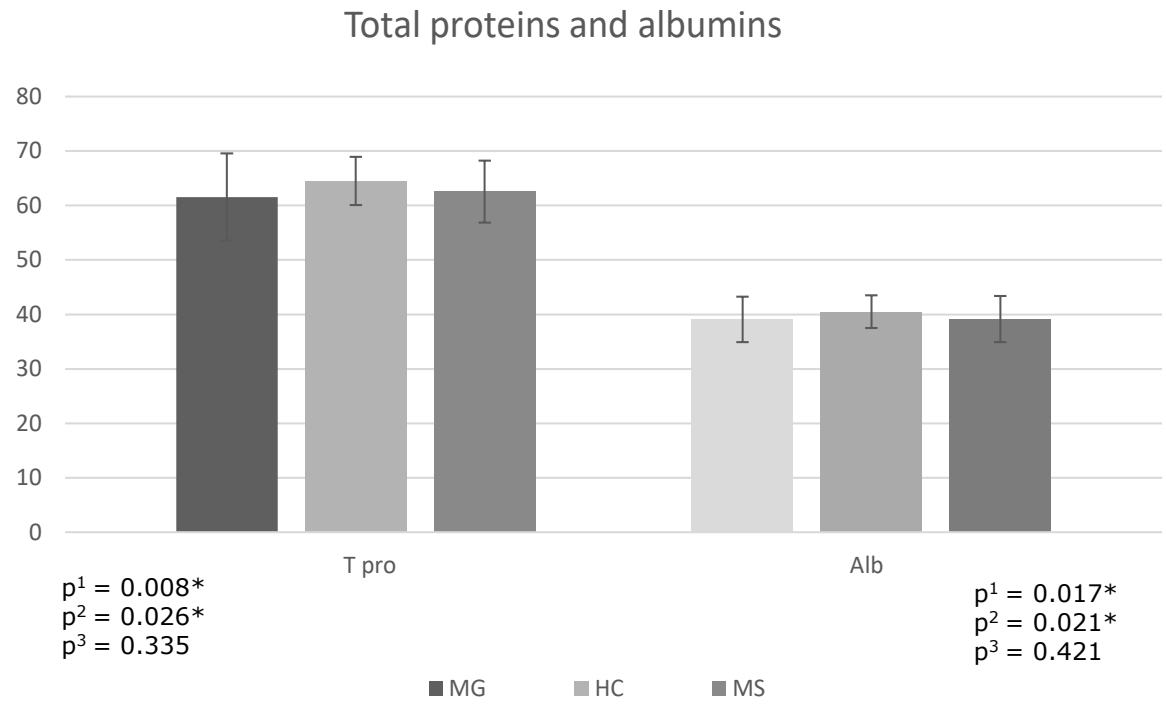
Class	Description	Number of patients
Class I	Any ocular muscle weakness. May have weakness of eye closure. All othermuscle strength is normal.	19
Class II	Mild weakness affecting other than ocular muscles. May also have ocular muscle weakness of any severity.	
IIa	Predominantly affecting limb, axial muscles, or both. May also have lesser involvement of oropharyngeal muscles.	22
IIb	Predominantly affecting oropharyngeal, respiratory muscles, or both. May alsohave lesser or equal involvement of limb, axial muscles, or both.	14
Class III	Moderate weakness affecting other than ocular muscles. May also have ocular muscle weakness of any severity	
IIIa	Predominantly affecting limb, axial muscles, or both. May also have lesser involvement of oropharyngeal muscles.	15
IIIb	Predominantly affecting oropharyngeal, respiratory muscles, or both. May also have lesser or equal involvement of limb, axial muscles, or both.	12
Class IV	Severe weakness affecting other than ocular muscles. May also have ocular muscle weakness of any severity.	
IVa	Predominantly affecting limb and/or axial muscles. May also have lesser involvement of oropharyngeal muscles.	4
IVb	Predominantly affecting oropharyngeal, respiratory muscles, or both. May also have lesser or equal involvement of limb, axial muscles, or both.	4
Class V	Defined by intubation, with or without mechanical ventilation, except when used during routine postoperative management.	2

Table 4. Values of total and direct bilirubin, albumin, total proteins and creatinine in serum

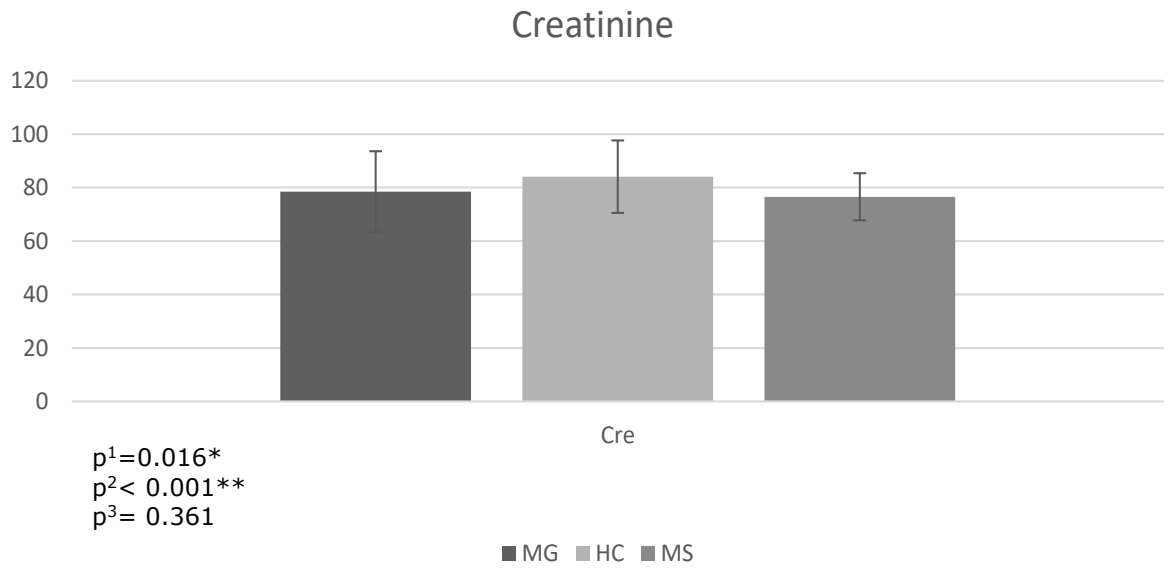
	Total bilirubin (μmol/L)	Direct bilirubin (μmol/L)	Albumin (g/L)	Total protein (g/L)	Creatinine (μmol/L)
MG	11.09 ± 3.72	2.14 ± 1.34	61.56 ± 8.13	39.08 ± 4.16	78.50 ± 15.11
HC	14.23 ± 5.02	2.63 ± 1.30	64.5 ± 44.44	40.50 ± 3.02	84.13 ±13.55
MS	10.49 ± 2.75	1.80 ± 0.91	62.56 ± 5.71	39.14 ± 4.26	76.68 ± 8.82
P^1(MG vs. HC)	< 0.001	0.022	0.008	0.017	0.016



Grapf 1. Serum total and direct bilirubin levels (μmol/L) in MG and MS patients and in healthy control (HC) group (p1= MG versus HC; p2 = HC versus MS; p3 = MS versus MG; p < 0.05*; p < 0.001**)



Grapf 2. Serum albumin, and total protein levels (g/L) in MG and MS patients and in healthy control (HC) group (p1= MG versus HC; p2= HC versus MS; p3= MS versus MG; p<0.05*; p<0.001**)



Grapf 3. Serum creatinine levels (μmol/L) in MG and MS patients and in healthy control (HC) group (p1= MG versus HC; p2= HC versus MS; p3= MS versus MG; p<0.05*; p<0.001**)

Table 5. Correlation between serum total and direct bilirubin levels with the age of onset, sex, MGFA clinical classification, and thymus pathology in patients with MG

	Total bilirubin (μmol/L)			Direct bilirubin (μmol/L)		
Variables	Mean	SD	P	Mean	SD	P
Age of onset			0.119			0.024*
< 50 (n = 50)	11.7	3.4		2.5	1.9	
≥ 50 (n = 42)	10.7	4.3		1.8	1.1	
Sex			0.074			0.013*
male (n = 36)	12.0	3.7		2.8	2.1	
female (n = 56)	10.8	4.0		1.8	1.1	
MGFA			0.275			0.058
I (n = 19)	11.3	2.8		2.2	1.1	
IIa (n = 22)	11.4	5.6		2.1	1.5	
IIb (n = 14)	10.9	2.1		2.0	0.9	
IIIa (n = 15)	9.9	2.8		1.7	1.1	
IIIb (n = 12)	10.5	2.5		2.2	1.8	
IVa (n = 4)	13.3	6.4		3.0	2.2	
IVb (n = 4)	12.0	2.5		2.4	1.1	
V (n=2)	16.3	5.4		5.3	5.6	
Thymus pathology			0.054			0.020*
present (n = 68)	10.0	2.3		1.65	0.7	
absent (n = 24)	11.7	4.3		2.43	1.8	

Table 6. Correlation between serum creatinine, albumin and protein levels with the age of onset, sex, MGFA clinical classification, and thymus pathology in patients with MG

	Creatinine (μmol/L)			Proteins (g/L)			Albumin (g/L)		
Variables	Mean	SD	P	Mean	SD	P	Mean	SD	P
Age of onset			0.011*			0.013*			0.004*
< 50 (n = 50)	82.0	14.4		59.6	7.8		37.9	4.0	
≥ 50 (n = 42)	74.8	12.0		63.8	8.0		40.4	3.9	
Sex			> 0.001*			0.902			0.212
male (n = 36)	85.3	13.4		61.7	7.8		38.4	4.0	
female (n = 56)	74.5	12.4		61.5	8.4		39.5	4.2	
MGFA			0.327			0.545			0.158
I (n = 19)	78.3	13.3		61.8	9.5		39.1	3.8	
IIa (n = 22)	75.7	12.8		61.8	7.6		39.9	3.9	
IIb (n = 14)	81.0	10.9		60.3	10.0		37.8	4.2	
IIIa (n = 15)	76.7	16.4		64.0	6.3		40.9	4.2	
IIIb (n = 12)	86.1	24.9		59.7	5.9		38.7	3.1	
IVa (n = 4)	81.3	30.1		55.8	7.0		33.7	3.1	
IVb (n = 4)	81.2	22.0		66.7	9.6		39.8	6.2	
V (n=2)	99.8	14.0		59.5	10.4		37.7	6.0	
Thymus pathology			0.398			0.082			0.102
present (n = 68)	78.0	13.1		60.7	8.1		28.6		
absent (n = 24)	80.8	15.8		64.0	7.8		40.2		

Discussion

The link between MG and OS has not yet been established, but there is evidence of low antioxidative status in MG, including lower levels of creatinine, bilirubin, albumin, uric acid compared to healthy controls (13).

Lower serum bilirubin concentrations were noted in patients with MG compared to the healthy subjects, and significantly lower values in the females were observed, but no differences in respect to the modified Osserman classification (14) which corresponds to our results (Table 5). In our study, the value of direct bilirubin showed significantly lower values in women, late onset patients with MG and in patients with pathologically altered thymus.

The research conducted by Weng et al. showed that values of serum albumin were lower in patients with MG in correlation with values of healthy subjects and that it correlated with the degree of clinical presentation, so they could serve as a biomarker for the severity of the disease (15). Our research has not demonstrated a statistically significant difference in the value of the albumin related to the MGFA classification (Table 6). The ratio of serum albumin and clinical manifestation could be considered in the context of monitoring course of the disease and response rate for an individual patient. In here presented study, serum albumin and total proteins were significantly lower in late onset patients, which can indicate the significance of aging process for the mechanism of the total antioxidant defense decrease.

The contribution of creatinine in total antioxidant capacity is recorded (16). Our results showed lower values of creatinine in the patients suffering from MG and MS, indicating the reduced antioxidant capacity in these autoimmune neurological diseases (Table 4). Creatinine values were significantly lower in the female group, which was expected. In our study, only the creatinine values were lower in the early onset patients (for total bilirubin there was no statistical significance between the groups and for direct bilirubin, total proteins and albumins the values were lower in the late onset patients) (Table 5 and 6), which is consistent with the results of some previous studies on the impact of aging on the serum creatinine values (17).

In this study, the analysis of total bilirubin, albumin, total proteins and creatinine showed no statistically significant difference between the patients with and without changes in the thymus, which indicates that antioxidant protection is not correlated with the presence of pathology of the thymus (Table

5 and 6). It should be emphasized that this was not the case with all tested biomarkers. Direct bilirubin levels were significantly lower in the group of patients with pathologically altered thymus. The question arises as to whether the altered bilirubin values are phenomenon associated with thymus pathology within the myasthenia gravis, or may have a primary pathogenetic significance in the development of thymus pathology. In this regard, further research is needed.

Yang et al. (13) found a statistically significant difference in the antioxidant status between patients classified by MGFA classification, while the research conducted by Fuhua et al. (18) has not recorded the same differences. Our results showed no significant statistical difference in any of the tested parameters compared to MGFA classification (Table 5 and 6). The lack of correlation could be explained by insufficient statistical pattern (the small number of patients in IVa, IVb and V group according to MGFA classification).

Oxidative stress has an impact on the activation of the complement system, which is the most important pathophysiological pathway that compromises neuromuscular transmission in patients with MG (19). Further studies on the connection between MG and oxidative stress can be guided in that direction. The recent studies have shown elevated values of oxidative and gluco-oxidative modifications in serum proteins of patients with MG (20). Further monitoring of oxidative stress parameters in these patients is necessary, especially in order to define appropriate biomarkers of the severity of the disease. As an advantage of our research, we emphasize the fact that to our knowledge, this is the first trial of creatinine, bilirubin, albumins and total proteins as antioxidant parameters (and the parameters of OS in general) in patients with MG, which has been done on a group of patients outside the Asian continent.

Conclusion

The results of our research showed significantly lower values of all investigated parameters in MG group related to the healthy control group, which could suggest a potential role of oxidative process in MG pathology. Among the analyzed parameters, direct bilirubin showed significantly lower value in women, in patients with the late onset of the disease, as well as in the group of MG patients with pathologically altered thymus gland. None of the investigated parameters correlated with severity of the clinical manifestation, so further research is needed in order to define a biomarker of oxidative stress as a potential marker of clinical progression in patients suffering from autoimmune acquired myasthenia gravis.

References

1. Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br J Pharmacol 2004; 142:231-55. [CrossRef] [PubMed]
2. Brambilla D, Mancuso C, Scuderi MR, Bosco P, Cantarella G, Lempereur L, et al. The role of antioxidant supplement in immune system, neoplastic, and neurodegenerative disorders: a point of view for an assessment of the risk/benefit profile. Nutr J 2008; 7:29-37. [CrossRef] [PubMed]
3. Gironi M, Borgiani B, Mariani E, Cursano C, Mendozzi L, Cavarretta R, et al. Oxidative stress is differentially present in multiple sclerosis courses, early evident and unrelated to treatment. J Immunol Res 2014; 2014: 961863 [CrossRef] [PubMed]
4. Gilhus NE, Vrschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. Lancet Neurol 2015; 14(10):1023-36. [CrossRef] [PubMed]
5. Venkatesham A, Sharath BP, Vidya SJ, Krishna D. Effect of reactive oxygen species on cholinergic receptor function. Indian J Pharm 2005; 6:366-70.
6. Krishnaswamy A, Cooper E. Reactive oxygen species inactivate neuronal nicotinic acetylcholine receptors through a highly conserved cysteine near the intracellular mouth of the channel: implications for diseases that involve oxidative stress. Physiol 2012; 590 (1): 39-47. [CrossRef] [PubMed]
7. Stuerenburg HJ. The roles of carnosine in aging of skeletal muscle and in neuromuscular diseases. Biochemistry (Mosc) 2000; 65:862-5. [PubMed]

8. Jangi S, Otterbein L, Robson S. The molecular basis for the immunomodulatory activities of unconjugated bilirubin. Int J Biochem Cell Biol 2013; 45:2843-5. [CrossRef] [PubMed]
9. Peng F, Deng X, Yu Y, Chen X, Shen L, Zhong X, et al. Serum bilirubin concentrations and multiple sclerosis. J Clin Neurosci 2011; 18:1355-9. [CrossRef] [PubMed]
10. Roche M, Rondeau P, Singh NR, Tarnus E, Bourdon E. The antioxidant properties of serum albumin. FEBS Lett 2008; 582:1783-7. [CrossRef] [PubMed]
11. Tetik S, Kilic A, Aksoy H, Rizaner N, Ahmad S, Yardimci T. Oxidative stress causes plasma protein modification. Indian J Exp Biol 2015; 53(1):25-30. [PubMed]
12. Jansen EH, Beekhof PK, Cremers JW, Viezeliene D, Muzakova V, Skalicky J. Long-term stability of parameters of antioxidant status in human serum. Free Radic Res 2013; 47:535-40. [CrossRef] [PubMed]
13. Yang D, Su Z, Wu S, Bi Y, Li X, Li J, et al. Low antioxidant status of serum bilirubin, uric acid, albumin and creatinine in patients with myasthenia gravis. Int J Neurosci 2016; 126:1120-6. [CrossRef] [PubMed]
14. Zhou X, Sun ZW. Changes of serum bilirubin and uric acid in patients with myasthenia gravis. Zhonghua Yi Xue Za Zhi 2013; 93:1287-91. [PubMed]
15. Weng Y, Yang D, Qian M, Wei MM, Yin F, Li J, et al. Low serum albumin concentrations are associated with disease severity in patients with myasthenia gravis. Medicine 2016; 95(39):e5000. [CrossRef] [PubMed]
16. Nyasavajjala SM, Phillips BE, Lund JN, Williams JP. Creatinine and myoglobin are poor predictors of anaerobic threshold in colorectal cancer and health. J Cachex Sarcopenia Muscle 2015; 6:125-31. [CrossRef] [PubMed]
17. Tiao JY, Semmens JB, Masarei JR, Lawrence-Brown MM. The effect of age on serum creatinine levels in an aging population: relevance to vascular surgery. Cardiovasc Surg 2002; 10(5):445-51. [CrossRef] [PubMed]
18. Fuhua P, Xuhui D, Zhiyang Z, Ying J, Yu Y, Feng T, et al. Antioxidant status of bilirubin and uric acid in patients with myasthenia gravis. Neuroimmuno-modulation 2012; 19:43-9. [CrossRef] [PubMed]
19. Collard CD, Lekowski R, Jordan JE, Agah A, Stahl GL. Complement activation following oxidative stress. Molecular Immunology 1999; 36:941-8. [CrossRef] [PubMed]
20. Adamczyk-Sowa M, Bieszczad-Bedrejczuk E, Galiniak S, Rozmiłowska I, Czyżewski D, Bartosz G, et al. Oxidative modifications of blood serum proteins in myasthenia gravis. Journal of Neuroimmunol 2017; 305: 145-53. [CrossRef] [PubMed]

Originalni rad **UDC: 616.8-009.1:[577.334:542.943`78**
doi:10.5633/amm.2018.0401

STATUS SERUMSKIH ENDOGENIH ANTIOKSIDATIVNIH MARKERA: BILIRUBINA, ALBUMINA, UKUPNIH PROTEINA I KREATININA KOD BOLESNIKA OBOLELIH OD MIJASTENIJE GRAVIS

Aleksandar Stojanov[1], Gordana Đorđević[1,2], Srđan Ljubisavljević[1,2], Jelena Stojanov[3]

[1]Klinika za neurologiju, Klinički centar Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[3]Specijalna bolnica za psihijatrijske bolesti "Gornja Toponica", Niš, Srbija

Kontakt: Aleksandar Stojanov
Ozrenska 32, 18000 Niš, Srbija
E-mail: astojanov1986@gmail.com

Postoje brojni dokazi koji potvrđuju etiopatogenetski značaj oksidativnog stresa kod autoimunih bolesti, uključujući i neke imuno-posredovane neurološke bolesti, poput multiple skleroze. Međutim, uloga oksidativnog stresa i oksidativni status kod bolesnika sa mijastenijom gravis i dalje je nedovoljno istraženo područje.

Cilj našeg rada bio je uporediti nivoe antioksidativnih markera u serumu: ukupnog i direktnog bilirubina, albumina, ukupnih proteina i kreatinina, kod obolelih od mijastenije gravis (MG) sa rezultatima dobijenim u kontrolnoj grupi zdravih ispitanika i bolesnika obolelih od multiple skleroze (MS).

Ispitanici su podeljeni u tri grupe (92 bolesnika sa MG, 68 zdravih ispitanika i 74 bolesnika sa MS). Svi bolesnici sa MG su bili novodijagnostikovani, klasifikovani prema MGFA klasifikaciji i bili su podeljeni u grupe u odnosu na godine početka tegoba (rani početak < 50 godina, kasni početak ≥ 50 godina), polu i patološki izmenjenom timusu (prisutno / odsutno).

Nivoi ispitivanih antioksidativnih markera u serumu bili su značajno niži u grupi bolesnika sa MG i MS u odnosu na zdrave kontrole ($p < 0,05$). Nije bilo statistički značajne razlike između MG i MS grupe. U odnosu na MGFA klasifikaciju, nije evidentirana korelacija sa serumskim nivoima ispitavnih parametara.

Naši rezultati ukazuju na potencijalnu ulogu oksidativnog stresa u patogenezi mijastenije gravis. Među ispitivanim parametrima kod bolesnika obolelih od mijastenije gravis vrednosti direktnog bilirubina su bile značajno niže kod žena, starijih bolesnika i bolesnika sa patološki izmenjenim timusom.

Acta Medica Medianae 2018;57(4):05-13.

Ključne reči: *mijastenija gravis, serumski antioksidansi, oksidativni stres*

Original article

UDC: 537-962:[612.438:636.028
doi:10.5633/amm.2018.0402

THE EFFECT OF MELATONIN ON THE CATABOLISM OF POLYAMINES IN THE RAT THYMUS DURING THE EXPOSURE TO MICROWAVE RADIATION

Dušan Sokolović[1], Boris Djindjić[1,2], Dejan Krstić[3], Vera Marković[4], Goran Ristić[4], Danka M. Sokolović[5], Mladjan Golubović[2], Branka Djordjević[1], Momir Dunjić[6], Dejan Popović[1], Tamara Karuntanović[1], Nikola Tatar[7], Petar Babović[8]

The toxic effect of microwave radiation (MW) causes the change in the metabolism of polyamines. Polyamines (spermine and spermidine) and their precursor, diamin putrescine, are non-protein nitrogenous bases and they are essential to the function of the cell. Spermine and spermidine are catabolized by the enzyme polyamine oxidase (PAO), while the catabolism of putrescine is under the effect of the diamine oxidase (DAO). The neurohormon melatonin participates in maintaining the normal function of the immune system. The aim of this study was to analyze the effect of melatonin on the catabolism of polyamines in the rat thymus, following the chronic microwave exposure. Wistar rats were divided into four experimental groups: 1) control group, 2) Mel - the animals which were given melatonin daily (2mg/kg), 3) MW - the animals which were exposed to MW (4h daily), 4) the animals which were exposed to MW and were given melatonin daily. The animals were sacrificed after 20, 40 and 60 days of the experiment. There was an increase in the PAO activity and decrease in the DAO activity (already after 20 days) in comparison to the control in the thymus of rats exposed to microwave radiation. There was also a statistically significant positive correlation ($p < 0.05$) between malondialdhehyde levels and the activity of PAO during the MW exposure. A significant decrease in both PAO and DAO activity was found in the thymus of animals exposed to MW and treated with melatonin, in comparison to the irradiated animals not treated with melatonin.

Acta Medica Medianae 2018;57(4):14-21.

Key words: *melatonin, microwave radiation, thymus, polyamine oxidase, diamine oxydase*

[1]University of Niš, Faculty of Medicine, Serbia
[2]Clinical Center, Niš, Serbia
[3]University of Niš, Faculty of Occupational Safety, Serbia
[4]University of Niš, Faculty of Electronic Engineering, Serbia
[5]Institute for Blood Transfusion in Niš, Serbia
[6]European University, Novi Sad, Serbia
[7]University of Niš, Faculty of Philosophy, Serbia
[8]University of Niš, Faculty of Medicine, PhD Student, Niš, Serbia

Contact: Dušan Sokolović
Zoran Djindjić blvd. 81, 18000 Niš, Srbija
E-mail: soko@medfak.ni.ac.rs

Introduction

In recent years, the modern lifestyle has led to the increase in the number of microwave (MW) emitters, not only in the workplace but also in the proximate home environment. The most common MW emitters in our environment are mobile phones, cellular repeaters, WI-FI devices, satellite mobile communication devices, radio transmitters, microwave heating devices and radar facilities. The effects on the human health caused by exposure to electromagnetic fields from mobile phones are not visible in a short period because they are small and cumulative. The independent expert group on mobile phones formed by the British Government has, in its report, recommended a limited mobile phone talk time during the day, relocating radio and cellular repeaters away from populated areas, as well as a limited mobile phone use by children. These recommendations were the result of numerous epidemiological and experimental studies that indicated numerous harmful effects of microwave radiation in animals and humans. It has been proven that MW exposure leads to the promotion of cancer cell growth in the brain, impaired DNA molecule structure, an increase in the leukemia prevalence in children after 2-6 years of radiation exposure, REM sleep cycle disorders, headaches and irritability, as well as a significant disruption of the immune status of the organism. The results of numerous studies have pointed out that the increase in oxidative stress intensity is one of the

pathogenetic mechanisms involved in the cellular damage after the MW exposure (1, 2).

Polyamines spermine and spermidine and their precursor diamine putrescine are non-protein nitrogenous low molecular weight bases present in all living systems (3). An abundance of data from the literature suggests that polyamines are prevalent in almost all tissues and organs, therefore we can say that they are essential to the function of the cell (4). In mammalian cells, polyamines are found in milimolar concentrations, and their highest concentration is spotted in the tissues characterized by an intense protein synthesis (thymus, pancreas, prostate and liver). The importance of polyamines is reflected by their indispensable role in the vital processes of growth, division and cell differentiation (due to the interaction with nucleic acids) (5), as well as their role in regulating the permeability and stability of the cell membrane (by interacting with phospholipids) (6). Recent research on polyamines speak of their antioxidant effects, as well as the inhibitory effect on the lipid peroxidation process (7).

Conversion of L-arginine to L-ornithine by the enzyme arginase is considered as the first step in the polyamine synthesis. In mammalian tissues, initial and rate-limiting reaction in polyamine biosynthesis is decarboxylation of L-ornithine by specific ornithine decarboxylase (ODC) and formation of putrescine. Transfer of the aminopropyl group from 5'-S-methyl-5'-thioadenosine (MTA) is regulated by spermidine synthase. Further condensation of spermidine with another MTA molecule leads to formation of spermine (8). In mammalian cells, biochemical pathway of polyamine catabolism is carried out as an intracellular conversion of polyamine. While spermidine / spermine byosinthesis is an irreversible process, polyamine back-conversion to putrescine is possible and regulated by two enzymes: spermidine / spermine N1 -acetyltransferase (SSAT) and polyamine oxydase (PAO). This interconversion is known as putrescine cycle (4). The first step in the polyamine intercon-version is acetylation of polyamine molecule at N1 position by SSAT using acetyl-coenzyme A and forming N1-acetyl spermine. It acts as a substrate for PAO in the next step, whereas PAO catalyzes its oxidative deamination producing spermidine, 3-acetamidopro-panal and hydrogen peroxide. These two enzymes are also responsible for back-conversion of spermidine to spermine. An enzyme which catalyzes conversion of spermine back to spermidine without acetylation was discovered in 2002 and it is known today as spermine oxydase (SMO) (9). Amino group of putrescine may further undergo oxydative deamination by diamino oxydase (DAO) producing 4-aminobutanal (gamma-aminobutyraldehyde) which is converted to gamma-aminobutyric acid (GABA).

High activity of PAO and DAO in hepatic, splenic, and thymus tissue shows that these two enzymes have the key role in the maintenance of total content of polyamines in mammalian tissues (10). Polyamine oxydase (EC 1.4.3.4) is a flavin-contraining enzyme with a molecular weight of 60,000 D. To date, there were four PAO isoenzymes discovered, each having different substrate specificity (11). The highest PAO activity is present in the liver, testes, kidneys, and thymus, whereas the preferred substrate is N1-acetyl spermine. High PAO activity is observed in serum of gravid women during the second and third trimester. It is noted that an activity of the PAO is higher in tissues with high polyamine biosynthesis, indicating that PAO might have an important role in polyamine level regulation in mammalian tissues. It is considered that PAO generates putrescine when there is the need to lower the cell content of higher polyamines (spermine and spermidine).

Diamine oxydase (EC 1.4.3.6) is a key enzyme in terminal polyamine catabolism. It catalyzes the oxidative deamination of putrescine, cadaverine, and histamine, consequently producing amino aldehydes, hydrogen peroxide, and ammonia. Optimal pH for enzyme activity is 7.0-7.4, and half-life is 14 h. Diamino oxydase is a copper-containing enzyme, therefore copper-binding agents, such as diethyldithiocarbamate, inhibit the enzyme activity. Hydrogen peroxide and amino aldehydes produced during polyamine degradation exert cytotoxic properties and may be included in the apoptosis initiation (12).

It has been shown that microwave radiation disrupts the activity of the enzyme ODC and reduces the concentration of ODC mRNA. Exposure to microwave radiation causes a disturbance of polyamine metabolism, which severity depends on the frequency of the radiation used, the exposure time and the type of irradiated tissue. It has been shown that ODC activity decreases 3 to 4 hours after MW exposure in the muscle cells, however, there are results indicating an increase in ODC activity in the brain tissue.

Synthesis of neurohormone melatonin is performed in the pineal gland. The synthesis is controlled by light from the outside environment, which inhibits its biosynthesis. Melatonin mediates the function of numerous hormones and participates in the maintenance of the normal function of the immune system. It shows immunostimulatory effect, prevents the onset of cancer, neurodegenerative diseases and diabetes complications, regulates the level of mRNA for certain proteins (13). Melatonin has significant antioxidant effects in the brain and thymus tissue (14, 15). It neutralizes the hydroxyl radical more efficiently than reduced glutathione and mannitol (16), stimulates the mRNA synthesis of superoxide-dismutase and glutathione peroxidase (17). It has been shown that it prevents oxidative damage to DNA molecules. The activity of the pineal gland and the secretion of melatonin are not only influenced by external light sources, but are also dependent on the electromagnetic fields. It has been shown that the change in the magnetic field causes a decrease in the secretion of melatonin (18).

Aim

The aim of this study was to analyze the effect of melatonin on the catabolism of polyamines (by measuring the enzyme activity of DAO and PAO) in the rat thymus after chronic exposure to microwave radiation.

Material and methods

Experimental model

Experiment was performed on white rats of Wistar species, weighing about 200 g, grown at the Institute for Biomedical Research, Faculty of Medicine, Niš. For the purpose of the experiment, an experimental model for the exposure to microwave radiation was created, consisting of a mobile test phone and a PC measuring controller. Using this PC measuring device, the mobile phone is brought into a state of emission that corresponds to the normal mode of operation during a telephone conversation. The mobile test phone was located in a plexiglass box that was placed in the middle of the cage at the floor level. All animals were in plexiglass cages in size 30x40x40 cm. Animals were exposed to microwave radiation in all experimental groups 4 hours a day, then moved to a room without sources of the electromagnetic field. Exposure to microwave radiation lasted for 20, 40 and 60 days.

Laboratory animals were divided into 4 experimental groups: I group (Control) – animals were injected with 1,0 ml of saline intraperitoneally per day; II group (Mel) – animals were injected with melatonin at a dose of 2 mg/kg body weight intraperitoneally per day; III group (MW) – the animals were exposed to the microwave radiation of the mobile phone 4 hours a day; IV group (MW+Mel) – the animals were exposed to the microwave radiation of the mobile phone 4 hours a day, and 30 minutes before radiation were injected with melatonin at a dose of 2 mg/kg body weight intraperitoneally per day. Seven animals from each group were successively sacrificed after 20, 40 and 60 days of the experiment.

The animals were sacrificed after the experiment, in ketamine anesthesia (2 ml/kg body weight). After sacrificing experimental animals, the thymus tissue was washed multiple times in a cold isotonic NaCl solution, immediately frozen at -20 °C and kept until homogenization. A 10% homogenate was then prepared in distilled water at 0 °C (on ice) using a homogenizer.

Biochemical methods

Determination of the activity of polyamino oxidase (PAO) and diamino oxidase (DAO). The spectrophotometric determination of the activity of PAO and DAO was performed by measuring the amount of the formed amino aldehyde under the action of these enzymes in the presence of the corresponding substrate (19). Putrescin dihydrochloride was used as a substrate for DAO (20), and spermine tetrahloride was used as a substrate for PAO (21). The reaction takes place in the TRIS-HCl buffer pH 7.2 for polyamine oxidase and 7.7 for diamine oxidase, with the addition of 0.4% 3-methyl-2-benzothiazolone hydrazone and 0.2% $FeCl_3$ to give the colored compound. The unit of activity was that amount of enzyme, which causes an increase in optical density by 0,100 at a wavelength of 660 nm (21). The enzyme activity was expressed in U/mg protein.

Determination of malondialdehyde concentration (MDA). The lipid peroxidation intensity in the tissues was measured using the spectrophotometric method based on the use of thiobarbituric acid (TBA), according to the method of Ohkawa et al. (1979). The concentration of MDA, as the final product of lipid peroxidation, was expressed in nmol / mg protein, using a molar extinction coefficient for MDA (1.56 x 10-5 M cm^{-1}) (22).

Determination of protein concentration. The amount of total protein in the rat thymus was determined by the Lowry method (1951), with bovine serum albumin as standard (23).

Statistical analysis

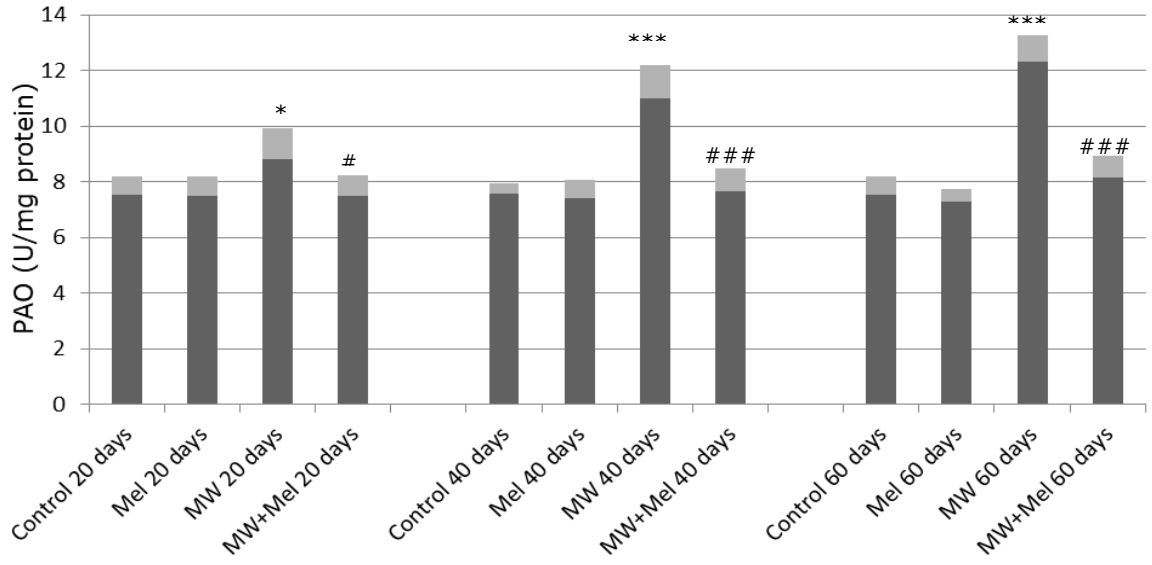
Statistical analysis was done with Excel 7.0 and SPSS 11.0 in the Windows 2000 environment, with results displayed graphically.

Results

There was an increase in PAO activity in the thymus of rats exposed to microwave radiation, when compared to the control and Mel group (MW_{20} vs. $Control_{20}$ and Mel_{20} $p < 0.05$; MW_{40} vs. $Control_{40}$ and Mel_{40} $p < 0.001$; MW_{60} vs. $Control_{60}$ and Mel_{60} $p < 0.001$). The application of melatoninin in dose of 2 mg/kg body weight to animals exposed to microwave radiation caused a decrease in PAO activity in the thymus tissue, when compared to MW exposed animals not treated with melatonin ($MW + Mel_{20}$ vs. MW_{20} $p < 0.05$; $MW + Mel_{40}$ vs. MW_{40} $p < 0.001$; $MW + Mel_{60}$ vs. MW_{60} $p < 0.001$) (Graph 1).

A decrease of DAO activity was observed in the thymus tissue of rats exposed to microwave radiation when compared to the control and Mel group (MW_{20} vs. $Control_{20}$ and Mel_{20} $p < 0.05$; MW_{40} vs. $Control_{40}$ and Mel_{40} $p < 0.001$; MW_{60} vs. $Control_{60}$ and Mel_{60} $p < 0.001$). The application of melatonin to animals exposed to microwave radiation resulted in a significant decrease of DAO activity in the thymus of MW exposed animals not treated with melatonin, after 40 days of exposure ($MW + Mel_{40}$ vs. MW_{40} $p < 0.05$; $MW + Mel_{60}$ vs. MW_{60} $p < 0.05$) (Table 1).

Graph 2 shows the correlation between the concentration of malondialdehyde (MDA) and activity of the enzyme PAO in the thymus tissue, during the exposure to microwave radiation. Linear correlation coefficient of $C = 0.61$ indicates that there is a strong and statistically significant positive correlation ($p < 0.05$) between the values of the MDA compared to the PAO activity during the exposure to microwave radiation.



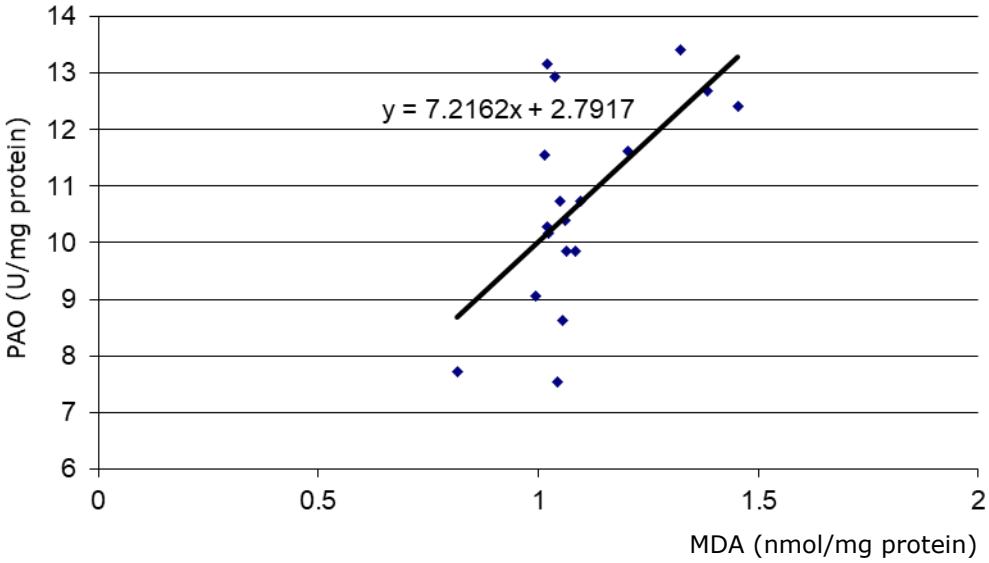
*p < 0,05 vs. Control and Mel, ***p < 0,001 vs. Control and Mel, #p < 0,05 vs. MW, ###p < 0,001 vs. MW

Graph 1. The effect of melatonin and microwave radiation on the enzyme polyamine oxidase (PAO) in rat thymus

Table 1. The effect of melatonin and microwave radiation on the enzyme diamine oxidase (DAO) in rat thymus

Groups	Control	Mel	MW	MW + Mel
20 days	9.08 ± 1.69	8.94 ± 1.61	7.60 ± 1.19	6.78 ± 0.43
40 days	9.42 ± 1.20	9.18 ± 1.07	8.09 ± 0.86*	6.97 ± 0.55#
60 days	9.96 ± 0.90	9.70 ± 0.83	7.93 ± 0.87***	6.54 ± 1.52#

*p < 0,05 vs. Control and Mel, ***p < 0,001 vs. Control and Mel, #p < 0,05 vs. MW



Graph 2. Linear regression between PAO activity (U/mg protein) and MDA (nmol/mg protein) levels in the rat thymus during exposure to microwave radiation

Discussion

Polyamine metabolism disturbance in the MW-irradiated rat thymus tissue is reflected in intensified catabolism. The exposure of animals to microwave radiation causes a significant, time-dependent increase in PAO activity in the thymus tissue relative to control (Graph 1), which points to intensification of the polyamine interconversion pathway. An intense increase in the PAO activity in the thymus tissue after the exposure to microwave radiation may be a result of polyamine metabolism disturbance that reduces spermine and spermidine (potent cell proliferative ability indicators) and increases the amount of putrescine (a biologically less potent indicator of proliferative and regenerative cell ability). This is probably one of the possible mechanisms for cells survival or their controlled transition to the apoptosis process in conditions of exposure to microwave radiation.

Hydrogen peroxide and amino aldehydes formed during the catabolism of spermine and spermidine by the PAO activity have cytotoxic properties and can participate in the initiation of thymocyte apoptosis (12). The combined action of SSAT and PAO in the polyamine acetylation and oxidation leads to the production of H_2O_2 which acts as an inducer of the SSAT activity and apoptosis causing a clear death signal. Also, the H_2O_2 formed in the process of polyamine catabolism, due to the ability of interaction with O_{2-} or further degradation in the presence of free iron, can lead to the production of OH radical – the immediate initiator of lipid peroxidation and further increment of this process. The results of the Tadolini study from 1988 indicated the possibility of polyamine role as a modulators of cell damage by free radicals, with the antioxidant effect of polyamine being attributed to the formation of the complex between Fe^{2+}/spermin/phospholipids, which limits the possibility of the Fenton reaction occurrence and the formation of OH• (24).

Graph 2 shows the linear regression analysis between the activity of PAO enzymes and levels of malondialdehyde in the thymus tissue of the radiated animals, and it shows a statistically significant positive correlation ratio (C = 0.61). This may indicate that the increase in PAO activity significantly contributes to an increase in the MDA level (secondary lipid peroxidation product), in the thymus tissue during microwave radiation exposure. The toxic effects of polyamine catabolism intermediates are related to the MDA concentration (25). It has been proven that 3-aminopropion aldehyde, from which MDA may be formed, is created by the action of PAO on spermine and spermidine. The toxicity of MDA as the final product of lipid peroxidation and its ability to modify macromolecules has been confirmed in numerous studies (26). Therefore, it could be expected that the increase in the total concentration of MDA in the thymus of animals exposed to microwave radiation, is partly influenced by the increased catabolism of spermine and spermidine.

Exposure to microwave radiation leads to a significant reduction in the degradation of putrescine by DAO enzyme in thymus tissue (Table 1). This can be explained by the striving of thymus cells to preserve the putrescine concentration, as its conversion to GABA leads to the production of toxic reactive oxygen radicals (ROS). Decrease in DAO activity can be considered as a limiting step towards the thymocyte apoptosis. Putrescine has special significance in the regulation of thyroid growth and proliferation in conditions resulting from the action of various immunosuppressants (glucocorticoids and androgens) that lead to a reduction in the amount of polyamines. This is achieved by the interconversion of spermine and spermidine into putrescine (27). On the other hand, putrescine is the most important regulator of T lymphocyte activity, unlike spermine and spermidine (28). The effect of microwave radiation completely resembles the effects of the immunosuppressant, as there is a decrease in the levels of spermine and spermidine and an increase in the amount of putrescine, followed by an increase in apoptosis and a reduction in proliferation in the thymus.

Spermine and spermidine stabilize chromatin and nuclear enzymes due to their ability to construct complexes with negative groups on proteins and the DNA molecule (29). Therefore, reduction of these polyamines leads to significant changes in the structure of the chromatin and the DNA molecule. It has been shown that spermine and spermidine prevent fragmentation of the DNA, by stabilizing its helix and protecting the thymocytes from apoptotic depletion (30). The effect of microwave radiation that reduces the amount of spermine and spermidine by increasing the activity of PAO significantly contributes to its apoptotic effect.

The results obtained in our experiment show that melatonin exhibits a strong modulatory effect on the disturbed metabolic pathways of the polyamines, in the tissue of the irradiated animals. Administration of melatonin led to a significant reduction in the catabolism of spermine, spermidine and putrescine in the thymus tissue of rats, during exposure to microwave radiation. This can be explained by the striving of thymus cells to preserve the concentration of spermine, spermidine and putrescine, which have been shown to have significant protective effects. It has been proven that polyamines participate in processes of growth, division and differentiation of cells, and therefore in regenerative, reparative and proliferative processes. In addition, spermine, spermidine and putrescine might be important for the stabilization of the cell membrane, antioxidative and antiapoptotic activity (31). Reduction of PAO and DAO activity aims to the growth of polyamine pools and the preservation of the regenerative and proliferative ability of the thymus cells, as demonstrated in this study.

Conclusion

By analyzing the obtained experimental results, it can be concluded that in condition of chronic exposure to microwave radiation, there are significant changes in the catabolism of polyamines in the thymus tissue of rats. The application of melatonin led to a normalization of the disturbed metabolism of spermine and spermidine (by lowering the activity of

polyamine oxidase), as well as the additional increase in the level of putrescine (by lowering the activity of diamine oxidase) in the irradiated animals.

Acknowledgment

This work was funded by the Ministry of Education, Science and Technological Development of Serbia (grant No. III 43012).

References

1. Sokolovic D, Djordjevic B, Kocic G, Jevtovic Stoimenov T, Stanojkovic Z, Sokolovic DM et al. The effects of melatonin on oxidative stress parameters and DNA fragmentation in testicular tissue of rats exposed to microwave radiation. Adv Clin Exp Med 2015; 24 (3): 429-36. [CrossRef][PubMed]
2. Köylü H, Mollaoglu H, Ozguner F, Naziroglu M, Delibas N. Melatonin modulates 900 Mhz microwave-induced lipid peroxidation changes in rat brain. Toxicol Ind Health 2006; 22(5):211-6. [CrossRef][PubMed]
3. Tabor H, Tabor CW. Spermidine, spermine and related amines. Pharmacol Rev 1964; 16:245-300. [CrossRef][PubMed]
4. Thomas T, Thomas TJ. Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. Cell Mol Life Sci 2001; 58(2):244-58. [CrossRef][PubMed]
5. Xiao L, Swank RA, Matthews HR. Photoaffinity polyamines: sequence specific interactions with DNA. Nucleic Acids Res 1991; 19(13):3701-8. [CrossRef]
6. Schuber F. Influence of polyamines on membrane functions. Biochem J 1989; 260(1):1-10. [CrossRef][PubMed]
7. Gaboriau F, Vaultier M, Moulinoux JP, Delcros JG. Antioxidative properties of natural polyamines and dimethylsilane analogues. Redox Rep 2005; 10(1):9-18. [CrossRef][PubMed]
8. Williams-Ashman HG, Seidenfeld J, Galletti P. Trends in the biochemical pharmacology of 5′-deoxy-5′-methylthioadenosine. Biochem Pharmacol 1982; 31(3): 277-88. [CrossRef][PubMed]
9. Vujcic S, Diegelman P, Bacchi CJ, Kramer DL, Porter CW. Identification and characterization of a novel flavin-containing spermine oxidase of mammalian cell orgin. Biochem J 2002; 367(Pt 3):665-75. [CrossRef][PubMed]
10. Bjelakovic G, Kocic G, Pavlovic D, Nikolic J, Stojanovic I, Bjelakovic GB et al. Effects of folic acid on polyamine concentrations and polyamine oxidase activity in regenerating rat liver. Pteridines 2003; 14(4):109-13. [CrossRef]
11. Bolkenius FN, Seiler N. Acetylderivates as intermediates in polyamine catabolism. Int J Biochem 1981; 13 (3):287-92. [CrossRef][PubMed]
12. Parchment RE, Pierce GB. Polyamine oxidation, programmed cell death, and regulation of melanoma in the murine embryonic limb. Cancer Res 1989; 49(23): 6680-86. [CrossRef][PubMed]
13. Baydas G, Canatan H, Turkoglu A. Comparative analysis of the protective effects of melatonin and vitamin E on streptozocin-induced diabetes mellitus. J Pineal Res 2002; 32(4):225-30. [CrossRef][PubMed]
14. Sokolovic D, Djindjic B, Nikolic J, Bjelakovic G, Pavlovic D, Kocic G et al. Melatonin reduces oxidative stress induced by chronic exposure of microwave radiation from mobile phones in rat brain. J Radiat Res 2008; 49(6):579-86. [CrossRef][PubMed]
15. Sokolovic D, Djordjevic B, Kocic G, Veljkovic A, Marinkovic M, Basic J et al. Melatonin protects rat thymus against oxidative stress caused by exposure to microwaves and modulates proliferation/apoptosis of thymocytes. Gen Physiol Biophys 2013; 32(1):79-90. [CrossRef][PubMed]

16. Poeggeler B, Reiter RJ, Tan DX, Chen LD, Manchester LC. Melatonin, hydroxyl radical-mediated oxidative damage, and aging: a hypothesis. J Pineal Res 1993; 14(4):151-68. [CrossRef][PubMed]
17. Antolín I, Rodríguez C, Saínz RM, Mayo JC, Uría H, Kotler ML et al. Neurohormone melatonin prevents cell damage: effect on gene expression for antioxidant enzymes. FASEB J 1996; 10(8):882-90. [CrossRef][PubMed]
18. Brendel H, Niehaus M, Lerchl A. Direct suppressive effects of weak magnetic fields (50 Hz and 16 2/3 Hz) on melatonin synthesis in the pineal gland of Djungarian hamsters (Phodopus sungorus). J Pineal Res 2000; 29(4):228-33. [CrossRef][PubMed]
19. Bachrach U, Reches B. Enzymatic assay for spermine and spermidine. Anal Biochem 1966; 17(1):38-48. [CrossRef][PubMed]
20. Quash G, Calogero H, Fossar N, Ferdinand A, Taylor D. Modification of diamine oxidase activity in vitro by metabolites of asparagine and differences in asparagine decarboxylation in normal and virus-transformed baby hamster kidney cells. Biochem J 1976; 157(3): 599-608. [CrossRef][PubMed]
21. Quash G, Gresland L, Delain E, Huppert J. Antipolyamine antibodies and cell lysis. The inhibitory effect of putrescine. Exp Cell Res 1972; 75(2):363-8. [CrossRef][PubMed]
22. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95(2):351-58. [CrossRef][PubMed]
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193(1):265-75. [CrossRef][PubMed]
24. Tadolini B. Polyamine inhibition of lipid peroxidation. The influence of polyamines on iron oxidation in the presence of compounds mimicking phospholipid polar heads. Biochem J 1988; 249(1):33-36. [PubMed]
25. Quash G, Taylor DR. Serum β-aminopropionaldehyde: Identification and origin. Clin Chim Acta 1970; 30(1): 17-23. [CrossRef]
26. Nair V, Cooper CS, Vietti DE, Turner GA. The chemistry of lipid peroxidation metabolites: crosslinking reactions of malondialdehyde. Lipids 1986; 21 (1):6-10. [CrossRef][PubMed]
27. Ferioli ME, Pinotti O, Pirona L. Gender-related differences in polyamine oxidase activity in rat tissues. Amino Acids 1999; 17(2):139-48. [CrossRef][PubMed]
28. Seiler N, Atanassov CL. The natural polyamines and the immune system. In: Jucker E, editor. Progress in Drug Research. Basel: Birkhauser 1994. p. 87-141. [CrossRef]
29. Heby O, Persson L. Molecular genetics of polyamine synthesis in eukaryotic cells. Trends Biochem Sci 1990; 15(4):153-8. [CrossRef][PubMed]
30. Redman C, Xu MJ, Peng YM, Scott JA, Payne C, Clark LC, et al. Involvement of polyamines in selenomethionine induced apoptosis and mitotic alterations in human tumor cells. Carcinogenesis 1997; 18(6):1195-202. [CrossRef][PubMed]
31. Hu RH, Pegg AE. Rapid induction of apoptosis by deregulated uptake of polyamine analogues. Biochem J 1997; 328(Pt 1):307-16. [CrossRef][PubMed]

Originalni rad **UDC: 537-962:[612.438:636.028**
doi:10.5633/amm.2018.0402

EFEKAT MELATONINA NA KATABOLIZAM POLIAMINA U TIMUSU PACOVA TOKOM IZLAGANJA MIKROTALASNOM ZRAČENJU

Dušan Sokolović[1], Boris Đinđić[1,2], Dejan Krstić[3], Vera Marković[4], Goran Ristić[4], Danka M. Sokolović[5], Mlađan Golubović[2], Branka Đorđević[1], Momir Dunjić[6], Dejan Popović[1], Tamara Karuntanović[1], Nikola Tatar[7], Petar Babović[8]

[1]Univerzitet u Nišu, Medicinski fakultet, Srbija
[2]Klinički centar Niš, Srbija
[3]Univerzitet u Nišu, Fakultet zaštite na radu, Srbija
[4]Univerzitet u Nišu, Elektronski fakultet, Srbija
[5]Institut za transfuziju krvi, Niš, Srbija
[6]Evropski Univerzitet, Novi Sad, Srbija
[7]Univerzitet u Nišu, Filozofski fakultet, Niš, Srbija
[8]Univerzitet u Nišu, Medicinski fakultet, Student doktorskih studija, Niš, Srbija

Kontakt: Dušan Sokolović
Bul. Dr Zoran Đinđić 81, 18000 Niš, Srbija
E-mail: soko@medfak.ni.ac.rs

Toksično dejstvo mikrotalasnog zračenja (MW) utiče na promenu metabolizma poliamina. Poliamini (spermin i spermidin) i njihov prekursor diamin putrescin, predstavljaju neproteinske azotne baze i oni su esencijalni za život ćelije. Katabolizam spermina i spermidina obavlja se dejstvom enzima poliamin oksidaze (PAO), a putrescina diamin oksidaze (DAO).Neurohormon melatonin učestvuje u održavanju normalne funkcije imunog sistema. Cilj ovog istraživanja bio je da se analizira efekat melatonina na katabolizam poliamina u timusu pacova, nakon hronične ekspozicije mikrotalasnom zračenju.Wistar pacovi su bili podeljeni u četiri eksperimentalne grupe: 1) kontrola, 2) Mel -životinjama je svakodnevno davan melatonin (2mg/kg), 3) MW -životinje su izlagane MW (4h/dnevno), 4) MW+Mel -pacovi kojima je aplikovan melatonin izlagani su MW. Životinje su žrtvovane nakon 20, 40 i 60 dana eksperimenta. U timusu pacova koji su izlagani mikrotalasnom zračenju došlo je do porasta aktivnosti PAO i sniženja aktivnosti DAO (već nakon 20 dana) u odnosu na kontrolu. Postoji i statistički značajna pozitivna korelacija ($p < 0,05$) između nivoa malondialdehida i aktivnosti PAO, u toku izlaganja MW. Kod životinja koje su izlagane MW i kojima je aplikovan melatonin, došlo je do značajnog sniženja aktivnosti PAO i DAO u tkivu timusa, u odnosu na ozračene životinje koje nisu tretirane melatoninom.

Acta Medica Medianae 2018;57(4):14-21.

Ključne reči: *melatonin, mikrotalasno zračenje, timus, poliamin oksidaza, diamin oksidaza*

Original article

UDC: 616.718.5-001.5-089.23
doi:10.5633/amm.2018.0403

PRIMARY WOUND CARE AND EXTERNAL SKELETAL FIXATION IN SURGICAL TREATMENT OF OPEN TIBIAL FRACTURES

Ivana Golubović[1], Predrag Stojiljković[1,2], Ivan Golubović[2], Zoran Radovanović[1,4], Milan Radojković[1,5], Aleksandar Mitić[1], Zoran Baščarević[3], Katarina Kutlešić[2], Andrija Krstić[1], Stevo Najman[1], Zoran Golubović[1,2]

Open tibial fractures are the most severe orthopaedic injuries. The lower leg is often injured due to its position in the locomotor system. The injuries of the lower leg skin and soft tissues, bone comminution and threatening infections make the treatment of these fractures particularly complex. The management of open tibial fractures is potentially associated with numerous complications.

The data on treatment outcomes of 36 patients operatively treated for the open tibial fractures in Clinic for Orthopaedic surgery and traumatology, Clinical Center Nis in Serbia during the period from January 1, 2012 to June 31, 2014 were retrospectively analyzed and compared. In all the patients, after thorough wound rinsing, removal of the foreign bodies, debridement and delayed wound closure, fractured bone segments were repositioned and stabilized using external fixator.

In 28 (77.78%) patients fractures healed without major complications, while in 8 (22.22%) major complications occurred, including tibial osteomyelitis in 3 (8.33%) and fracture malunion in 5 (13.88%) patients.

Primary wound care, external fixation, antibiotic and antitetanus prophylaxis are crucial in treatment of open tibial fracture.

Acta Medica Medianae 2018;57(4):22-28.

Key words: *Open tibial fractures, primary wound care, external skeletal fixation*

[1]University of Niš, Faculty of Medicine, Serbia
[2]Clinic for Orthopaedic Surgery and Traumatology, Clinical center Niš, Serbia
[3]Institute for Orthopaedic Surgery 'Banjica', Belgrade, Serbia
[4]Radiology Center, Clinical Center Niš, Niš, Serbia
[5]Surgery Clinic, Clinical Center Niš, Niš, Serbia

Contact: Zoran Golubović
Gutenbergova 37, 18000 Niš, Serbia
E-mail: doktorzorangolubovic@gmail.com

Introduction

Open tibial fractures are the most severe orthopaedic injuries. The lower leg is often injured due to its position in the locomotor system. Tibial diaphysis fractures are the most common of all long bone diaphyseal fractures in 40%, while the open tibial fractures are the most common open bone fractures (1). Open tibial fractures are closed in 77 % and open in 33% (2). Open tibial fracture occurs in approximately two per 10 000 persons per year in the developed country (3). Open lower leg fractures most commonly occur in traffic accidents and during agricultural and sport activities (1). The injuries of the lower leg skin and soft tissues, bone comminution and threatening infections make the treatment of these fractures particularly complex. The treatment includes thorough wound rinsing, removal of foreign bodies, debridement, bone segments reposition, external fixation or internal fixation, antibiotic therapy and antitetanus prophylaxis (4).

The management of open lower leg fractures is potentially associated with numerous complications including: soft tissue infection, osteomyelitis, fracture malunion/nonunion and the loss of the extremity (5). The management success depends on both the severity of injury, patient's general condition and comorbidities and treatment modality applied.

The aim of the study

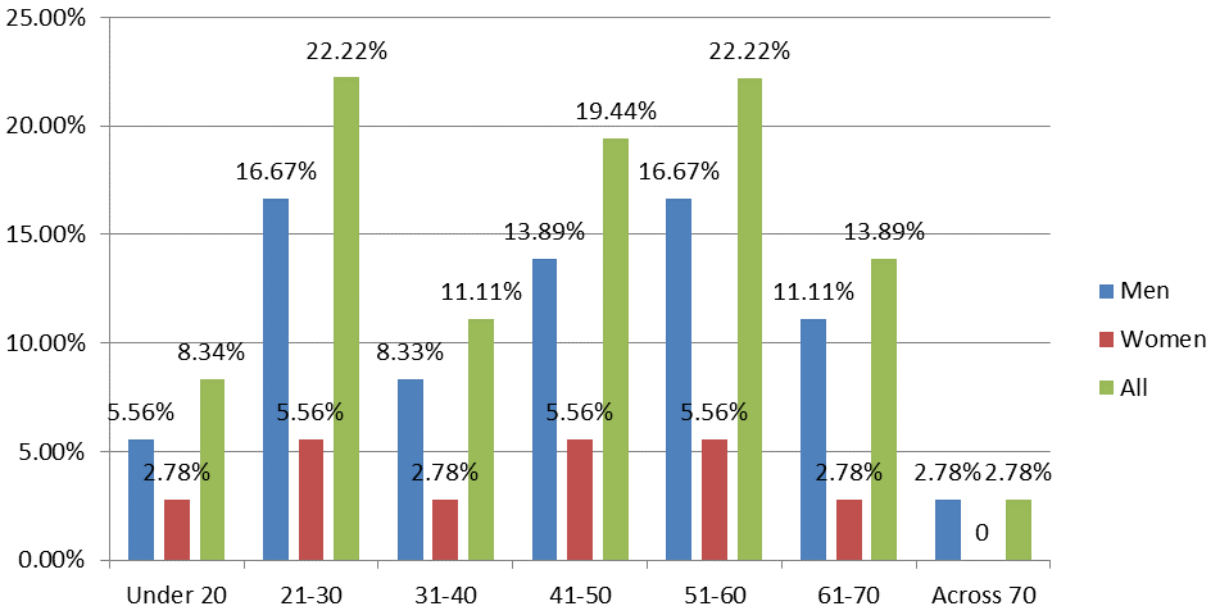
The aim of our study was to present the results of the open lower leg fracture treatment with primary wound closure, external fixation, antibiotic and antitetanus prophylaxis.

Materials and methods

The data on treatment outcomes of 36 patients operatively treated for the open tibial fractures

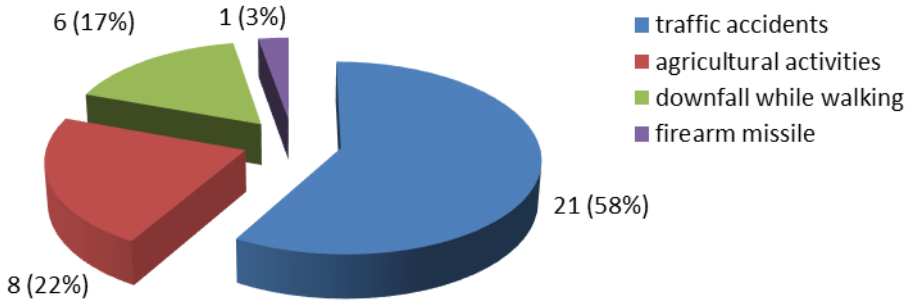
in Clinic for Orthopaedic surgery and traumatology, Clinical Center Nis in Serbia during the period from January 1, 2012 to June 31, 2014 were retrospectively analyzed and compared.

There were 27 (75%) men and 9 (25%) women, most of which being in the third 8 (22.22%), fifth 7 (19.44%) and sixth 8 (22.22%) decade of life (Graph 1).



Graph 1. Gender and age distribution of the patients

Most of the patients were injured in the traffic accidents 21 (58%), while 8 (22%) of them were injured during the agricultural activities. The downfall while walking caused the injury in 6 (17%) pati-ents and 1 (3%) was injured by a firearm missile (Graph 2).

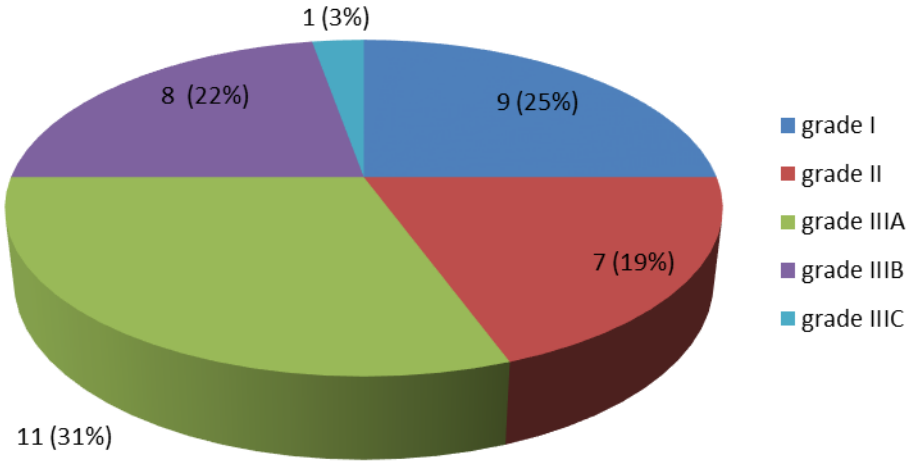


Graph 2. The mechanisms of injury

For the analysis we used the open fracture classification system established by Ramon Gustilo (6) according to which there were 9 (25%) patients with grade I, 7 (19%) with grade II, 11 (31%) with grade IIIA, 8 (22%) with grade IIIB and one (3%) with grade IIIC open lower leg fracture (Graph 3).

In all the patients, after thorough wound rinsing, removal of the foreign bodies, debridement and delayed wound closure, fractured bone segments were repositioned and stabilized using Mitkovic external fixator.

Immediately upon admission, all patients were administered antimicrobial therapy that included the combination of a third-generation cephalosporin (ceftriaxone, 2gr/24h) and semi-synthetic aminoglycoside (amikacin, 1gr/24h).



Graph 3. The severity of injuries of our patients according to the Gustilo open fracture classification

Extremely contaminated wounds, for example with soil, with increased risk of clostridial infection and gas gangrene, required the administration of additional antibiotic (penicillin G sodium, 4 to 6 million units/day or clindamycin). All patients received antitetanus prophylaxis according to the protocol.

Results

In 28 (77.78%) patients fractures healed without major complications (Figure 1), while in 8 (22.22%) major complications occurred including tibial osteomyelitis in 3 (8.33%) and fracture malunion in 5 (13.88%) patients (Graph 4). Minor complications such as soft tissue infection, surrounding the pins of external fixator and soft tissue infection were detected in 6 (16.67%) and 4 (11.11%) patients, respectively.

Infection of the soft tissue surrounding the pins and soft tissue infection were successfully treated with thorough daily debridement and wound care and antimicrobial medication according to the antibiogram results. Tibial osteomyelitis developed in one patient with grade IIIA and 2 patients with grade IIIB open fractures. Fracture malunion and pseudoarthrosis occurred in one patient with grade II, 2 patients with grade IIIA and 2 patients with grade IIIB fractures. In 2 (5.56%) patients primary bone comminution caused nonunion, while in 3 (8.33%) patients open fractures resulted in tibial hypertrophic pseudoarthrosis.

Discussion

Open lower leg fractures most commonly occur in traffic accidents, with the direct mechanism of injury. The indirect mechanism of injury implies the rupture of lower leg soft tissue and skin from the inside with sharp segment(s) of fractured bone(s) resulting in open fracture. Indirect mechanism often causes less severe injuries compared to the direct mechanism (1).

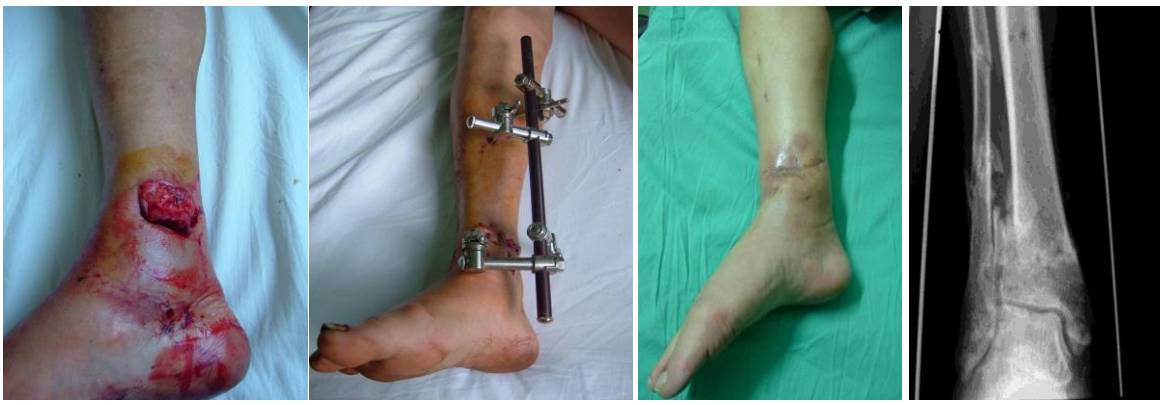
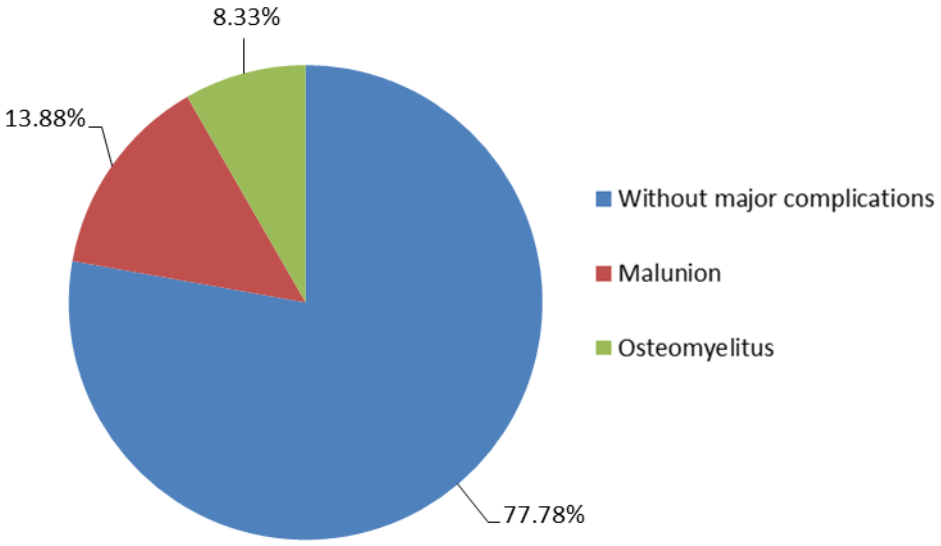


Figure 1. Open tibial fracture treated with external fixator Mitkovic



Graph 4. Open tibial fracture treatment results

All open fractures are considered primarily contaminated. Among numerous classifications the most commonly used is the one proposed by Ramon Gustilo based on the strength of the force that caused the lower leg bone fracture and soft tissue damage (6).

Lower leg open fractures require urgent surgical treatment in case of satisfactory general condition of the patient. The "6-hour rule", long-accepted guideline for dealing with open fractures, stating that, to prevent infection, open fractures should be fully managed within a 6-hour time frame, should be applied as its relevance is supported by the evidence provided by Kindsfater and Jonassen (7) and Kreder and Armstrong (8) in 1995.

Early parenteral antibiotic administration is associated with the infection incidence decrease and should be applied immediately upon admission. Antibiotic treatment is to be administered during 48 to 76 hours in patients with grade I and II and up to 120 hours in patients with grade III open fractures (9).

Primary wound care is essential for the prevention of osteomyelitis, gas gangrene and tetanus. Before detailed debridement, thorough wound rinsing using saline and hydrogen and removal of foreign bodies, including the parts of soil and clothing and bone fragments, should be done. Sometimes, more than 10L of liquid are required for quality wound rinsing. It includes the removal of damaged skin, subcutaneous fat, fascia, and muscles, as well as removal of small periosteum bone fragments. Necrotic muscle tissue is a good host for both anaerobic and aerobic bacteria. When assessing the muscle vitality, its colour, consistency, bleeding and contractility should be taken into consideration (1).

Widely accepted surgical treatment of the Gustilo-Anderson types IIIB and IIIC open fractures these days is a routine treatment involving the use of external fixation (10, 11).

External fixation is a standard method of all lower leg fracture stabilization. It provides good biomechanical conditions for fracture healing, allows good approach for wound care and it does not interfere with the knee and ankle movements. Problems associated with this fixation method include frequent soft tissue and bone infections around the pins when the fixator is applied longer than 6 months and higher incidence of fracture malunion and nonunion (12).

Satisfactory clinical function has been achieved with external fixation at the 89% of the patients with open tibial fractures Grade III with 93% of the fractures healing well and as expected, which was noted in a study case conducted by Edward in 1988 (13).

Intramedullary nailing in the therapy of Grade IIIB and IIIC fractures was leading to infection in 20.3% of the cases and therefore was proclaimed the risky treatment by Yokoyama K (14).

Moreover the conclusion is that using secondary nailing after delayed primary one or primary external fixator is increases the risk of infection (15).

Fracture management with intramedular nailing and external fixation was compared through several clinical trials; both ways were applied to treat the open tibial shaft fractures. The selection of the treatment depended both on surgical experience of the doctor as well as on patient presentation. Advantages of external fixation treatment are shorter time and its suitability in polytrauma patients, but on the other hand it has many disadvantages such as high incidence of complications like refracture, nonunion or delayed union and not always being well tolerated by the patients. While on the other side, intramedullary nailing includes less time for healing, earlier load-bearing and extremely low risk of complications (15-17).

Osteosynthesis with plate should be avoided in treatment of open tibial fractures due to high risk of infection (18).

Open fracture wound should not be primary closed, but left open and closed using secondary sutures or some of the plastic surgery techniques (depending on the severity of the soft tissue damage/defect such as fasciocutaneous flap, microvascular transplantation etc.) when certain that no infection occurred (1). Closing the wound as early as possible, according to contemporary orthopaedic principles, requires previous thorough debridement and absence of contamination. Caudle and Stern reported that early aggressive soft tissue reconstru ction during the first 7 days after the injury, in order to cover the fractured bone segments in patients with grade III open fractures, significantly reduces the risk of infection, fracture malunion/nonunion and amputation (19).

Conclusion

Primary wound care, external fixation, antibiotic and antitetanus prophylaxis, delayed wound closure are crucial in treatment of open tibial fracture. Most common complications of open tibial fracture are deep bone infection – osteomyelitis and fracture malunion or nonunion.

Acknowledgment

This work is part of the project "Virtual human osteoarticular system and its application in preclinical and clinical practise" (No. III 41017), supported bywas funded by the Ministry of Edu-cation, Science and Technological Development of Serbia.

References

1. Golubović Z, Stojiljković P, Mačukanović-Golubović L, Milić D, Milenković S, Kadija M, et al. External fixation in the treatment of open tibial shaft fractures. Vojnosanitet Pregl 2008; 65(5):343-7. [CrossRef] [PubMed]
2. Court-Brown CM, McBirnie J. The epidemiology of tibial fractures. J Bone Joint Surg 1995; 77B:417-21. [CrossRef] [PubMed]
3. Court-Brown CM, Bugler KE, Clement ND, Duckworth AD, McQueen MM. The epidemiology of open fractures in adults: a 15-year review. Injury 2012; 43:891-7. [CrossRef] [PubMed]
4. Cross WW, Swiontkowski MF. Treatment principles in the management of open fractures. Indian J Orthop 2008; 42(4):377-86. [CrossRef] [PubMed]
5. Kohlprath R, Assal M, Uc¸kay I, Holzer N, Hoffmeier P, Suva D. Open fractures of the tibia in the adult: surgical treatment and complications. Rev Med Suisse 2011; 7(322):2482-4. [PubMed]
6. Gustilo RB, Mendoza RM, Williams DN. Problems in the management of type III (severe) open fractures. A new classification of type III open fractures. J Trauma 1984; 24:742-6. [CrossRef] [PubMed]
7. Kindsfater K, Jonassen EA. Osteomyelitis in grade II and III open tibia fractures with late debridement. J Orthop Trauma 1995; 9(2):121-7. [CrossRef] [PubMed]
8. Kreder HJ, Armstrong P. A review of open tibia fractures in children. J Pediatr Orthop 1995; 15(4): 482-8. [CrossRef] [PubMed]
9. Golubovic I, Vukasinovic Z, Stojiljkovic P, Golubovic Z, Stamenic S, Najman S. Open segmental fractures of the tibia treated by external fixation. Srp Arh Celok Lek 2012; 140(11-12):732-7. [CrossRef] [PubMed]
10. McKee MD, Yoo DJ, Zdero R, Dupere M, Wild L, Schemitsch EH, et al. Combined single-stage osseous and soft tissue reconstruction of the tibia with the

Ilizarov method and tissue transfer. J Orthop Trauma 2008; 22(3):183-9. [CrossRef] [PubMed]
11. Kataria H, Sharma N, Kanojia RK. Small wire external fixation for high-energy tibial plateau fractures. J Orthop Surg 2007; 15(2):137-43. [CrossRef] [PubMed]
12. Mitković M, Bumbaširević M, Golubović Z, Mićić I, Mladenović D, Milenković S, et al. New concept in external fixation. Acta Chir Iugosl 2005; 52(2): 107-11. [CrossRef] [PubMed]
13. Edwards CC, Simmons SC, Browner BD, Weigel MC. Severe open tibial fractures. Results treating 202 injuries with external fixation. Clin Orthop Relat Res 1988; 230:98-115. [PubMed]
14. Yokoyama K, Itoman M, Uchino M, Fukushima K, Nitta H, Kojima Y. Immediate versus delayed intramedullary nailing for open fractures of the tibial shaft: a multivariate analysis of factors affecting deep infection and fracture healing. Indian J Orthop 2008; 42(4): 410-9. [CrossRef] [PubMed]
15. Blachut PA, Meek RN, O'Brien PJ. External fixation and delayed intramedullary nailing of open fractures of the tibial shaft. J Bone Joint Surg Am 1990; 72(5):729-35. [CrossRef] [PubMed]
16. Koval KJ, Clapper MF, Brumback RJ, Stribling Ellison P, Poka A, Bathon H, et al. Complication of reamed intramedullary nailing of the tibia. J Orthop Trauma 1991; 5:184-9. [CrossRef] [PubMed]
17. Court-Brown CM, Keating JF, McQueen MM. Infection after intramedullary nailing of the tibia. J Bone J Surg 1992; 74(B):770-4. [PubMed]
18. Melvin JS, Domrizikabroski DG, Torbert JT, Kovach SJ, Esterhai JL, Mehta S. Open tibial shaft fractures: II. Definitive management and limb salvage. J Am Acad Orthop Surg 2010; 18:108-17. [CrossRef] [PubMed]
19. Caudle RJ, Stern PJ. Severe open fractures of the tibia. J Bone Joint Surg Am 1987; 69(6):801-7. [CrossRef] [PubMed]

Originalni rad **UDC: 616.718.5-001.5-089.23**
doi:10.5633/amm.2018.0403

PRIMARNA OBRADA RANE I SPOLJNA SKELETNA FIKSACIJA U LEČENJU OTVORENIH PRELOMA TIBIJE

Ivana Golubović[1], Predrag Stojiljković[1,2], Ivan Golubović[2], Zoran Radovanović[1,4], Milan Radojković[1,5], Aleksandar Mitić[1], Zoran Baščarević[3], Katarina Kutlešić[2], Andrija Krstić[1], Stevo Najman[1], Zoran Golubović[1,2]

[1]Univerzitet u Nišu, Medicinski fakultet, Srbija
[2]Klinika za ortopedsku hirurgiju i traumatologiju, Klinički centar u Nišu, Srbija
[3]Specijalna ortopedska bolnica Banjica, Beograd, srbija
[4]Centar za radiologiju, Klinički centar Niš, Niš, Srbija
[5]Klinika za digestivnu hirurgiju, Klinički centar Niš, Niš, Srbija

Kontakt: Zoran Golubović
Gutenbergova 37, 18000 Niš, Srbija
E-mail: doktorzorangolubovic@gmail.com

Otvoreni prelomi tibije spadaju u najteže ortopedske povrede. Zbog svog položaja u lokomotornom sistemu, potkolenica se često povređuje. Povrede kože i mekog tkiva potkolenice, kominukacija kosti i prateće infekcije čine lečenje ovih preloma naročito složenim.

Lečenje otvorenih preloma potkolenice je potencijalno povezano sa brojnim komplikacijama. U radu se prikazuju rezultati operativnog lečenja 36 pacijenata sa otvorenim prelomom potkolenice, koji su lečeni u Klinici za ortopediju i traumatologiju Kliničkog centara u Nišu u periodu od 1. januara 2012. do 31. juna 2014. godine. Kod svih pacijenata, nakon primarne obrade rane, odstranjivanja stranih tela, debridmana i odloženog zatvaranja rane, polomljeni koštani fragmenti su reponirani i stabilizovani korišćenjem spoljašnjeg skeletnog fiksatora.

Kod 28 pacijenata (77,78%) prelom je zarastao bez većih komplikacija, dok je kod 8 (22,22%) pacijenata došlo do većih komplikacija, uključujući osteomijelitis tibije kod tri (8,33%) i nezarastanje preloma kod 5 (13,88%) pacijenata.

Primarna obrada rane, spoljna skeletna fiksacija, antibiotska i antitetanusna profilaksa su ključni u lečenju otvorenih preloma tibije.

Acta Medica Medianae 2018;57(4):22-28.

Ključne reči: *otvoreni prelomi potkolenice, primarna obrada rane, spoljna skeletna fiksacija*

Original article

UDC: 616.132.2-089.843-085.8:613.73
doi:10.5633/amm.2018.0404

THE INFLUENCE OF EXERCISE TRAINING ON QT DISPERSION AND RISK FACTORS FOR CARDIOVASCULAR DISEASES IN PATIENTS AFTER CORONARY ARTERY BYPASS GRAFT SURGERY

Viktor Stoičkov[1,2], Sandra Šarić[1], Stanoje Andonov[1], Svetlana Kostić[1], Milan Lović[1], Marija Sekulović[1]

The aim of this study was to determine the impact of exercise training on QT dispersion and risk factors for cardiovascular disease in patients after coronary artery bypass graft surgery (CABG).

143 patients after CABG, in a sinus rhythm, without atrioventricular or branch blocks, average age 57.5 years, were involved in the study. Patients were randomly divided into the exercise training group (TG: 107 patients) and non-training group (NTG: 36 patients). In addition to clinical examination and laboratory analysis, all the subjects had standard ECGs out of which, QTd was calculated and QT dispersion (QTdc) was corrected. The patients performed the exercise test according to Bruce's protocol, after that the participants of the training group were involved in the exercise training. According to the results of the exercise test, the TG of patients was subjected to a certain degree of physical activity (gymnastic exercises, using the bicycle ergometer and walking). During the follow-up period, medication therapy was not changed. After the observed follow-up period of 21 days, the standard ECG and the exercise test were performed, once again.

In TG of patients, after treatment with exercise training, there was a significant reduction in QTd and QTDc ($p < 0.005$ for both parameters). In TG of patients, after 3 weeks, there was a significant reduction in systolic and diastolic blood pressure, heart rate, double product, total and LDL cholesterol ($p < 0.001$ for all parameters). TG of patients, who were on the second exercise test, achieved significantly longer time, while the non-training group showed no significant changes.

The study showed that exercise training has favourable effects on QT dispersion in patients after CABG. Exercise training led to significant reduction in blood pressure, heart rate, double product, cholesterol, as well as significantly improved physical exercise capacity, which has a beneficial effect on the prognosis in these patients.

Acta Medica Medianae 2018;57(4):29-35.

Key words: *exercise training, coronary artery bypass graft surgery, QT dispersion, risk factors for cardiovascular disease*

[1]Institute for Treatment and Rehabilitation "Niška Banja", Niš, Serbia
[2]University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Viktor Stoičkov
Blvd Nemanjića 34A/8, 18000 Niš, Serbia
E-mail: viktorstoickov67@gmail.com

Introduction

Patients after coronary artery bypass graft surgery (CABG) are at risk of new cardiovascular and arrhythmic events, cardiac death and sudden cardiac death (1). After CABG, patients have a significant reduction in fitness, caused by myocardial damage and long bedding. As a result of prolonged bedding and long physical inactivity, the weakness of skeletal muscle, damage to peripheral circulation, and dysfunction of autonomic nervous system occur. A significant reduction in fitness is clinically manifested by a marked reduction in tolerance to physical effort, the presence of postural hypotension, tachycardia in rest, and mental disorders, most often in the form of depression or anxiety. The degree of reduction of fitness depends mostly on the length of the stay in the bed and the degree of damage to the left ventricular function. Decreased tolerance to effort is due to reduced left ventricular function and decreased skeletal muscle strength, due to decreased perfusion of skeletal muscle and increased peripheral resistance. Tachycardia in rest and disproportionate increa-

se in the heart rate with less effort is due to the dysfunction of the autonomic nervous system, reduced parasympathetic tone and increased sympathetic tone (2).

After CABG, good control of the risk factors of coronary disease is necessary for the purpose of secondary prevention of coronary disease. After CABG in patients who did not have physical activity, about 15% of restenosis were recorded in a year, and 88% of patients experienced adverse cardiovascular events: myocardial infarction, stroke, revascularization procedures and hospitalization due to angina pectoris (3).

Arterial hypertension is a risk factor for myocardial infarction, stroke and myocardial left ventricular hypertrophy, and is a serious threat to the development of heart failure and cardiac death. Arterial hypertension is the cause of 2/3 of stroke and 1/2 of ischemic heart disease. Thus, arterial hypertension remains the leading cause of mortality and represents a major health problem, as well (4). Poor nutrition and physical inactivity are in favour of atherosclerosis (5). It has been noted that in individuals with low physical activity, intima of carotid arteries is intensified, while significant changes in individuals with higher physical activity do not appear (6).

The rate of development of atherosclerosis under the influence of risk factors for cardiovascular disease depends on whether one or more risk factors, values of certain risk factors are present as well as the length of its duration. In addition to physical training, reduction of LDL cholesterol is easily achieved by statins and they should be administered immediately and intensively at high doses (7-11).

QT dispersion (QTd) represents the heterogeneity in the repolarization of the ventricular myocardium and the marker is ischemia and electrical instability of the myocardium, reflecting the increased tendency towards ventricular arrhythmias. Several studies have shown that QTd is a significant prognostic marker for arrhythmic events, cardiac mortality and sudden cardiac death in coronary patients (12-17).

Considering the fact that QT dispersion is a significant prognostic marker in coronary patients and that patients after CABG are at risk of new cardiovascular events, the aim of this study is to determine the impact of exercise training on QT dispersion and risk factors for cardiovascular disease in patients after CABG.

Material and methods

The research was carried out at the Institute for Treatment and Rehabilitation Niška Banja, Niš.

One hundred forty three patients after CABG, in a sinus rhythm, without atrioventricular or branch blocks, the average age 57.5 years, 38 women and 109 men, were involved in the study. Patients were randomly divided into the exercise training group (107 patients) and non-training group (36 patients). Patients were of similar age and baseline stress test duration. Patients were included in the study within 3 months after CABG.

In addition to the clinical examination and laboratory analysis, all the subjects had standard ECGs out of which QTd was calculated and corrected QT dispersion (QTdc) as well. The patients were subjected to the exercise test, after that the participants of the training group were involved in the exercise training.

According to the results of the exercise test, the training group patients were subjected to the degree of physical activity (gymnastic exercises, using the bicycle ergometer and walking). During the follow-up period, medication therapy was not changed. After the observed follow-up period of 21 days, the standard ECG and the exercise test were performed once again.

QT interval was determined according to ECG, from the starting point of the Q or R peak to the end of the T wave-where the down-slope of the T wave merged with the isoelectric line. The QT interval was determined in each offset from three consecutive sinus cycles as a mean value. The values of QT intervals were corrected for the frequency of heart rate according to Bazett's formula (18). QT dispersion was determined as the difference derived from the maximal and minimal value of QT interval found in any of the 12 offsets. Out of the corrected value of QT interval, where the minimal value was subtracted from the maximum value found in any of the ECG offsets, the corrected value for QT dispersion (QT dc) was obtained.

All participants in the study were subjected to treadmill exercise test according to Bruce's protocol (19). The criteria for discontinuation of the test were the following: 1) sub-maximal heart frequency (defined as 85% of heart frequency); 2) depression of ST segment greater than 2mm; 3) reduction of systolic blood pressure for 10 or more mmHg during the exercise; 4) increased values of blood pressure greater than 240/120 mmHg; 5) occurrence of significant symptoms or arrhythmias. ECG criteria for a positive exercise test were the following: the presence of the horizontal or downstream ST depression≥1mm than isoelectric line lasting longer than 0.08 seconds in three consecutive cycles; ischemic elevation of ST segment ≥ 1mm in offsets without Q peak.

Statistical Analyses

Characteristics of study and control group were expressed as mean ± SD (continuous variables), with number and % in brackets (categorical variables). We compared clinical and biochemical data of patients and the control group using Student t-test for normally distributed data (expressed as mean ± SD). All analyses were performed with SPSS statistical analysis software, version 10.0 (SPSS, Chicago, IL, United States) at the significance level set at $p < 0.05$.

Results

In patients after CABG, the baseline values of the monitored parameters did not differ between the two groups of subjects, Table 1.

In the training group of patients, after treatment with exercise training, there was a significant reduction in QTd and QTDc parameters, systolic and diastolic blood pressure, heart rate, double product, total and LDL cholesterol, Table 2. The training group of patients who were on the second exercise test achieved significantly longer time, Table 2.

In the non - training group of patients, after the 21 - day follow - up period, there were no significant changes in the monitored parameters, Table 3.

Table 1. Baseline values of monitored parameters in examined groups of patients, compared with Student t-test

Monitored parameters	Training group of patients	Non - training group of patients	P
N	107	36	-
QTd (ms)	48.2 ± 15.9	47.8 ± 14.5	NS
QTdc (ms)	50.5 ± 18.2	50.2 ± 16.5	NS
Systolic blood pressure (mmHg)	138.7 ± 13.9	137.6 ± 14.4	NS
Diastolic blood pressure (mmHg)	88.5 ± 8.4	87.9 ± 9.3	NS
Heart rate (beats/min)	77.3 ± 7.8	76.9 ± 6.9	NS
Double product (beat/min x mmHg)	11546.7 ± 912.5	11498.8 ± 1047.3	NS
Total cholesterol (mmol/L)	5.1 ± 1.6	5.2 ± 2.1	NS
LDL cholesterol (mmol/L)	3.1 ± 0.9	3.1 ± 1.1	NS
Glycemia (mmol/L)	5.3 ± 2.1	5.1 ± 1.9	NS
Time achieved on the exercise test (min)	5.3 ± 1.4	5.5 ± 1.4	NS

Data are expressed as X ± SD-compared with Student-t test.
QTd: QT dispersion; QTdc: corrected QT dispersion

Table 2. Comparison of monitored parameters in the training group of patients before and after treatment with exercise training, compared with Student t-test

Monitored parameters	Before exercise training	After exercise training	P
N	107	107	-
QTd (ms)	48.2 ± 15.9	42.1 ± 14.2	0.005
QTdc (ms)	50.5 ± 18.2	43.6 ± 15.7	0.005
Systolic blood pressure (mmHg)	138.7 ± 13.9	129.2 ± 9.3	0.001
Diastolic blood pressure (mmHg)	88.5 ± 8.4	83.6 ± 5.9	0.001
Heart rate (beats/min)	77.3 ± 7.8	68.8 ± 6.9	0.001
Double product (beat/min x mmHg)	11546.7 ± 912.5	10227.6 ± 628.4	0.001
Total cholesterol (mmol/L)	5.1 ± 1.6	4.7 ± 1.2	0.025
LDL cholesterol (mmol/L)	3.1 ± 0.9	2.9 ± 0.6	0.05
Glycemia (mmol/L)	5.3 ± 2.1	4.6 ± 1.2	0.005
Time achieved on the exercise test (min)	5.3 ± 1.4	8.2 ± 1.9	0.001

Data are expressed as X ± SD-compared with Student-t test.
QTd: QT dispersion; QTdc: corrected QT dispersion

Table 3. Comparison of monitored parameters in the non - training group of patients before and after follow up period (three weeks), compared with Student t-test

Monitored parameters	Before exercise training	After exercise training	P
N	36	36	-
QTd (ms)	47.8 ± 14.5	47.6 ± 13.9	NS
QTdc (ms)	50.2 ± 16.5	49.8 ± 15.9	NS
Systolic blood pressure (mmHg)	137.6 ± 14.4	134.2 ± 13.9	NS
Diastolic blood pressure (mmHg)	87.9 ± 9.3	86.2 ± 8.8	NS
Heart rate (beats/min)	76.9 ± 6.9	74.2 ± 7.8	NS
Double product (beat/min x mmHg)	11498.8 ± 1047.3	11258.4 ± 1273.5	NS
Total cholesterol (mmol/L)	5.2 ± 2.1	5.1 ± 2.3	NS
LDL cholesterol (mmol/L)	3.1 ± 1.1	3.1 ± 1.2	NS
Glycemia (mmol/L)	5.1 ± 1.9	5.0 ± 2.1	NS
Time achieved on the exercise test (min)	5.5 ± 1.4	5.9 ± 1.7	NS

Data are expressed as X ± SD-compared with Student-t test.
QTd: QT dispersion; QTdc: corrected QT dispersion

Discussion

In our patients after CABG, a significant reduction in blood pressure was found after treatment with exercise training. Reducing the high blood pressure leads to a significant reduction in the risk of stroke, heart and renal failure, aortic dissection, adverse cardiac events and total mortality rate, as well (5, 7). Reducing high blood pressure due to physical training in our patients after CABG contributes to a significant reduction in the risk of new cardiovascular events. Long-term resistant training contributes to the maintenance of the normal structure and function of the heart, maintains the elasticity of the aorta and maintains lower blood pressure, too (20). Physical training in cardiovascular patients improves survival and reduces hospitalization (7, 21).

After a physical exercise was performed in our patients after CABG, a significant reduction in total and LDL cholesterol was found. In coronary patients, lipid reduction due to statin therapy leads to a significant increase in survival and decreases cardiac mortality, as well. In patients at very high risk, LDL cholesterol reduction is required below 1.8mmol / L (8, 11). Reducing total and LDL cholesterol in our patients after CABG indicates the significant importance of exercise training. The rate of development of atherosclerosis under the influence of risk factors for cardiovascular disease depends on whether one or more risk factors are present as well as the length of its duration. Comparing patients receiving a high dose of statins with those receiving a regular dose after CABG, the medium - and long-term efficacy of a high dose showed significant reduction in LDL-C, lower occurrence of adverse cardiac events, and reduction in graft restenosis (9).

After treatment with exercise training, in our training group of patients, apart from significantly reduced heart rate, blood pressure and cholesterol, a significant reduction in QTd and QTdc was recorded. The most important factor responsible for reducing QTd parameters is the improvement of the function of the autonomic nervous system, as a significant reduction in the heart rate has been found. It is probable that an improvement in collateral myocardial circulation contributed to the reduction of QTd parameters, as many studies have shown that ischemia increases QTd (15, 22, 23).

The function of the autonomic nervous system affects the values of QTd parameters, as circadian variation is observed in healthy individuals. These variations are probably the result of the change in the tone of the autonomic nervous system. There was a significant increase in QTd in the early morning hours. This morning rise in QTd values coincides with the time when there is increased myocardial vulnerability to ventricular tachycardia and fibrillation as well as sudden cardiac death. The increased QTd value is a significant marker for the development of malignant ventricular arrhythmias when sympathetic tone is increased. However, the same degree of QTd increase will not indicate the same degree of risk for malignant ventricular arrhythmias when the tone of the vagus is increased (24). Administration of insulin, which leads to hypoglycaemia and increased sympathetic tone, causes an increase in QTd values (25). It has been shown that administration of noradrenaline causes a significant increase in QTd values (26). Considering that a significant reduction in blood pressure and heart rate has occurred in our patients after a treatment with exercise training, we can conclude that there has been an improvement in the function of the autonomic nervous system, reducing sympathetic tone and increasing the tone of the vagus.

In our patients after CABG, after the treatment with exercise training, a significant increase in physical exercise capacity was observed. Training

group of patients on the second exercise test achieved a significantly longer time and higher load level. Also, training group of patients experienced a significant reduction in double product. Exercise training leads to an extension period of time for the appearance of angina pain at the time of loading or even eliminates it, since it increases VO max by reducing the heart rate and systolic blood pressure at a certain load. This reduction in the double product leads to the reduction of myocardial oxygen uptake, which prolongs the time until anginosis appears (3).

Conclusion

The study showed that exercise training has favourable effects on QT dispersion in patients after CABG. In patients after CABG, exercise training led to a significant reduction in blood pressure, heart rate, double product, and cholesterol, and significantly improved physical exercise capacity, which has a beneficial effect on the prognosis in these patients.

References

1. Kulik A. Secondary prevention after coronary artery bypass graft surgery: a primer. Curr Opin Cardiol 2016; 31(6):635-43. [CrossRef] [PubMed]
2. Dennis C. Rehabilitation of patients with coronary artery disease. In: Braunwald E, editors. Heart disease. Philadelphia: W.B. Saunders Company; 1997. p. 1392-401.
3. Thompson PD. Exercise based, comprehensive cardiac rehabilitation. In: Mann D, Zipes DP, Libby P, Bonow RO, editors. Heart Disease. Philadelphia: Saunders Elsevier; 2015. p. 1015-20.
4. Victor RG. Systemic hypertension: Mechanisms and diagnosis. In: Mann D, Zipes DP, Libby P, Bonow RO, editors. Heart Disease. Philadelphia: Saunders Elsevier; 2015. p. 1934-51.
5. Victor RG, Libby P. Systemic hypertension: Management. In: Mann D, Zipes DP, Libby P, Bonow RO, editors. Heart Disease. Philadelphia: Saunders Elsevier; 2015. p. 1953-75.
6. Kozakova M, Palombo C, Morizzo C, Nolan JJ, Konrad T, Balkau B, et al. Effect of sedentary behaviour and vigorous physical activity on segment-specific carotid wall thickness and its progression in a healthy population. Eur Heart J 2010; 31:1511-9. [CrossRef] [PubMed]
7. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur Heart J 2016; 37:2315-81. [CrossRef] [PubMed]
8. Catapano AL, Graham I, De Backer G, Wiklund O, Chapman MJ, Drexel H, et al. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. Eur Heart J 2016; 37(39):2999-3058. [CrossRef] [PubMed]
9. Bin C, Junsheng M, Jianqun Z, Ping B. Meta-Analysis of Medium and Long-Term Efficacy of Loading Statins after Coronary Artery Bypass Grafting. Ann Thorac Surg 2016; 101:990-5. [CrossRef] [PubMed]
10. Braunwald E. Reduction of LDL-cholesterol: important at all ages. Eur Heart J 2016; 37:1982-4. [CrossRef] [PubMed]
11. Morrow DA, Boden WE: Stable ischemic heart disease. In: Mann D, Zipes DP, Libby P, Bonow RO, editors. Heart Disease. Philadelphia: Saunders Elsevier; 2015. p. 1182-227.
12. Okin PM, Devereux RB, Fabsitz RR, Lee ET, Galloway JM, Howard BV. Principal component analysis of the T wave and prediction of cardiovascular mortality in American Indians: the Strong Heart Study. Circulation 2002; 105:714-9. [CrossRef] [PubMed]

13. Padmanabhan S, Silvet H, Amin J, Pai RG. Prognostic value of QT interval and QT dispersion in patients with left ventricular systolic dysfunction: Results from a cohort of 2265 patients with an ejection fraction of </=40%. Am Heart J 2003; 145:132-8. [CrossRef] [PubMed]
14. Sheehan J, Perry IJ, Reilly M, Salim A, Collins M, Twomey EM, et al. QT dispersion, QT maximum and risk of cardiac death in the Caerphilly Heart Study. Eur J Cardiovasc Prev Rehabil 2004; 11:63-8. [CrossRef] [PubMed]
15. Mirbolouk F, Arami S, Salari A, Shad B, Kazemnejad E, Moladoust H. Corrected QT-interval and dispersion after revascularization by percutaneous coronary intervention and coronary artery bypass graft surgery in chronic ischemia. J Invasive Cardiol 2014; 26(9): 444-50. [PubMed]
16. Scott PA, Rosengarten JA, Shahed A, Yue AM, Murday DC, Roberts PR, et al. The relationship between left ventricular scar and ventricular repolarization in patients with coronary artery disease: insights from late gadolinium enhancement magnetic resonance imaging. Europace 2013; 15(6):899-906. [CrossRef] [PubMed]
17. Piranfar MA. The relationship between QT dispersion and ischemic injuries in myocardial isotope scan. Acta Med Iran 2014; 52(5):345-51. [PubMed]
18. Bazett HC. An analysis of the time-relations of electrocardiograms. Heart 1920; 7:353-70.
19. Bruce RA, Fisher LD, Cooper MN, Gey GO. Separation of effects of cardiovascular disease and age on ventricular function with maximal exercise. Am J Cardiol 1974; 34:757-63. [CrossRef]
20. Morra EA, Zaniqueli D, Rodriguez S, El-Aouar LM, Lunz W, Mill JG, et al. Long-term intensive resistance training in man is associated with preserved cardiac structure/function, decreased aortic stiffness and lover central augmentation pressure. J Hypertens 2014; 32(2):286-93. [CrossRef] [PubMed]
21. Martin BJ, Hauer T, Arena R, Austford LD, Galbraith PD, Lewin AM, et al. Cardiac rehabilitation attendance and outcomes in coronary artery disease patients. Circulation 2012; 126(6):677-87. [CrossRef] [PubMed]
22. Stankovic I, Putnikovic B, Janicijevic A, Jankovic M, Cvjetan R, Pavlovic S, et al. Myocardial mechanical and QTc dispersion for the detection of significant coronary artery disease. Eur Heart J Cardiovasc Imaging 2015; 16(9):1015-22. [CrossRef] [PubMed]
23. Zhang F, Zhang X, Zhang X, Chen B, Liu Y, Yue WW, et al. Coronary Revascularization Improves QT Dispersion in Patients with Chronic Coronary Artery Total Occlusion. Cell Biochem Biophys 2015; 72:127-30. [CrossRef] [PubMed]
24. Molnar J, Rosental JE, Weiss JS, Somberg JC. QT interval dispersion in healthy subjects and survivors of sudden cardiac death: Circadian variation in a twenty four hour assessment. Am J Cardiol 1997; 79(9): 1190-3. [CrossRef] [PubMed]
25. Robinson RT, Harris ND, Ireland RH, Lee S, Newman C, Heller SR. Mechanisms of abnormal cardiac repolarization during insulin-induced hypoglycemia. Diabetes 2003;52:1469-74. [CrossRef] [PubMed]
26. Sun ZH, Swan H, Viitasalo M, Toivonen L. Effects of epinephrine and phenylephrine on QT interval dispersion in congenital long QT syndrome. J Am Coll Cardiol 1998; 31:1400-5. [CrossRef] [PubMed]

Originalni rad **UDC: 616.132.2-089.843-085.8:613.73**
doi:10.5633/amm.2018.0404

UTICAJ FIZIČKOG TRENINGA NA QT DISPERZIJU I FAKTORE RIZIKA ZA KARDIOVASKULARNE BOLESTI KOD BOLESNIKA NAKON HIRURŠKE REVASKULARIZACIJE MIOKARDA

Viktor Stoičkov[1,2], Sandra Šarić[1], Stanoje Andonov[1], Svetlana Kostić[1], Milan Lović[1], Marija Sekulović[1]

[1]Institut za lečenje i rehabilitaciju "Niška Banja", Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

Kontakt: Viktor Stoičkov
Bulevar Nemanjića 34A/8, 18000 Niš, Srbija
E-mail: viktorstoickov67@gmail.com

Cilj rada bio je da se ispita uticaj fizičkog treninga na QT disperziju i faktore rizika za kardiovaskularne bolesti kod bolesnika nakon hirurške revaskularizacije miokarda (CABG).

Studijom je obuhvaćeno 143 bolesnika nakon CABG, u sinusnom ritmu, bez AV blokova i blokova grana, prosečne starosti 57,5 godina. Bolesnici su podeljeni u grupu sa fizičkim treningom (TG: 107 bolesnika) i ne-trening grupu (NTG: 36 bolesnika). Bolesnici su imali sličnu starost i trajanje testa opterećenja. Svim ispitanicima je pored kliničkog pregleda i laboratorijskih analiza urađen standardni EKG iz kojeg je izračunavana QTd i korigovana QT disperzija (QTdc). Bolesnicima je takođe urađen test fizičkim opterećenjem na pokretnoj traci po Bruceovom protokolu, nakon čega su ispitanici TG uključeni u tretman fizičkim treningom. Na osnovu rezultata testa opterećenja, ispitanicima TG je određivan stepen fizičke aktivnosti (gimnastičke vežbe, terenske staze, vožnja na ergobiciklu). Tokom perioda praćenja ispitanicima nije menjana medikamentna terapija. Nakon sprovedenog perioda praćenja, koji je trajao 21 dan, bolesnicima je ponovo rađen standardni EKG i test fizičkim opterećenjem.

Kod ispitanika TG, nakon sprovedenog tretmana fizičkim treningom, došlo je do značajnog smanjenja vrednosti parametara QTd i QTdc ($p < 0,005$ za oba parametra) i do značajnog smanjenja sistolnog i dijastolnog arterijskog pritiska, frekvencije srčanog rada, dvojnog proizvoda, ukupnog i LDL holesterola ($p < 0,001$ za sve parametre). Ispitanici TG na drugom testu opterećenja postigli su značajno duže vreme ($p < 0,001$). Kod ispitanika NTG parametri se nisu značajno promenili nakon perioda praćenja.

Studija je pokazala da je kod bolesnika nakon CABG, fizički trening izazvao značajno smanjenje parametara QTd, arterijskog pritiska, frekvencije srčanog rada, dvojnog proizvoda i holesterola, a značajno je poboljšan funkcionalni radni kapacitet, što povoljno utiče na prognozu bolesti.

Acta Medica Medianae 2018;57(4):29-35.

Ključne reči: *fizički trening, hirurška revaskularizacija miokarda, QT disperzija, faktori rizika za kardiovaskularne bolesti*

Original article

UDC: 616.71-001.5-089.881
doi:10.5633/amm.2018.0405

RABBIT BONE TISSUE RESPONSE TO THE DEFECTS TREATED WITH DIFFERENT FIXATION METHODS

Ivan Micić[1,2], Miloš Petrović[3], Predrag Stojiljković[1,2], Sanja Stojanović[4,5], Stevo Najman[4,5], Nebojša Vacić[1]

Blood supply and stabilization at the fracture site are two essential factors in fracture healing. Different fixation methods may enable different conditions of stability needed for fracture healing.

The aim of this study was to determine, by histological tissue analysis, whether different methods of fixation and biomechanical characteristics of osteosynthetic material have an effect on the bridgeable tissue characteristics and the rate of the healing of defects of long bones in experimental animals.

Experimental procedure was performed on twenty-one Chinchilla rabbits. Artificially created bone defects were treated in one of the following ways: with a plate, an internal fixator and an external fixator.

Six weeks after surgery, the results of histological analysis showed that union was started in all examined samples. When a plate was used for fixation, fibro-cartilaginous healing stage was marked clearly with high levels of activity. In the defects treated with selfdynamisable internal fixator, the woven bone tissue was seen indicating the bony callus formation. When external fixator was used for fixation, the effect was comparable with that seen in the defect side treated with selfdynamisable internal fixator.

Based on the results obtained in our study, we can conclude that biomechanical characteristics of internal and external fixators are more superior than biomechanical characteristics of a plate in the treatment of bone defects in experimental animals.

Acta Medica Medianae 2018;57(4):36-42.

Key words: *bone defects, plate, selfdynamisable internal fixator, external fixator, bone healing*

[1]Clinic for Orthopaedic Surgery and Traumatology, Niš, Serbia
[2]University of Niš,Faculty of Medicine, Niš, Serbia
[3]Institute of Veterinary Medicine, Niš, Serbia
[4]University of Niš, Faculty of Medicine, Scientific Research Center for Biomedicine, Niš, Serbia
[5]University of Niš, Faculty of Medicine, Department of Biology and Human Genetics, Niš, Serbia

Contact: Micić Ivan
Clinic for Orthopaedic Surgery, Clinical Center Niš
Bul. Dr Zorana Djindjića 48, 18000 Niš, Serbia
E-mail: ivanmicic2000@yahool.com

Introduction

At the moment of fracture, physiological load of the bone is completely damaged, whereby mechanical feedback for adaptive response of the bone on load is also disrupted.

After formation of callus, its remodeling demands reestablishment of normal cortical architecture with the help of common adaptive mechanisms. This load through fracture is important for stimulation of adaptive response and complete healing of the fracture.

The union of bone fractures means a complicated cascade of processes on cellular and biochemical levels, which ends with a complete structural and functional recovery of a damaged bone. Although the potential of bone tissue regeneration is exceptionally high, the process of bone healing is disrupted in 5 to 10 percent of cases, which results in delayed healing or the lack of it (1). If we take such a big number of fractures into account, this high incidence of healing dysfunction surely poses a significant problem in modern traumatology.

Damage of local blood vessels is a consequence of the fracture, especially in periosteum (2, 3). Considering that forming of callus depends on vascularization of periosteum, as well as surrounding soft tissue, damage of these structures delays normal formation of callus (4, 5).

Natural process of fracture healing, including formation of stabilizing callus on the healing site, is stimulated by load and movements, although excessive movement may cause damage of bone union on

the fracture site (6, 7). Movements and load stimu-late inflammatory phase of bone healing and especi-ally revascularization (8). This biomechanical appea-rance is responsible for differentiation of healing dir-ection, between primary and secondary bone healing and it leads to right differentiation of tissue into cal-lus (9). If movements between fragments are elimi-nated and if load is transferred only via applied in-ternal fixation, union of bone can be disrupted. In the presence of axial micro movements and rigid fixation via rigid fixation apparatus, biological and mechanical bone healing can be improved, which leads to faster and histological more qualitative heal-ing (10). In practice, this means that greater rigidity of applied osteofixation material, which disables axial micro movements, may reduce the size of the heal-ing process (11). Also, reduction of fixation rigidity especially after first 4 weeks of healing may fasten and cause greater healing (12). This idea enabled rigidity decrease of the external fixator frame and invention of new osteofixation materials, which have been applied in practice in the process of dynamisation at the fracture site (13).

The aim of this work was to establish, by analysis of histological preparations, whether different methods of fixation and biomechanical characteristics of osteosynthetic materials have influence on bridgeable tissue characteristics and dynamics of union of long bone defects in experimental animals.

Material and methods

Animals

Twenty-one Chinchila rabbits were used in this study with average weight of 3.3 kg (3.2-3.5 kg). All the rabbits were sexually mature and all were parasite-free and had normal hematological profiles. All animals were acclimated to the environment for two weeks before the operation. Ethical committee of The Faculty of Medicine, the University of Niš, approved the study design.

Osteosynthetic material

As osteosynthetic material we used:

1) A plate with 4 screws which was made to resemble the classic AO plate used for fixation of long bone diaphysis.

2) A model of Mitkovic Selfdynamisable internal fixator with 4 clamps and 4 screws (14). The constituent parts of a mini internal fixator are: an oval clamp, a bar bearing the clamp and pins. Clamps with pins can be positioned in parallel position or under 90o angle.

3) A model of Mitkovic external fixator with 4 pins (15). The parts of a mini external fixator are: an oval clamp, the carrier of the clamp, the bar and the pin for fixation 2 mm in diameter. The fixator enables putting pins in parallel and convergent position up to 90 degrees.

All osteosynthetic material was made in "Trafix" Company, Niš, Serbia and all was made of 316L steel.

Surgical procedure

The surgery was performed according to principles of asepsis and antisepsis. Anesthesia of experimental animals was performed by giving Zoletil 50R (Virbac, France) in dosage of 10 mg/Kg of weight. Before the surgery Gentamicin (Galenika, Serbia) in dosage of 2 mg/Kg of weight was applied.

Each experimental animal had its right femur operated. Incision was made by lateral approach of the thigh 5 centimeters long. In the middle third of femoral diaphysis ostectomy was done by electric saw and a bone defect 2 mm big was created. At the ends of proximal and distal fragment, 1 mm in length, electrocauterization was performed to destroy osteoblasts and create conditions for nonunion of ostectomy. In six rabbits, the wound was closed with Vicryl 3-0 string and the extremity left without immobilization.

In other experimental animals the bone defect of 2 mm was fixed alternately by application of the plate, internal fixator and external fixator so that each of them was applied in five experimental animals, respectively. In the case where plate was applied, the periosteum was removed to ensure appropriate support of the plate. Axial reposition of proximal and distal femoral fragments was performed so that already created bone defect was lapsed. The hole for screws was made by Kirschner wire, 1 mm in diameter, and two distal and two proximal screws were placed and fixed tightly. Internal fixator was placed without removing of the periosteum. After the holes were made by K-wires, 1 mm in diameter, the pins were placed through the clamps of internal fixator under 90 degrees angle and tightly fixed at the bone and at the bar of the fixator. Two pins were placed in the proximal and two in the distal fragment of the femur.

To apply the external fixator, two convergent oriented pins were put into proximal and two pins into distal fragment of the femur after holes drilling by K-wires 1 mm in diameter. The created bone defect was also exposed in the length of skin incision in order to provide identical condition in experiment. Afterwards, clamps and bar of the external fixator were placed. The pins were fixed to the clamps and clamps were fixed to the bar as well. (Figure 1)

After the surgery, the wound was closed in layers and sterile gauze and bandage were used. During three days post-surgery, injection of Gentamicin was given in dosage of 2 mg/body weights every twelve hours and to eliminate pain, 1 ml injection of Novalgetol (metamizole sodium) was given every twelve hours. The rabbits were kept in special cages, given standard food and water ad libitum. The support on to operated extremity was allowed.

Six weeks after surgery, material for histological analysis was taken from the place of previously created bone defect using the same skin incision.

Figure 1. Surgical procedure of creating defects and placing fixators.

Preparation for histological examination

All animals were sacrificed six weeks after surgery. After disarticulation in the hip joint the right femurs were harvested and the tissues in the bone defect side, as well as the bone at either end of the site, were processed for histological analysis. The tissue was fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 24 h at 4 ^{0}C. After fixation and decalcification, this tissue was processed with use of graded concentrations of alcohol and then it was embedded in paraffin, sectioned and stained with Hematoxylin and Eosin (HE). Tissue samples were cut at 2 microns on a microtome (Historange). Bone tissue sections were made in both longitudinal and transversal planes in relation to the place of ostectomy, owing to which the whole healing surface was obtained for examination.

Histological preparations were prepared and analyzed at the Scientific Research Center for Biomedicine at the Faculty of Medicine, University of Niš. Microscopic analysis was performed on light microscope Olympus BH, at 20x and 40x objective magnification.

Results

Six weeks after surgery the results of histological analysis showed that in all examined samples the union was started.

In the group in which osteosynthesis was not used, material forming in the site of the ostectomy consisted largely of a fibrovascular collagenous connective tissue presenting fibrocartilaginous union (Figure 2).

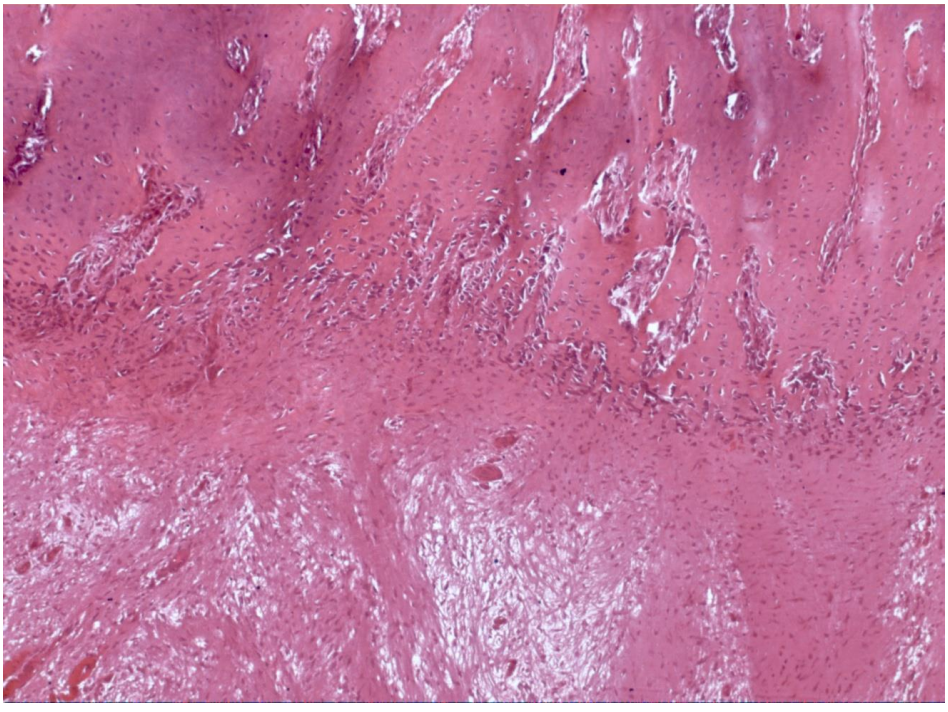


Figure 2. Histological images of the bone defect six weeks after the surgery in the group when osteosynthesis was not used. H&E staining; 20x objective magnification.

Fibrous tissue bridged residual part of ostectomy. At histological preparations where plate with screws was applied in experiments, all stadiums of tissue differentiation were visible within created defect such as granulation tissue, fibro-granulation tissue and highly differentiated fibrocartilaginous tissue in less amount. Fibrocartilaginous union was marked clearly with high levels of activity and more brisk than was noted in the absence of fixation (Figure 3). Residual part of the ostectomy was bridged from fibrous tissue with vascular capillary inside the tissue.

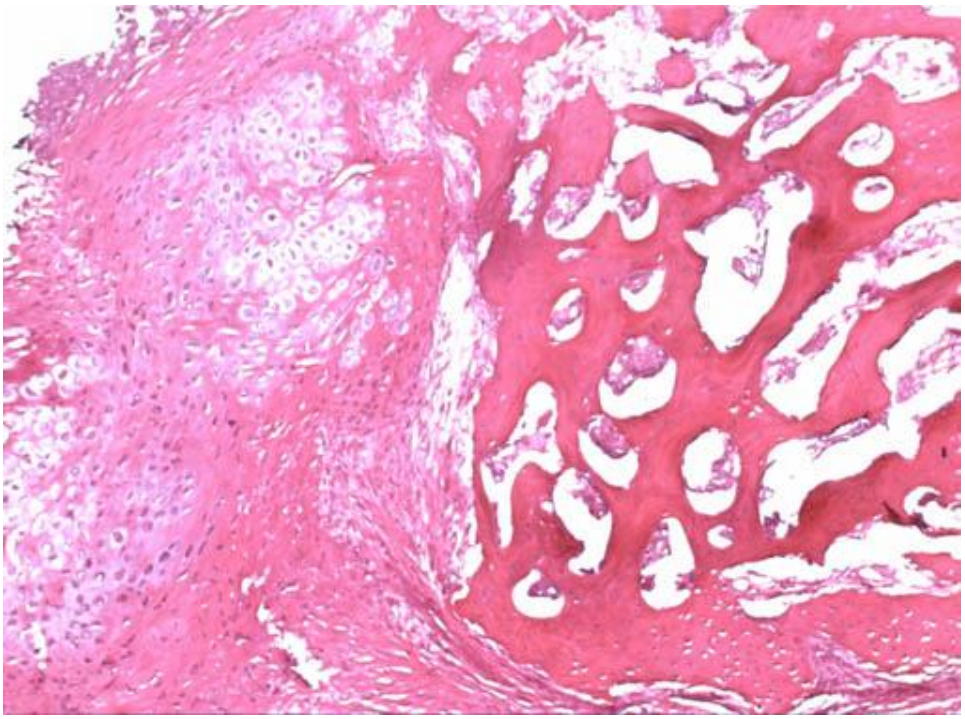
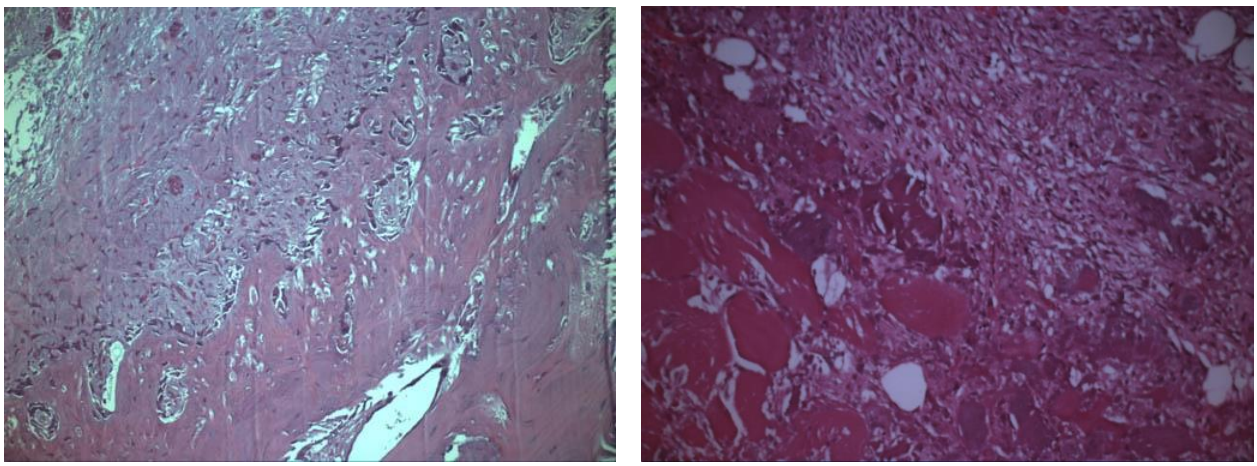


Figure 3. Histological images of the bone defect treated with plate osteosynthesis, six weeks after the surgery. H&E staining 20x objective magnification.

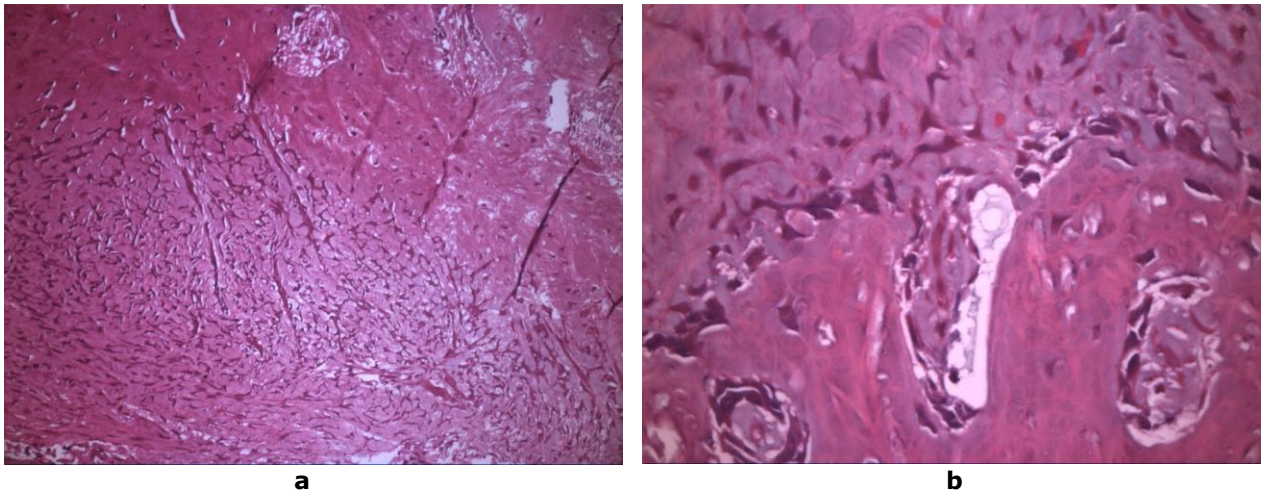
Histological preparations of the created defect site when internal fixator was applied showed the presence of newly formed woven bone with process of ossification and resorption (Figure 4a and 4b). This showed that osteogenic process in this case was much faster and more abundant in relation to osteogenic process with application of plate with screws.



a **b**

Figure 4. Histological images of the bone defect treated with internal fixator, six weeks after the surgery. H&E staining; a) 20x objective magnification; b) 40x objective magnification

Histological preparations of the created defect fixed with external fixator showed the presence of the woven bone with resorption of old and ossification of woven bone (Figure 5a and 5b). The effect was comparable to the one seen in defect treated with self-dynamisable internal fixator. In two rabbits, disintegration of fixation was seen, in one case from the distal screws of the plate and in another from the distal pins of the external fixator.



a **b**

Figure 5. Histological images of the bone defect treated with external fixator, six weeks after the surgery. H&E staining; a) 20x objective magnification; b) 40x objective magnification

Discussion

Blood supply and stabilization at the fracture site are two essential factors in fracture healing. Each of them can be partially damaged but both of them must be present to enable healing. If these factors are absent nonunion and pseudoarthrosis appear. Different fixation methods may enable different conditions of stability needed for fracture healing. However, each method damages blood supply of the bone in some degree, which has to be considered when choosing adequate method for treatment of specific fractures.

Vascular response of the bone to all biocompatible implants mainly depends on: the site of implants' placing, effect of implantation on normal blood supply, stability of implants and characteristic of their surface, as well as their functions. Bone physiology must be primarily taken into account by bioengineers who design implants and surgeons who use them.

Using a strong plate and screws for the fracture treatment anatomical reparation, faster recovery, mobilization of the joint and, to some degree, better bone healing could be achieved. Furthermore, it is known that drilling of holes, placing of screws, and application of plate and longer presence of the plate at the fracture site has a role in the development of bone osteopenia in different degree and different time (16). Plate fixation demands surgical incision through soft tissue, great deperiosting of the bone and drilling holes for the screws through cortex and medulla. All this leads to additional damage of already disturbed bone vascularization (17, 18). Stabilization of fracture with adequately placed compressive plate leads to transfer of compressed forces from proximal onto distal part of broken bone through fracture. When the bone is directly connected to the plate with screws, then the plate and the bone have the same division of load. The plate suffers the same amount of axial shortage as the bone. Fixation with plate and screws represents firm fixation on the fracture side without presence of axial or lateral micro movements (19).

The pins of external fixator lead to limited damage of bone at the spot of placing but they cannot damage blood supply of the site of healing. Placed vertically in relation to longitudinal bone axis, they don't damage primary or secondary vascularization of broken bone significantly. Reparative processes at the fracture site are determined only by primary damage of bone vascularization that has happened at the fracture site. Hematoma at the fracture site is small, which enables easier and faster healing of blood vessels through it. External fixation, although not sufficiently stable at the beginning of healing, afterwards enables dynamization at the nonunion site and finally adequate healing. Less rigid external fixation enables interfragmentary mobility and union of the fracture through the process of secondary healing.

The pins and screws of internal fixator also damage the bone in a limited way, as in the case of external fixator. The bar of internal fixator itself does not damage periosteal vascularization since it does not lean on the periosteum as tightly as in the case of plate application. Placing internal fixator prevents the damage of periosteum necessary for the process of bone reparation (14). Placing of internal fixator minimally damages the soft tissue as well, because it is applied through two minimal skin incisions (14).

The internal fixator minimally damages not only the soft tissue and the bone vascularization, it

also has specific biomechanical characteristics in that it behaves as "intelligent implant". This means that at the beginning of union, internal fixator provides stable fixation of fracture or the site of nonunion. Later, as the healing process develops, osteolysis appears around the pins, and clamps fixing the pins for the fixator bar give way, which enables compression at the fracture site along the fixator bar. Rotational movements which can damage vascularization at the fracture site are disabled by convergent orientation of pins (14). This enables dynamisation along fixator bar, the load of the bone gradually increases and the union process advances (14).

According to histological changes at created defect sites under different conditions of fixation, it has been shown that biomechanical characteristics of internal and external fixator are much better than biomechanical characteristics of plate with screws. Since stability among fragments is much better if they are fixed with a plate in comparison to fixation with external fixator, it can be said that this stability possibly has influence on healing at the beginning of osteogenesis. In later stages, dynamization at the site of union and minimal damage of the bone vascularization are much important, and that can be reached by application of external and internal fixator.

Conclusion

Histological analysis of tissue samples from experimental animals points out that stabilization of bone fragments by internal and external fixators allows the bony callus formation six weeks after surgery. Fixation by plate resulted in early stages of fracture healing and fibrocartilaginous tissue formation. Based on the results obtained in our study we can conclude that biomechanical characteristics of internal and external fixators are more superior than biomechanical characteristics of the plate in the treatment of bone defects in experimental animals.

Acknowledgement

This study was supported by the Ministry of Education, Science and Technological development of the Republic of Serbia (Grant No. III 41017) and by Faculty of Medicine, University of Niš, Serbia (internal project grant No. 11-14629-4/16).

References

1. Einhorn TA. Enhancement of fracture-healing. J Bone Joint Surg Am 1995; 77:940-56. [CrossRef] [PubMed]
2. Schweiberer L, Baumgart R, Deiler S. Die biologischen Bedingungen atropher und hypertropher Pseudoarthrosen der Schaftknochen. Ursachen und Erscheinungsbild. Chirurg 1999; 70:1193-201. [CrossRef] [PubMed]
3. Postacchini F, Gumina S, Perugia D, De Martino C. Early fracture callus in the diaphysis of human long bones. Histologic and ultrastructural study. Clin Orthop Relat Res 1995; 310:218-28. [PubMed]
4. Hulth A. Current concepts of fracture healing. Clin Orthop Relat Res 1999; 249:265-84. [PubMed]
5. Landry PS, Marino AA, Sadasivan KK, Albright JA. Effect of soft-tissue trauma on the early periosteal response of bone to injury. J Trauma 2000; 48:479-83. [CrossRef] [PubMed]
6. Sarmiento A, Sobol PA, Sew Hoy AL, Ross SD, Racette WL, Tarr RR. Prefabricated functional braces for the treatment of fractures of the tibial diaphysis. J Bone Joint Surg J Am 1984; 66:1328-39. [CrossRef] [PubMed]
7. Cornell CN, Lane JM. Newest factors in fracture healing. Clin Orthop Relat Res 1992; 277:297-311. [CrossRef] [PubMed]
8. Wallace AL, Draper ER, Strachan RK, McCarthy ID, Hughes SP. The effect of devascularisation upon early bone healing in dynamic external fixation. J Bone Joint Surg Br 1991; 73:819-25. [CrossRef] [PubMed]
9. Cheal EJ, Mansrnann KA, DiGioia AM, Hayes WC, Perren SM. Role of interfragmentary strain in fracture healing: ovine model of a healing osteotomy. Orthop Res 1991; 9(1):131-42. [CrossRef] [PubMed]
10. Kenwright J, Richardson JB, Cunningham JL, White SH, Goodship AE, Adams MA, et al. Axial movement and tibial fractures. A controlled randomised trial of treatment. J Bone Joint Surg Br 1991; 73:654-9. [CrossRef] [PubMed]
11. Goodship AE, Watkins PE, Rigby HS, Kenwright J. The role of fixator frame stiffness in the control of fracture healing. An experimental study. J Biomech 1993; 26(9):1027-35. [CrossRef] [PubMed]
12. Eggers EL, Histand MB, Norrdin RW, Konde LJ, Schwarz PD. Canine osteotomy healing when stabilised with decreasingly rigid fixation compared to constantly rigid fixation. Vet Comp Orthop Traumatol 1993; 6:182. [CrossRef]
13. Behrens F, Searls K. External fixation of the tibia. Basic concepts and prospective evaluation. J Bone Joint Surg Br 1986; 68(2):246-54. [CrossRef] [PubMed]
14. Mitkovic M. New biological method of internal fixation of the femur. Facta Universitatis 2001; 8(1):50-3.
15. Mitkovic M. New Concepts in External Fixation. Nis: Prosveta; 1993.
16. Terjensen T, Norby A, Arnulf V. Bone atrophy after plate fixation. Computed tomography of femoral shaft fractures. Acta Orthop Scand 1985; 56:416-8. [CrossRef] [PubMed]

17. Barron SE, Robb RA, Taylor WF, Kelly PJ. The effect of fixation with intramedullary rods and plates on fracture-site blood flow and bone remodeling in dogs. J Bone Joint Surg 1977; 59:376-85. [CrossRef] [PubMed]
18. Lewallen GD, Chao EY, Kasman RA, Kelly PJ. Comparison of the effects of compression plates and external fixators on early bone-healing. J Bone Joint Surg Am 1984; 66:1084-91. [CrossRef] [PubMed]
19. Perren SM. The biomechanics and biology of internal fixation using plates and nails. Orthopedics 1989; 12(1):21-34. [PubMed]

Originalni rad

UDC: 616.71-001.5-089.881
doi:10.5633/amm.2018.0405

ODGOVOR KOŠTANOG TKIVA KUNIĆA NA DEFEKTE TRETIRANE RAZLIČITIM METODAMA FIKSACIJE

Ivan Micić[1,2], Miloš Petrović[3], Predrag Stojiljković[1,2], Sanja Stojanović[4,5], Stevo Najman[4,5]

[1]Klinika za ortopediju itraumatologiju, Klinički centar, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[3]Veterinarski institut Niš, Niš, Srbija
[4]Univerzitet u Nišu, Medicinski fakultet, Naučnoistraživački centar za biomedicinu, Niš, Srbija
[5]Univerzitet u Nišu, Medicinski fakultet, Odeljenje za biologiju i humanu genetiku, Niš, Srbija

Kontakt: Ivan Micić
Klinika za ortopediju i traumatologiju, Klinički cetar Niš
Bul. dr Zorana Đinđića 48, 18000 Niš, Srbija
E-mail: ivanmicic2000@yahoo.com

Vaskularizacija i stabilizacija preloma su dva najvažnija faktora za zarastanje. Različite metode fiksacije daju različitu stabilnost potrebnu za zarastanje preloma.

Cilj rada bio je utvrditi da li različite metode fiksacije i biomehaničke karakteristike osteosintetskog materijala imaju uticaja na karakteristike premošćavajućeg tkiva i brzinu zarastanja na mestu defekata dugih kostiju kod eksperimentalnih životinja, metodom histološke analize tkiva.

Eksperimentalno istraživanje je vršeno na Chinchilla kunićima. Veštački kreirani koštani defekti kod 21 kunića tretirani su na jedan od sledećih načina: pločom, unutrašnjim fiksatorom i spoljnim fiksatorom. Histološka analiza tkiva je nakon 6 nedelja pokazala da je u slučajevima fiksacije pločom prisutno fibro-hrskavičavo zarastanje sa visokom aktivnošću. Na mestu defekata tretiranih unutrašnjim fiksatorom, uočava se formiranje mlade zrele kosti, kao i u slučajevima primene spoljašnjeg fiksatora. Na osnovu dobijenih rezultata, možemo zaključiti da biomehaničke karakteristike unutrašnjeg i spoljašnjeg fiksatora omogućavaju brže zarastanje defekata unutar 6 nedelja kod eksperimentalnih životinja.

Acta Medica Medianae 2018;57(4):36-42.

Ključne reči: *koštani defekt, ploča, samodinamizirajući unutrašnji fiksator, spoljni fiksator, zarastanje preloma*

Original article

UDC: 615:614.2
doi:10.5633/amm.2018.0406

ACCESS TO ORPHAN DRUGS: A CROSS COUNTRY COMPARISON OF LEGISLATIVE APPROACH AMONG SERBIA, CROATIA AND MACEDONIA

Dušanka Krajnović[1], Jasmina Arsić[2], Ljiljana Tasić[1], Guenka Petrova[3], Svetlana Milijić[4]

Access to orphan drugs (In EU regulation Orphan Drugs are refered as Orphan Medicinal Products (OMP)) is a key role in determining whether patients with rare diseases (RDs) will receive adequate and efficient treatment. The objective of this article is to identify differences in patient access to orphan drugs in 3 pharmaceutical markets: Serbia, Croatia and Macedonia. Patient access was defined: as the market access (availability) and affordability (financial accessibility). We analysed the legislative requirements for the authorisation process and made a cross country comparison. Retrospective cross-sectional analysis was done on drug lists in selected countries and a cross-comparison between the List of Orphan Drugs in Europe (LODE) for a six-month period (May 2014-October 2014). We included all 179 OMPs marketed in EU in our analysis, which had received market authorization in Croatia upon its membership in the EU. Total number of marketed drugs in Serbia was 59 (32.96%) drugs and in Macedonia 52 (29.05%) drugs. However, market authorization does not guarantee patient access to any given drug, so only 39.11% of OMPs could be accessed by Croatian patients (70 drugs).The number of refunded drugs in Serbia and Macedonia was smaller (32 and 20, respectively) which makes respectively, 17.88% and 11.17% of drugs on the LODE. The present study showed some variations between countries in selected indicators of availability and access to orphan drugs. Patients in Croatia had greater number of registered and refunded drugs, but in Serbia more than a half of registered OMPs could be refunded from National Health Insurance Fund. Macedonia had smaller number of inhabitants and also had the smaller number of patients from certain RDs which results in lower total number of OMPs.

Acta Medica Medianae 2018;57(4):43-51.

Key words: *orphan medicinal product (OMP), affordability, availability, rare diseases, legislative requirements*

[1]University of Belgrade, Faculty of Pharmacy, Department of Social Pharmacy and Pharmaceutical Legislation, Belgrade, Serbia,
[2]At the time of the research student at postgraduates specialization programme, University of Belgrade, Faculty of Pharmacy, Department of Social Pharmacy and Pharmaceutical Legislation, Belgrade, Serbia
[3]Medical University of Sofia, Faculty of Pharmacy, Department of Social Pharmacy, Sofia, Bulgaria
[4]Republic Fund for Health Insurance, Niš, Serbia

Contact: Dušanka Krajnović
Faculty of Pharmacy
450 Vojvode Stepe Str, 11221 Belgrade, Serbia
E-mail: parojcic@pharmacy.bg.ac.rs
dusica.krajnovic@pharmacy.bg.ac.rs

Introduction

According to the Regulation on Orphan Medicinal Products of the European Parliament and the European Council (EC) N° 141/2000 Orphan Medicinal Products (OMPs, also known as Orphan drugs-ODs[1]) are used for the diagnosis, treatment and prevention of rare diseases (RDs) occurring with a prevalence of at least 0.05%, i.e. affecting no more than 5 in 10,000 people (1). In Europe, the status of rare diseases may refer to all diseases without a specific treatment while in the USA, regardless of their prevalence, rare diseases include those without a proven efficient treatment with existing drugs. In both regions of the world, a two-step licensing system is applied by regulatory bodies to market drugs including orphan designation (OD) and marketing authorisation (MA) for an OMP. RD are characterised not only by their low prevalence but also by the fact that the rarity concept can be considered from several aspects (2): the perspective of the centre of expertise (clinical centres of excellence), a diagnostic reference centre, clinical studies, as shown in Figure 1.

[1] We shall use interchangeable the terms Orphan Medicinal Products (OMPs) and orphan drugs (ODs) in this text.

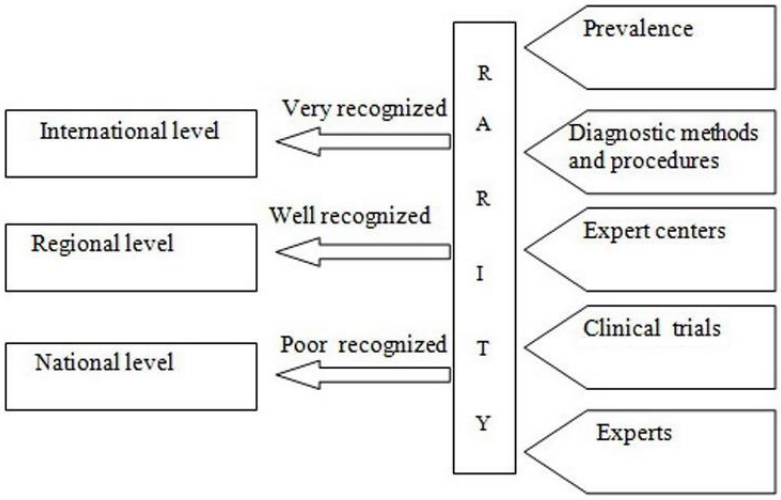


Figure 1. Rarity concept

The process of obtaining OD includes the centralized procedure of the EU or national procedures for countries that are not EU members. There is a special Committee for Orphan Medicinal Products (COMP) within EMA whose task is to control criteria connected to the significant benefit for acquiring orphan status and to the guidelines for clinical research in small groups which are used for assessment of clinical proof, while acquiring permission (3).

Besides classical drugs for rare diseases which passed the complete procedure for getting the OD and marketing authorisation (MA) EMA recognises additional group of OMPs, without OD and with MA. This group of drugs includes all those drugs which possess drug permission for use with one or more indications for rare diseases, but did not pass the procedure for acquiring OD, or the status was removed. OMPs with MA and without OD cannot be of the same name like the name dedicated to other indications.

Aim

This paper aimed to evaluate the market access (availability) to OMPs and make a comparison in three neighbouring countries (Serbia, Macedonia and Croatia) with regulatory approach. A further aim was tto evaluate affordability (financial accessibility) of OMP in selected coutries.

Material and methods

The following methods were used: a comparative analysis of applications for MA of drugs in Serbia, Macedonia, and Croatia. Serbia and Macedonia are non-Eu countries and Croatia is EU country with EU/EMA procedures accepted. The manual document analysis of secondary data was conducted on October 2014, comparing the national legislative files with the Regulation on OMP (Regulation (EC) no. 141/2000. Furthermore, in order to study potential differences between OMP availability and affordability in Serbia, Macedonia and Croatia, we performed a comparative study of the authorised OMP and OMP included in the reimbursement drug lists in the selected countries.

First, we obtained information about the OMPs with MA in Europe from the List of Orphan Drugs in EU (LODE) for a six-month period (May 2014-October 2014), including OMP with and without prior OD (4). Then, we searched the officially issued sources for authorised drugs and reimbursed drugs in the three countries. Information on the OMP availability in these countries was systematized by INN and ATC code from the databases of the national authorities for National drug registers (NDRs) and National Reimbursement Lists (NRLs). The market access of OMPs was analyzed by crossing OMPs identified in the NDRs and LODE; the affordability was evaluated by crossing NRLs with LODE among the three countries.

The data were collected from the NDRs in Serbia, Macedonia and Croatia. The data of reimbursed OMP were collected from the NRLs in selected countries.

The sources of documents were collected from the official website of the Medicines and Medical Devices Agency of Serbia (MMDAS) and from the National Health Insurance Fund (NHIF) of the Republic of Serbia (5, 6), from the official website of the Ministry of Health, and NHIF of the Republic of Macedonia (7) and from the official website of the Medicines and Medical Devices Agency of Croatia (MMDAC) and NHIF of Croatia (8, 9).

Results

The results connected with the regulatory availability refer to the analysis of legislative approaches for the acquiring and renewal of the MA, in the sense of time limits for procedures and types of MA procedures. The availability analysis refers to the registered drugs authorised at national drug lists, while the analysis refers to reimbursed drugs, meaning

financial affordability of the drugs in the selected countries. All the results are given for each country as follows (Serbia, Macedonia and Croatia) and then the comparison between them was made.

Analysis of the legislative and policies to OMP in the selected countries

All three selected countries define similar regulatory requirements for the MA of OMP. The analysis of the application for MA will be presented in the following sections whereas the comparison of the results obtained in the selected countries is presented in Table 1.

Serbia - regulatory approach

According to the National Organization for Rare Diseases of Serbia (NORDS), about 500.000 people are suffering from some of the RD out of 7.2 million of inhabitants in Serbia (10). In the Republic of Serbia, the MA of OMP is included in the national procedure for MA of all medicinal products. A drug is launched onto the market with a predetermined intensity of effect, pharmaceutical form and package based on a drug licence issued by the MMDAS which confirms that all the regulatory requirements for MA have been fulfilled and that a drug fulfils the standards of high quality, safety and efficiency. Moreover, the latest amendments introduced by the pharmaceutical legislature in the Republic of Serbia in 2010, taken over from the European directives on medicines, pertain directly and indirectly to OMPs: i) a new definition of drugs was introduced so as to include all the progressive forms of therapy, gene or cell therapy, ii) the drug licence is to be renewed after five years after which period it is issued for an indefinite period of time, iii) a new period for data protection related to the results of pre-clinical and clinical studies, the so called "8+2+1" period (data exclusivity period), was introduced by a new directive, iv) the introduction of new categories for drug marketing authorisation and licensing accelerated approval licence is to be granted within 150 days after receiving the complete application (Table 1.). A conditional approval may be granted for drugs used for the treatment, prevention or diagnosis of RDs and for drugs already licensed under a centralised procedure as well as other drugs of great importance for public health. Upon agreement with an applicant, MMDAS may issue a drug licence under certain conditions, which the applicant is expected to fulfil and which are revised and checked by MMDAS once in 12 months following the date the adaptive licence was issued. The licence is valid for 12 months and may be renewed until the necessary requirements for a proper licence are fulfilled in case the benefits of the drug in question surpass the risks that may occur due to insufficient data on a clinical research. An adaptive licence may be issued under an accelerated procedure.

Macedonia - regulatory approach

It is estimated that in Macedonia from 2.1 million people, there are about 100.000 patients suffering from RDs. The Macedonian Law on Medicines and Medical Devices governs the use of medicines and medical devices in human medicine, their quality, safety and effectiveness, their production, testing, marketing, sale, prices, quality control, advertising and inspection supervision (11). The Medicines and Medical Devices Agency of Macedonia (MMDAM) issues approvals for drugs authorised in at least three EU countries within 15 days after a complete application was submitted on the basis of their quality, safety and effectiveness estimated in the procedure of MA in the EU and upon the proposal of the Committee for Medicines. In case of submitting an application for a drug authorised in fewer than three EU countries, the MMDAM either accepts or rejects the application within 90 days after the application was submitted on the basis of the drug's quality, safety and effectiveness estimated in the MA in the EU and upon the proposal of the Committee for Medicines. MA is granted for a period of eight years.

An adaptive licence is granted by the Agency in case the drug in question is unavailable, yet essential for a patient's welfare and treatment (rare diseases, ethical aspects, life-threatening diseases). An adaptive licence is granted for one year at most (12).

Croatia - regulatory approach

It is estimated that in Croatia there are about 250.000 patients suffering from RDs (4.2 million populations) The Republic of Croatia placed OMPs on the "List of exceptionally expensive drugs" in 2006. OMPs include all drugs that pursuant to regulations in the European Union have been granted the status of drugs used for the treatment of serious and rare diseases. Since 2010, Medicines and Medical Devices of Croatia (MMDAC) has been publicising a list of OMPs with MA and OD in the EU and which have been granted a European marketing authorisation (EMA) (13). In accordance with the Law on Medicines, OMP include all drugs that pursuant to EU regulations have been granted the status of drugs used for the treatment of serious and rare diseases (13). Since 2010, the MMDAC has been posting on its Internet pages a list of OMPs with an OD in the EU and MA (13). The Managing Board of the Croatian Health Insurance Fund determines which drug is to be placed on the "List of exceptionally expensive drugs" after deliberation by the Committee for Medicines. The financing of treatment by a drug from the "List of expensive drugs" is financed by a separate fund part of the state budget allocated to expensive drugs under supervision paying particular attention to the control of drugs use increase. Compassionate use of drugs is also possible from the moment of diagnosis until the drug is authorised for use. OMPs

availability depends on import procedures and treatment financing. Drugs which are not on the "List of expensive drugs" are financed by hospital funds, which might impose a significant financial burden upon an institution. Table 1 shows legislation and policies in the area of rare diseases and orphan drugs in Serbia, Macedonia and Croatia.

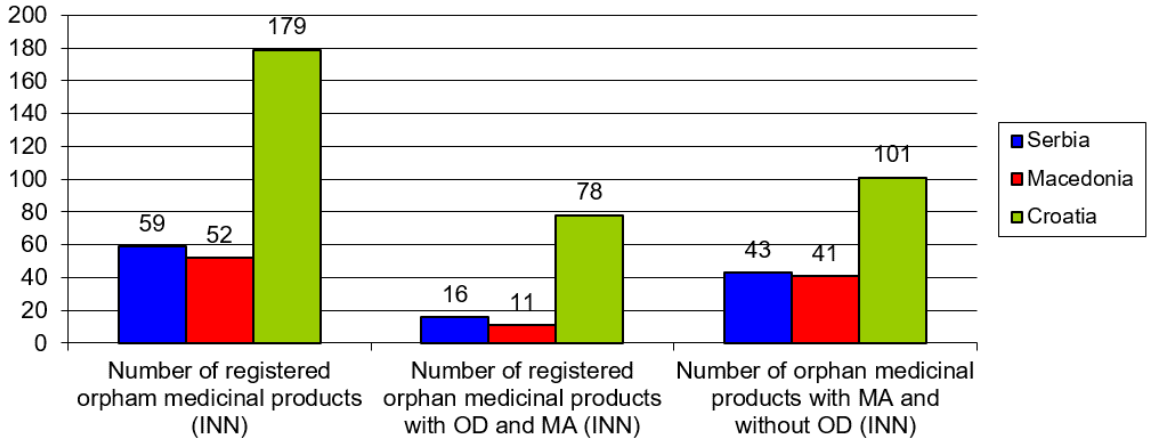
Table 1. Legislation and policy in the area of rare diseases and orphan drugs in Serbia, Macedonia and Croatia

	Serbia	Macedonia	Croatia
Population (mill) GDP per capita in 2014 (Euro)	7.2 3772	2.11 2642	4.3 9620
Number of patients with rare diseases	500.000	100.000	250.000
Key incentives of the orphan drug legislation			
The time limit for the valid application for MA (days)	210	210	210
The time limit for the reduced application for MA (days)	150	15 days if MP is avaliable in 3 countries in EU 90 days if MP is avaliable in < 3 countries in EU	150
Orphan Drug MA (years) Normal approvals	5	5	5
Orphan Drug MA (years) Conditional approvals	1	1	1
Orphan Drug MA (years) Exeptional circumstances	/	/	1

Analysis of market access of OMPs in the selected countries

In October 2014, 78 orphan drugs were on the LODE in Europe with OD and with MA in the EU and 101 drugs with MA and without OD (14).

In Serbia, 32.96% of OMPs were on the LODE, while 29.05% of all drugs in Macedonia were on the LODE (Graph 1.). All 179 registered drugs on the LODE became available in the Republic of Croatia upon its membership in the EU (October 2014).



Graph 1. Total number of OMPs in the selected countries (October 2014)

Analysis of affordability of OMPs in the selected countries

Serbia - affordability

The amendments to the Law on health insurance created legal grounds for providing distinct funds in the Serbian budget for financing the treatment of RD (15). The financial plans for patients were first drafted in 2012 on which occasion a separate fund was established. The fund is supposed to finance medical expenses caused by the treatment of RD with drugs not included in the NRLs. The financing of the fund is regulated by the Law of Health Insurance and the Law of Games of Chance so that 5% of the budget income is allocated to financing treatment of RD (15, 16). An amount of 335,322000 RSD (corresponding to about 2,733,781 EUR) was anticipated by the amendments to the financial plan for year 2014 to be transferred to the National Fund from the budget of Serbia intended for health protection and treatment RD patients (17). In Serbia, OMPs are on one of the five Lists of drugs of the National Health Insurance Fund of the Republic of Serbia (NHIFRS) (A, A1, B, C, D) and are subject to either complete or partial reimbursement. The Government of Serbia, on the 30th August 2014, founded the Budget fund for treatment and conditions or injures that could not be successfully treated in Serbia. The Fund is intended for the treatment of children up to 18 years suffering from RDs that are curable abroad if it is not possible to diagnose them in Serbia. The Fund is open in an indefinite period and managed by the Ministry of Health (18).

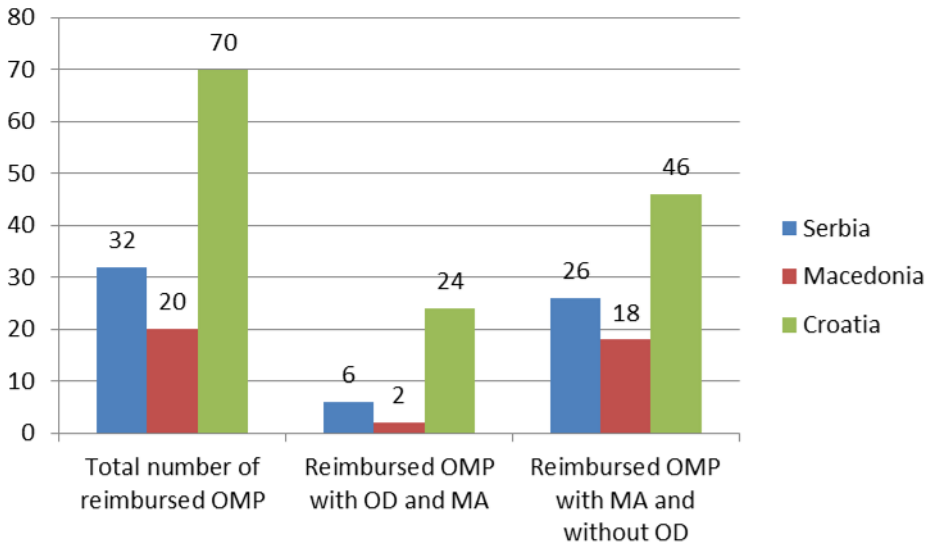
Macedonia - affordability

In Macedonia, OMPs are financed by funds from the budget of Macedonia. At the beginning of every year, the Government adopts the Programme for the treatment of RDs in the Macedonia defining how much money from that year's budget is allocated to this purpose and how it will be spent (19). The government of Macedonia adopts the Programme for the treatment of RDs pursuant to the Law of Health Insurance every year. In 2014, the Programme was financed with 80,000,000 MKD (corresponding to about 1,331,412 EUR). The newest amendments to the Law of Excise and the Law of Health Insurance adopted in December 2014 determined that an amount of 0053 MKD per cigarette pack was to be used for the financing of rare diseases, which is an extra amount of 250,000,000 MKD annually (corresponding to about 4,160,662 EUR). OMPs are purchased through the Ministry of Health in government procurement. In Macedonia, there is only one NRLs without any fees imposed on the patients. Unregistered drugs may be purchased on condition there is no other adequate therapy for certain patients and that these drugs have already been submitted to the licensing procedure or have been included in clinical research.

Croatia - affordability

The Croatian Law on Medicines is congruent to EU directives. The integral list of drugs for rare and serious diseases is being modulated and harmonised with the EU list. Medical expenses for the treatment of rare diseases are reimbursed by the state. The Croatian Ministry of Health is entitled to start an OD procedure. The national programme for RD predicted an amount of 360,689,000 HRK (47,155,052 EUR) to be spent for their treatment for the period from 2015 to 2020 (20).

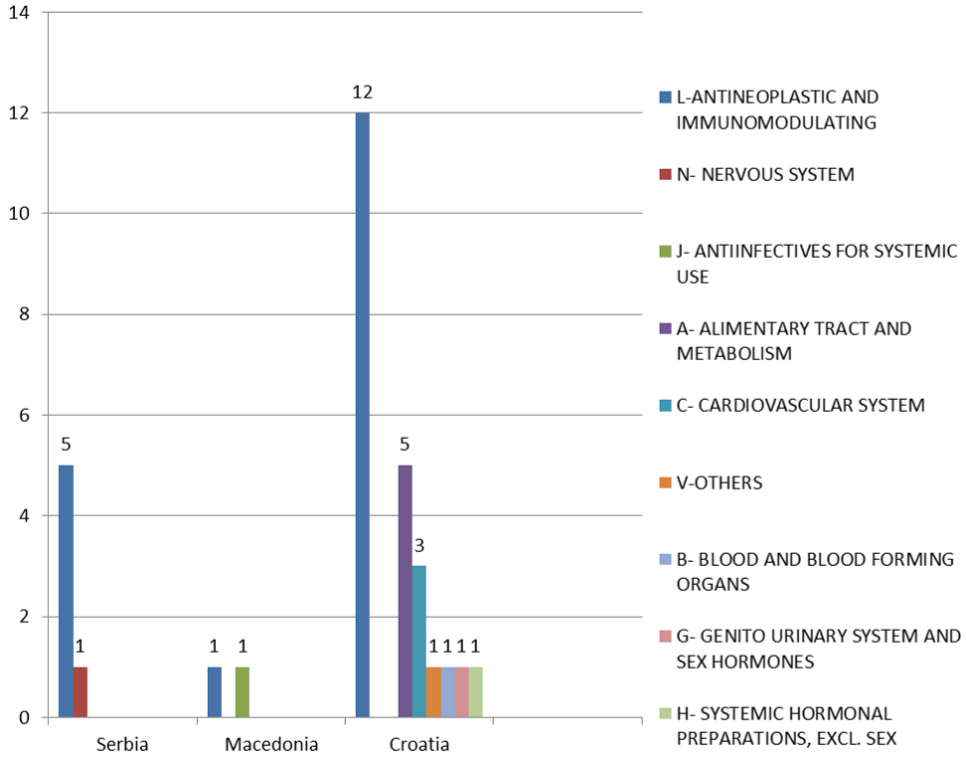
The representation of drugs which are being on NRLs relative to the complete number of registered OMPs in selected countries shows that in Serbia more than a half of drugs is financially affordable comparing with Macedonia and Croatia where the financially affordable drugs make a third of complete registered OMP (Graph 2.).



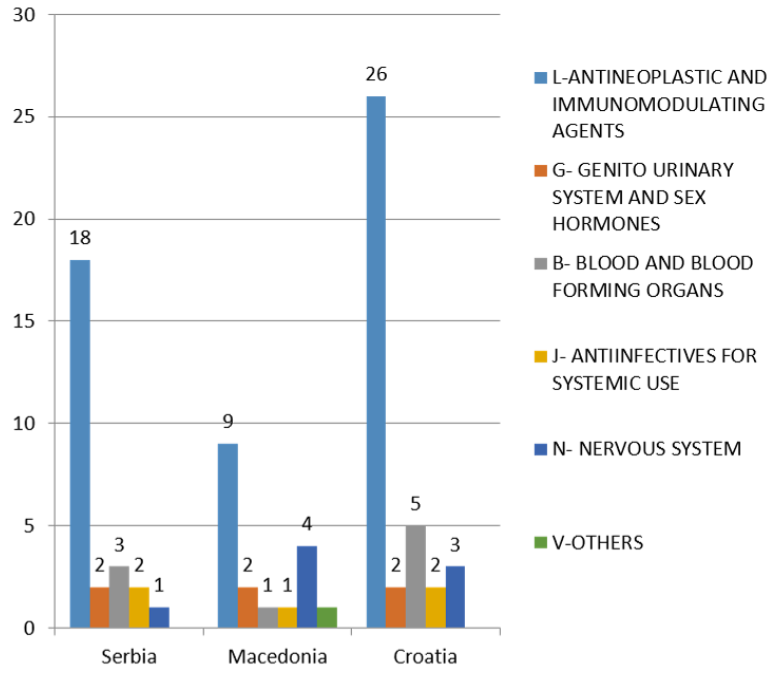
Graph 2. Total number of reimbursed OMPs (October 2014)

The financially most affordable OMPs with OD and MA, according to the first level of ATC classification were in group L – antineoplastics in all researched countries (Graph 3.).

The financially most affordable OMPs without OD and MA according to the first level of ATC classification were in group L – antineoplastics, in all studied countries (Graph 4.).



Graph 3. Number of reimbursed OMPs with OD and MA by ATC code (October 2014)



Graph 4. Number of reimbursed OMPs with MA and without OD by ATC code (October 2014)

Discussion

The mutual comparison of the financially affordable OMPs in Serbia, Macedonia and Croatia comparing with the complete number of registered drugs of this category represents the biggest financial affordability of these drugs is in Serbia (54.24% of registered drugs), while it is smaller in Macedonia and Croatia (38.46%, 39.10% respectively), no matter that Macedonia has the smallest number of registered drugs from the LODE while Croatia has all drugs from the LODE. This indicates to the fact that although there is the problem with the lack of funds for financing the treatment of patients with RD, more than a half of registered OMPs can be refunded from NHIF. Despite the fact that in Croatia, a member of the EU, a greater number of OMPs are registered, they are not completely affordable to the patients. Macedonia has the smallest number of inhabitants out of the three studied countries, which means that it also has the smallest number of patients from certain RD, which results in the lower total number of OMPs that are on the NRLs.There are very similar regulatory approaches in all three studied countries for MA for OMPs because they all came from the same health system and they harmonized the regulations for drugs with the European directive. A couple of authors have been analysing the affordability of OMP in Serbia and other countries (Macedonia, Bulgaria, Greece etc.) (21, 22). Besides, there were studies which compared the affordability of OMPs in other countries of the region, which represented that the affordability of drugs is different and subjected to dynamic changes (23).

The study of Zlatareva et al.(21) have shown the registered OMPs in January 2013 in Serbia, while our study represented the growth of 10 drugs in Serbia till October 2014. Comparing results of Zlatareva study with ours it is shown that the number of refunded drugs (on the cost of health insurance) is marginally raising so as for the 1 drug with OD and MA and 3 drugs with the MA and without OD. Pavlovic et al. (22) investigated 4 drugs financially affordable in Serbia with OD and MA and 17 drugs with MA and without OD in July 2011, revealing that the number of affordable drugs is slowly raising in Serbia. Zlatareva et al. represented that there was only one drug with the OD and MA affordable in Macedonia in January 2013 while 14 drugs with the MA and without OD were on the NRLs (21). In 2014, our results suggested that the number of financially affordable drugs in Macedonia raised from one to two drugs with OD and MA, while 18 drugs without OD and MA were on the NRLs.

Conclusion

From the perspective of health politics, regulative and legislative of OMPs within any country is different, the MA of ODs and the affordability through funds of health insurance is done in many different ways.

Different accessibility of OMPs in all three countries could be explained with the different capacity and modality for financing the treatment of RDs, and national health policies criteria for refunding of these drugs. The affordability and accessibility of OMPs are generally the problem both in developed and undeveloped countries. Our study showed that RD patients in Croatia have better accessibility of drugs than patients in Serbia and Macedonia, but in Serbia the affordability is rather better because half of the registered OMPs is refunded.

Acknowledgement

The research was conducted within the work of two scientific research projects financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia (project No. 41004 and No. 175036).

References

1. European Medicines Agency. Medicines for rare disease. [Internet] [updated-2017 Nov]. Avaliable from: URL:http://www.ema.europa.eu/ema/index.jsp?curl=pages/special_topics/general/general_content_000034.jsp.
2. Pariser AR, Yao LP. Rare Diseases and Orphan Drugs. In: Mulberg AE, Murphy D, Dunne J, Mathis LL, editors. Pediatric Drug Development. 2nd ed. New York: John Wiley & Sons Ltd; 2013. P. 130-48. [CrossRef]
3. European Commission. Volume 2A Procedures for marketing authorization Chapter 4 Centralized Procedure. [Internet]. [updated 2017 Nov]. Available from: URL: http://ec.europa.eu/health/files/eudralex/vol-2/a/ chap4rev200604_en.pdf.
4. Lists of medicinal products for rare diseases in Europe. [Internet]. [updated 2017 Nov]. Available from: URL: http://www.orpha.net/orphacom/cahiers/docs/GB/list_of_orphan_drugs_in_europe.pdf.
5. Medicines and Medical Devices Agency of Serbia. Availiable from: URL: http://www.alims.gov.rs/latin/2014/08/07/nacionalni-registar-lekova-2014/.
6. Serbian institute for health insurance. [Internet]. [updated 2017 Nov]. Available from: URL: http://www.rfzo.rs/index.php/osiguranalica/lekovi-info/pretraga-liste-lekova.
7. Ministry of Health of the Republic of Macedonia. [Internet]. [updated 2017 Nov]. Available from: URL: http://lekovi.zdravstvo.gov.mk/.
8. Agency for Medicinal Products and Medical Devices of Croatia. [Internet]. [updated 2017 Nov]. Available from: URL: http://www.halmed.hr/Lijekovi/Informacije-o-lijekovima/Lijekovi-za-lijecenje-rijetkih-i-teskih-bolesti/.
9. Croatian Health Insurance Fund. [Internet]. [updated 2017 Nov]. Available from: URL: http://www.hzzo.hr/zdravstveni-sustav-rh/trazilica-za-lijekove-s-vazecih-lista/arhiva-liste-lijekova.
10. National Organization for Rare Diseases. [Internet]. [updated 2017 Nov]. Available from: URL: http://www.norbs.rs/o-norbs-u/.
11. Official Gazette of the Republic of Macedonia: Law of drugs and medical devices. No 106, 2007. [Internet]. [updated 2017 Nov]. Available from: URL: https://www.wto.org/english/thewto_e/acc_e/mkd_e/WTACCMKD24A1_LEG_4.pdf.
12. European Medicines Agency. Orphan designation. [Internet]. [updated 2017 Nov]. Available from: URL: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000029.jsp.
13. Agency for Medicinal Products and Medical Devices of Croatia. [Internet]. [updated 2017 Nov]. Available from: URL:http://www.halmed.hr/Lijekovi/Informacije-o-lijekovima/Lijekovi-za-lijecenje-rijetkih-i-teskih-bolesti/.
14. Orphanet. Lists of medicinal products for rare diseases in Europe. [Internet]. [updated 2017 Nov]. Available from: URL: http://www.orpha.net/orphacom/cahiers/docs/GB/list_of_orphan_drugs_in_europe.pdf.
15. Official Gazette of the Republic of Serbia: Law of health insurance. No. 57, 2011. [Internet]. [updated 2017 Nov]. Available from: URL: http://www.skriningsrbija.rs/files/File/English/Republic_of_Serbia_Healthcare_Law.pdf.
16. Official Gazette of the Republic of Serbia: Law of Games of chance. No. 88, 2011. [Internet]. [updated 2017 Nov]. Available from: URL: http://www.skriningsrbija.rs/files/File/English/Republic_of_Serbia_Healthcare_Law.pdf.
17. Health insurance fund in the Republic of Serbia: The financial plan of the health insurance fund of the Republic of Serbia for the year 2014. [Internet]. [updated 2017 Nov]. Available from: URL: http://www.rfzo.rs/download/finplan2014.pdf.
18. Ministry of Health of the Republic of Serbia. [Internet]. [updated 2017 Nov]. Available from: URL: http://www.zdravlje.gov.rs/showpage.php?id=346.
19. Official Gazette of the Republic of Macedonia: Programme for treating rare diseases in the Republic of Macedonia for the year 2012. No. 8, 2012.
20. Ministry of Health of the Republic of Croatia: Decision on preparing a National orphan drugs programme for the period from 2015 - 2020. [Internet]. [updated 2017 Nov]. Available from: URL: https://zdravlje.gov.hr/UserDocsImages//Programi%20i%20projekti%20-%20Ostali%20programi//NACIONALNI-PROGRAM-ZA-RIJETKE-BOLESTI-2015-%202020.g.%20(1).docx. [CrossRef][PubMed]
21. Zlatareva A, Lakic D, Kamusheva M, Spaskov D, Momekov G, Petrova G. Analysis of Access to Orphan Drugs in Five Neighboring European Countries — Bulgaria, Greece, Macedonia, Romania and Serbia. World Journal of Pharmacy and Pharmaceutical Sciences 2013; 2:4415-34.
22. Pavlović N, Stanimirov B, Stojančević M, Paut-Kusturica M, Stoimenova A, Goločorbin-Kon S, at al. An insight on differences in availability and reimbursement of orphan medicines among Serbia, Bulgaria and Sweden. Biotechnology & Biotechnological Equipment 2012, 26(5): 3236-41. [CrossRef]
23. Kamusheva M, Stoimenova A, Doneva M, Zlatareva A, Petrova, G. A Cross Country Comparison of Reimbursed Orphan Medicines in Bulgaria, Greece, Macedonia. Biotechnology and Biotechnology Equipment 2013;27: 4186-93. [CrossRef]

Originalni rad **UDC: 615:614.2**
doi:10.5633/amm.2018.0406

DOSTUPNOST LEKOVA ZA RETKE BOLESTI: KOMPARATIVNA ANALIZA LEGISLATIVNIH ZAHTEVA IZMEĐU SRBIJE, HRVATSKE I MAKEDONIJE

Dušanka Krajnović[1], Jasmina Arsić[2], Ljiljana Tasić[1], Guenka Petrova[3], Svetlana Milijić[4]

[1]Univerzitet u Beogradu-Farmaceutski fakultet,Katedra za socijalnu farmaciju i farmaceutsko zakonodavstvo, Beograd, Srbija
[2]U vreme pisanja rada specijalizant na Univerzitetu u Beogradu-Farmaceutskom fakultetu,Katedri za socijalnu farmaciju i farmaceutsko zakonodavstvo, Beograd, Srbija
[3]Medicinski univerzitet u Sofiji, Farmaceutski fakultet,Katedra za socijalnu farmaciju, Sofija, Bugarska
[4]Republički fond za zdravstveno osiguranje, filijala Niš, Niš, Srbija

Kontakt: Dušanka Krajnović
Fakultet za farmaciju
Vojvode Stepe 450, 11221 Beograd, Srbija
E-mail: parojcic@pharmacy.bg.ac.rs
dusica.krajnovic@pharmacy.bg.ac.rs

Dostupnost lekova za retke bolesti (orfan lekovi, u EU regulativi koristi se izraz na engleskom Orphan medicinal products, OMP) igra važnu ulogu u tome da li će bolesnici sa retkim bolestima imati pristup efikasnoj i adekvatnoj terapiji. Ciljevi ovog rada su da se identifikuju razlike u pristupu bolesnika orfan lekovima u tri odabrane zemlje: Srbiji, Hrvatskoj i Makedoniji. Pristup orfan lekovima definisana je kao: tržišna pristupačnost (dostupnost) i priuštivost (finansijska pristupačnost). Analizirali smo legislativne zahteve u procesu stavljanja leka u promet i uradili komparaciju među posmatranim zemljama. Retrospektivnom studijom preseka poredili smo nacionalne liste lekova posmatranih zemalja i Listu orfan lekova u EU, za period od šest meseci (maj-oktobar 2014.). Od ukupno 179 OMP, koliko ih je u tom periodu bilo sa dozvolom za stavljanje u promet u EU, u Srbiji je bilo registrovano 59 (32,96%), u Makedoniji 52 (29,05%), dok su u Hrvatskoj učlanjenjem u EU svi registrovani lekovi postali tržišno dostupni. Međutim, dozvola za stavljanje u promet nije i garancija da bolesnik ima pristup datom leku, pa je samo 39,11% lekova sa dozvolom za stavljanje u promet bilo na listi lekova koje se refundiraju u Hrvatskoj (70 OMP). Broj lekova kojima bolesnici imaju pristup preko nacionalnih fondova zdravstvenog osiguranja u Srbiji i Makedoniji su manji (32 OMP i 20OMP, respektivno), što čini da je priuštivost lekova u Srbiji tek 17,88% , a u Makedoniji 11,17% od liste orfan lekova u EU. Broj lekova, fizička i finansijska pristupačnost u analiziranim zemljama nije ista, dok je u Hrvatskoj bolesnicima dostupan najveći broj registrovanih lekova, u Srbiji se više od polovine registrovanih OMP može refundirati o trošku RFZO. Makedonija zbog manjeg broja stanovnika ima manji broj obolelih od RB, što rezultira manjim brojem OMP.

Acta Medica Medianae 2018;57(4):43-51.

Ključne reči: *orfan lekovi (OMP), priuštivost, dostupnost, retke bolesti, legislativni zahtevi*

Original article

UDC: 618.146-006.6:615.276/277
doi:10.5633/amm.2018.0407

MOLECULAR MECHANISMS OF POTENTIAL SYNERGISTIC EFFECT OF KETOPROFEN AND MELOXICAM WITH CONVENTIONAL CYTOSTATICS IN HUMAN CERVIX CANCER CELL LINE

Ivana Damnjanović[1], Gordana Kocić[2], Stevo Najman[3], Sanja Stojanović[3], Katarina Tomović[1], Budimir Ilić[4], Andrej Veljković[2], Andrija Šmelcerović[1,4]

Cyclooxygenases clearly appear to be implicated in carcinogenesis. It has been reported that COX-2 is active throughout the entire process of cancer development and progression. Various molecular mechanisms may be responsible for this. Epidemiological and experimental studies have revealed that nonselective non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors can reduce the risk of cancer. Inhibition of COX provides a plausible explanation of the data on NSAIDs and cancer. However, the molecular pathways of this effect are still unclear, more complex and likely involve multiple COX-2-dependent and independent mechanisms, where pro- and anti-apoptotic Bcl-2 family members may take part.

We examined the effects of ketoprofen (KT), as nonselective COX-1/2, and meloxicam (MK), as selective COX-2 inhibitor, alone and combined with 5-fluorouarcil (FU) and cisplatin (CP), on the proliferation by MTT test and Bcl-2/Bax expression in HeLa cells (human cervical carcinoma cells).

MC alone or combined with conventional anticancer drugs, FU and CP, showed better cytotoxic and antiproliferative effect than KT. The levels of Bcl-2 were decreased while the levels of Bax were increased dose-dependently by KT and MC. A significant increase in the expression of Bax protein in HeLa cells was more pronounced for MC.

The synergy observed in the effects of ketoprofen and meloxicam with cisplatin and 5-fluorouracil on the cervical cancer cell line was generated by an enhancement of apoptosis. Therefore, ketoprofen and meloxicam may represent therapeutic candidates to improve access of cervical cancer chemoprevention and chemotherapy.

Acta Medica Medianae 2018;57(4):52-59.

Key words: *molecular mechanisms, synergistic effect, ketoprofen , meloxicam, HeLa cells*

[1]University of Niš, Faculty of Medicine, Department of Pharmacy, Nis, Serbia
[2]University of Niš, Faculty of Medicine, Department of Biochemistry, Niš, Serbia
[3]University of Niš, Faculty of Medicine, Department for Cell and Tissue Engineering, Institute of Biology and Human Genetics, Niš, Serbia
[4]University of Niš, Faculty of Medicine, Department of Chemistry, Niš, Serbia

Contact: Ivana Damnjanović
Department of Pharmacy, Faculty of Medicine, University of Niš
Blvd Dr. Zorana Djindjića 81, 18000 Niš, Serbia
E-mail: ivanad.ph@gmail.com

Introduction

Chronic inflammation and overexpression of cyclooxygenase enzymes (COX) take part in the development of epigenetic changes caused by environment/lifestyle factors that contribute to the accumulation of genetic mutations associated with cancer development and progression (1). COX enzymes clearly become dysregulated in cancers and all previous research indicate that these metabolic path-ways are involved in carcinogenesis and tumor progres sion (2). COX-1 is up-regulated in cervical and ovarian cancers (3). On the other hand, COX-2, which is normally undetectable in healthy tissue, is markedly overexpressed in colorectal (4), lung (5), prostate (6), cervical (7), ovarian (8), breast, gastric, pancreatic (9) and certain head and neck squamous cell cancers (10). COX-2 is commonly found in premalignant lesions, carcinoma in situ, invasive cancer, and metastatic disease. It is required thro-ughout the entire evolutionary process of cancer development and progression. Various molecular mechanisms may be responsible for the initiation and promotion of carcinogenesis by COX-2 (11). COX-2 expression in tumors is associated with aggressive tumor growth, increased propensity of tumors to metastasize, resistance

to standard radiotherapy and chemotherapy, and poor prognosis (12).

A large number of epidemiological and experimental studies have revealed that prolonged treatment with non-steroidal anti-inflammatory drugs (NSAIDs), which are COX inhibitors, can reduce the risk of cancer (13). NSAIDs inhibit cell proliferation and induce apoptosis of a number of cancer cells in vitro and in vivo, which is considered to be an important mechanism for the anti-tumor and chemopreventive activity of NSAIDs (14). However, the molecular pathways of this process are still unclear. Selective COX-1/2 and nonselective COX inhibitors modulate the cell cycle machinery at several sites, which may explain some of their antiproliferative / apoptotic effects (15). The proapoptotic effects and the chemopreventive potential of NSAIDs cannot be accounted only by COX inhibition alone. NSAIDs have been shown to inhibit proliferation and induce apoptosis in malignant cell lines which do not express either COX-1 or COX-2 (16). Overexpression of COX enzymes can be associated with changes in expression of members of Bcl-2 family which may influence the apoptosis (17).

The aim of this study was to examine the effects of ketoprofen (KT), as nonselective COX-1/2, and meloxicam (MK), as selective COX-2 inhibitor, alone and combined with 5-fluorouracil (FU) and cisplatin (CP), on the proliferation and Bcl-2/Bax expression in human cervix cancer cell line (HeLa).

Material and methods

Reagens

In this experiment we used commercial preparations of ketoprofen, meloxicam, cisplatin, and 5-fluorouracil, which are used in conventional clinical protocols for the treatment of colon and cervix carcinoma (18, 19). Ketoprofen was purchased from Sandoz Pharmaceuticals, Switzerland (Ketonal®, 100 mg/2 mL), meloxicam from Boehringer Ingelheim, Espana S.A. (Movalis®, 15 mg/1.5 mL), 5-fluorouracil from Pharmachemie BV - Netherlands (Fluorouracil-TEVA®, 50 mg/mL) and cisplatin from Ebewe Pharma Austria (Cisplatin Ebewe®, 10 mg/20 mL). DMEM (Dulbecco's Modified Eagle Medium), FBS (Fetal Bovine Serum), antibiotic/antimycotic solution, L-glutamine and Trypsin-EDTA solution were purchased from PAA Laboratories (PAA Laboratories, Austria) and 3-(4,5-dimethylthiazol-2-yl)-2,5 – diphenyltetrazolium bromide (MTT) was purchased from Carl Roth (Carl Roth, Germany). Trypan blue stain was purchased from Invitrogen. Primary anti-Bcl-2 and anti-Bax antibodies and secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Cell line

In this study we used HeLa S3 human cervical carcinoma cell line obtained from the Leibniz Institute DSMZ. Cells were cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine and antibiotic/antimycotic solution at 37 ºC in an atmosphere with 5% CO_2 and saturated humidity. Replacement of the culture medium was performed every 2 to 3 days.

Treatment of cells

Confluent culture of HeLa cells was harvested using Trypsin-EDTA solution, washed in buffer solution and the total number of cells was determined by Trypan blue dye exclusion test. Cells were seeded in 96-well plates (Greiner Bio-One, Germany) at density 3 x 104 cells per well and cultured for 24 h under standard cell culture conditions. After that, the examined compounds, alone or in combination, were added to the cells. KT, MC, CP, and FU were diluted in DMEM and three concentrations of each of these compounds were tested (group 1 – the lowest concentration, group 2 – middle concentration and group 3 – the highest concentration). Final concentrations of the tested compounds were the following: 2, 20, and 200 μM of KT, 10 μM, 50 μM, and 500 μM of MC, 1.66, 3.32, and 6.64 μM of CP and 10, 100, and 1000 μM of FU. We have also combined KT and MC with CP and FU, respectively. Combining was performed using compounds' concentrations from the same group as following: group 1 with group 1, group 2 with group 2 and group 3 with group 3 in the ratio 1:1 so the effective concentrations of compounds in combinations were twice less than the concentrations of compounds that were applied alone. As control, we used cells that were incubated only with completed cell culture medium, DMEM, without the assayed compounds. Cells were incubated with examined compounds for the next 48 h. After that, cell growth was examined by performing MTT test and the level of Bcl-2 and Bax proteins was also measured.

MTT test

Cell growth was examined by performing MTT test according to the protocol by Damnjanovic et al. (20). Results are presented as: the mean value of absorbance ± standard deviation from four to eight replications for the assayed compounds as well as control.

Measurement of Bcl-2 and Bax protein levels

For determining the levels of Bcl-2 and Bax proteins, the cells were treated as it was described in the section "Treatment of cells". After 48 h of incubation with the assayed compounds, cells were further processed according to the protocol by Kocic et al. (21) Briefly, the cells were washed with phosphate-buffered saline (PBS), fixed by using 70% methanol and permeabilized with 0.1% Triton in PBS. The cells were incubated with the primary anti-Bax and anti-Bcl-2 antibodies, washed three times and incubated with the FITC-conjugated secondary antibodies.

The mean fluorescence intensity (MFI; logarithmic scale) was determined and analyzed on a Victor™ multiplate reader (Perkin Elmer-Wallace, Wellesley, MA). The presented results were obtained following the subtraction of blank values obtained by the treatment with the secondary antibodies only.

Statistical analysis

The data were analyzed by the commercially available statistics software package (SPSS for Windows®, v. 17.0, Chicago, USA) using the Student's t-test and the ANOVA test. The results were presented as mean ± SD. The statistical significance was set to $p < 0.05$.

Investigations and results

The effect of different concentrations of KT and MC alone as well as combined with CP and FU on the growth of HeLa cells

As shown in Figure 1, HeLa cells treated with three different concentrations of CP and FU showed a statistically significant decrease in cell growth compared to the control group. MC reduced the growth of HeLa cells in a dose-dependent manner after 48 h. The combination of KT and CP showed a statistically significant dose-dependent decrease in cell growth in comparison with the control group, until the combination of KT and FU showed a statistically significant effect on cell growth only when middle examined concentration was used, as compared to the control group. The combination of MC and CP showed a statistical significance in the effect on cell growth in dose dependent manner, as compared to the control group. All tested concentrations of MC-FU combination showed a statistical significance in the effect on HeLa cells in comparison with the control group (Figure 1).

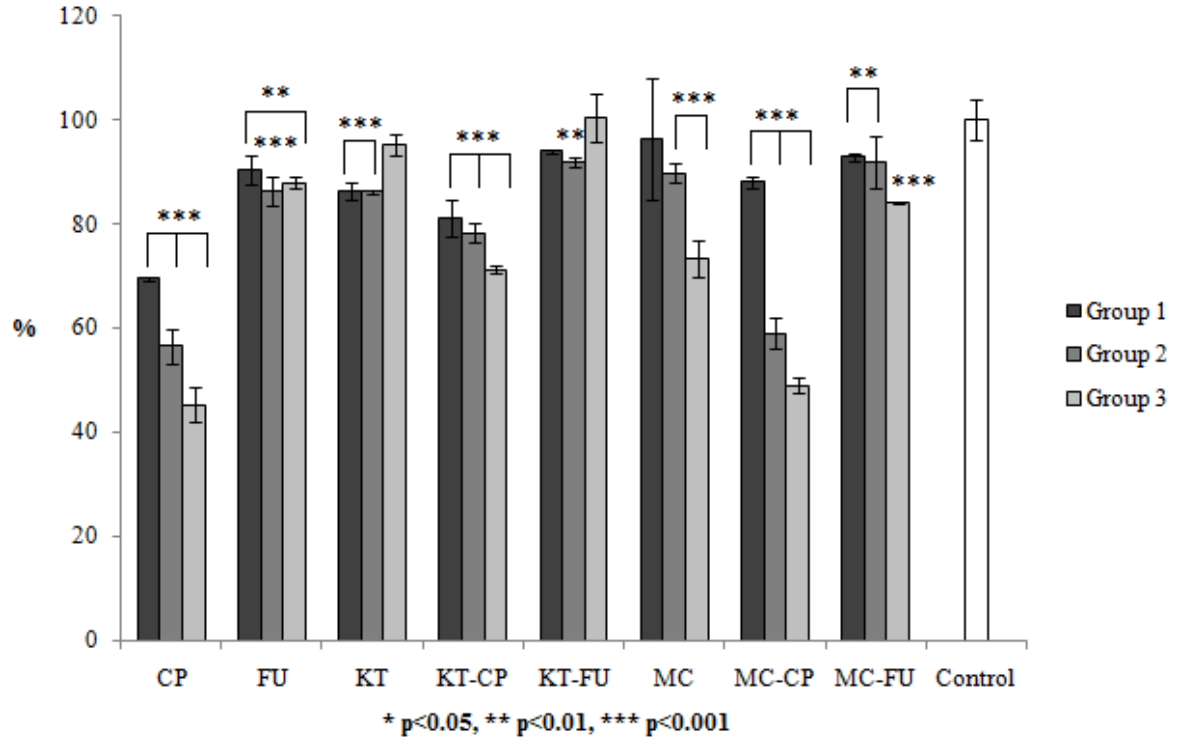


Figure 1: KT, MC alone as well as combined with CP and FU inhibit HeLa cells proliferation.
Final concentrations of the tested compounds were the following:
2, 20, 200 μM of KT; 10 μM, 50 μM, 500 μM of MC;
1.66, 3.32, 6.64 μM of CP; 10, 100, 1000 μM of FU.

The effect of different concentrations of KT and MC alone as well as combined with CP and FU on Bcl-2 expression in HeLa cells

To investigate the mechanism by which apoptosis was induced by combinations of KT or MC with cytostatic drugs (CP and FU), we evaluated the expression levels of Bcl-2 and Bax as apoptotic markers. The treatment of HeLa cells with the middle and the highest concentrations of KT-CP combination significantly decreased the expression of Bcl-2 (Figure 2a). The expression of Bcl-2 in HeLa

cells was significantly decreased after the treatment with the highest concentration of MC. The combination of MC and CP showed a statistically significant decrease in Bcl-2 expression level (Figure 2b).

The effect of different concentrations of KT and MC alone as well as combined with CP and FU on Bax expression in HeLa cells

HeLa cells were exposed to different con centrations of KT and MC alone, as well as combined with CP and FU, in order to investigate the effects on Bax expression. The expression of Bax was significantly increased after the treatment of HeLa cells only with the highest concentration of KT (Figure 3a). The treatment of HeLa cells with the MC showed a statistical significance in increasing the expression level of Bax in all analyzed groups. The MC-CP and MC-FU combinations, compared to the control group, showed a statistical significance in increasing the expression level of Bax in all analyzed groups (Figure 3b).

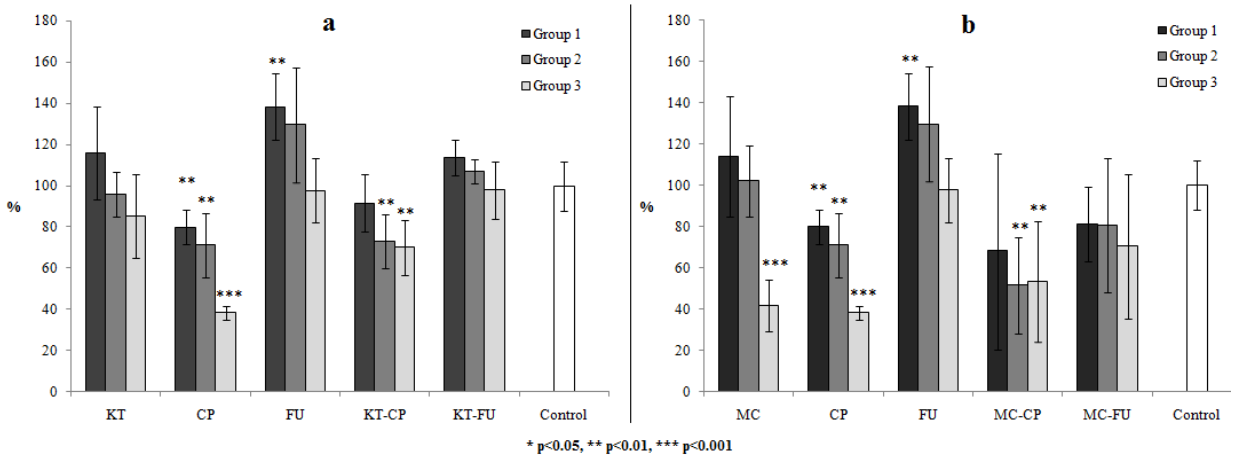


Figure 2: The effect of different concentrations of KT and MC alone as well as combined with CP and FU on Bcl-2 expression in HeLa cells.
Final concentrations of the tested compounds were the following:
2, 20, 200 μM of KT; 10 μM, 50 μM, 500 μM of MC;
1.66, 3.32, 6.64 μM of CP; 10, 100, 1000 μM of FU.

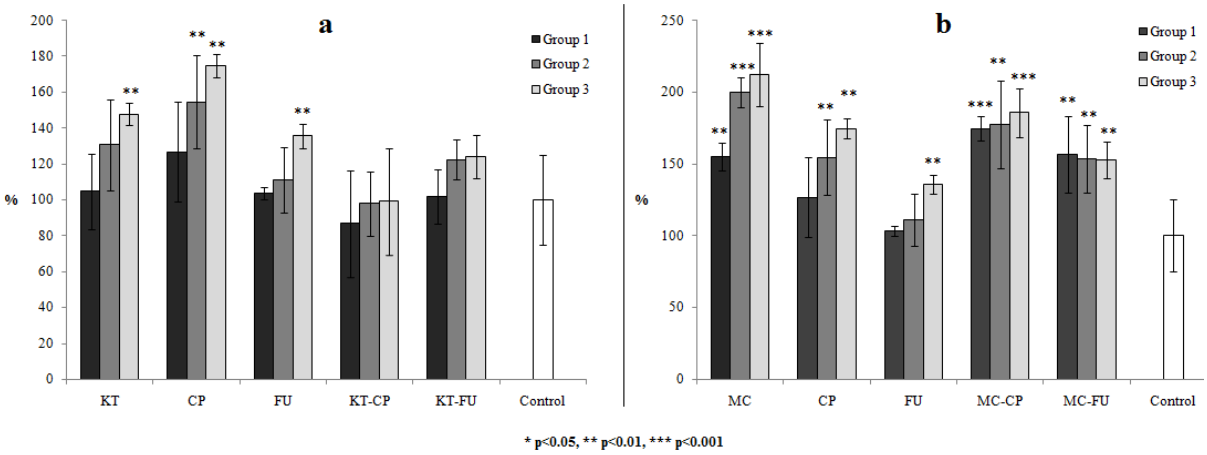


Figure 3: The effect of different concentrations of KT and MC alone as well as combined with CP and FU on Bax expression in HeLa cells.
Final concentrations of the tested compounds were the following:
2, 20, 200 μM of KT; 10 μM, 50 μM, 500 μM of MC;
1.66, 3.32, 6.64 μM of CP; 10, 100, 1000 μM of FU.

Discussion

The idea that NSAIDs could have a variety of molecular targets, not only provides a much-needed explanation of apparently disparate observations, but also underscores the opportunity which these tar-gets represent for cancer chemoprevention and for improving therapeutic efficacy in cancer therapy (15).

Our results show that KT and MC, in examined concentrations, exert some cytotoxic and antiproliferative effects on HeLa cells. The treatment of cells with examined NSAIDs alone or in combination with conventional anticancer drugs, FU and CP, showed better cytotoxic and antiproliferative effect in groups treated with MC than in groups treated with KT, especially when the combination of these concentrations compounds at the highest tested were applied (Figure 1). So the obtained results could potentially indicate the synergistic cytotoxic effect of MC and conventional cytostatics (Figure 1).

Currently, there is an evidence that nonselective NSAIDs and selective COX-2 inhibitors inhibit proliferation of different cancer cell types such as a highly invasive mouse CRC cell model-MC-26 (22), osteosarcoma MG-63 cells (23), prostate cancer models (24), HT-29 human colon cancer and HCT 15 cells (25). Our results are consistent with the data of other authors, who reported that various nonselective NSAIDs and highly selective COX-2 inhibitors decreased cancer cell proliferation (26, 27).

A body of evidence indicates a role of inflammation in the development and modulation of different steps of cancer progression (28). Amongst different mediators of inflammation, the cyclooxygenases clearly appear to be implicated in cancer development (29). COX-1, which is an isoform constitutively expressed in many tissues, is believed to function as a housekeeping enzyme (30). Conversely, COX-2 is a pro-inflammatory factor that shows rapid up-regulation in response to stimuli such as mitogens, cytokines, growth factors, and tumor promoters. Inhibition of COX is the best known biochemical effect of NSAIDs provided a plausible explanation of the epidemiological data on NSAIDs and cancer (15). NSAIDs and COX-2-specific inhibitors exert their effect in carcinogenesis by inhibiting COXs, especially COX-2, the isoform overexpressed in cancer (31). Our results suggest that KT and MC have distinct actions on cellular apoptosis and the growth of cervical cancer cells. Obtained results can be explained by different COX isoenzyme expression in vario-us cancer cells (29), the physicochemical properties of KT and MC (32), as well as their affinity for inhibiting COX isoenzyme (16).

It is important to say that the COX-independent effects of NSAIDs come from several lines of evidence. NSAIDs have antiproliferative and/or proapoptotic effects in cell lines that do not express either COX-1 or COX-2 (33). The cancer chemopreventive properties of NSAIDs are much more complex and likely involve multiple COX-2-independent effects where mitochondria and mitochondrial markers of apoptosis are key players (34).

In the mitochondrial pathway, apoptosis could be regulated in part by changes in the expression levels of various pro - and anti-apoptotic members of the Bcl-2 family. Among them, Bcl-2, Bcl-xL and Bcl-w effectively inhibit cellular apoptosis, while Bax, Bcl-xs and Bak promote it (35). Apoptosis deregulation in cancer cells appears to primarily affect the signaling pathways upstream of Bax/Bak and mitochondria, leaving the downstream core apoptotic machinery mostly intact (36). This presents a great opportunity for restoring apoptosis in cancer cells by manipulating the balance between the pro - and anti-apoptotic Bcl-2 family members (36).

Results of our research showed that the levels of anti-apoptotic marker Bcl-2 were decreased while the levels of pro-apoptotic marker Bax were increased dose-dependently by KT and MC in HeLa cells (Figure 2). The synergistic effect of the KT and CP in the culture of HeLa cells was observed. Decrease of Bcl-2 protein was more pronounced in HeLa cells treated with MC alone or combined with conventional cytotoxic drugs (CP and FU). Similar results were obtained in the study conducted by Gao et al (37).

In our study Bax levels were upregulated in the treatment with KT and MC, alone or in combination with CP and FU. KT, alone or combined with cytostatics, lead to a significant increase in the expression of pro-apoptotic Bax protein in HeLa cells, but the effect of MC was more pronounced (Figure 3). It should be noted that MC and FU showed the synergistic effect on Bax expression in HeLa cells.

The downregulation of Bcl-2 and upregulation of Bax induced by KT, MC and combinations with CP and FU in HeLa cells were also dose-dependent. Up to date there have been only a few studies to compare our results, especially when we want to analyze the effects of MC and KT on the proliferation and expression of Bcl-2 and Bax protein in HeLa cell culture. Zhou et al. showed that overexpression of Bax is closely involved in apoptosis induced by aspirin and indomethacin, without altering Bcl-2 and Bcl-xL expression (38). A study obtained by Liu et al. showed that celecoxib also triggered apoptosis in osteosarcoma MG-63 cells through downregulation of Bcl-2 (23), whereas Naruse et al. suggested that meloxicam at the concentration of 100 mM upregulated Bax in MG-63 cells, but had no significant effect on Bcl-2 expression (39). Similarly, Dong et al. found that treatment of HepG2 cells with meloxicam upregulated the expression of Bax, in a time-dependent manner, but had no effect on the expression of Bcl-xL and Bcl-2 (40). In the view of aforementioned facts, our results suggest that both KT and MC have distinct actions on cellular apoptosis and the growth of cervical cancer cells through a combination of COX-dependent and COX-independent pathways.

In conclusion, we propose that the synergy observed in the effects of ketoprofen and meloxicam with cisplatin and 5-fluorouracil on the growth of cervical cancer HeLa cell line was generated by an enhancement of apoptosis. The results of this study further emphasize the complexity of the role of the dysregulated expression of Bcl-2 family members in successful cancer therapy. According to our results,

ketoprofen and meloxicam may be classified as therapeutic candidates to improve access of cervical cancer chemoprevention and chemotherapy.

Acknowledgement

The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grants TR 31060, III 41017 and OI 172044).

References

1. Guillem-Llobat P, Dovizio M, Alberti S, Bruno A, Patrignani P. Platelets, cyclooxygenases, and colon cancer. Semin Oncol 2014; 41:385–96. [CrossRef] [PubMed]
2. Allaj V, Guo C, Nie D. Non-steroid anti-inflammatory drugs, prostaglandins, and cancer. Cell Biosci 2013; 3:8. [CrossRef] [PubMed]
3. Fürstenberger G, Krieg P, Müller-Decker K, Habenicht AJR. What are cyclooxygenases and lipoxygenases doing in the driver's seat of carcinogenesis? Int J Cancer 2006; 119:2247-54. [CrossRef] [PubMed]
4. Roelofs HM, Te Morsche RH, van Heumen BW, Nagengast FM, Peters WH. Over-expression of COX-2 mRNA in colorectal cancer. BMC Gastroenterol 2014; 14:1. [CrossRef] [PubMed]
5. Sung S, Park Y, Jo JR, Jung NK, Song DK, Bae J, et al. Overexpression of cyclooxygenase-2 in NCI-H292 human alveolar epithelial carcinoma cells: Roles of p38 MAPK, ERK-1/2, and PI3K/PKB signaling proteins. J Cell Biochem 2011; 112:3015-24. [CrossRef] [PubMed]
6. Kim BH, Kim CI, Chang HS, Choe MS, Jung HR, Kim DY, et al. Cyclooxygenase-2 overexpression in chronic inflammation associated with benign prostatic hyperplasia: is it related to apoptosis and angiogenesis of prostate cancer? Korean J Urol. 2011; 52: 253-9. [CrossRef] [PubMed]
7. Kulkarni S, Rader JS, Zhang F, Liapis H, Koki AT, Masferrer JL, et al. Cyclooxygenase-2 is overexpressed in human cervical cancer. Clin Cancer Res 2001; 7:429-34. [PubMed]
8. Wang M, He Y, Shi L, Shi C. Multivariate analysis by Cox proportional hazard model on prognosis of patient with epithelial ovarian cancer. Eur J Gynaecol Oncol 2011; 32:171-7. [PubMed]
9. Nie D. Cyclooxygenases and lipoxygenases in prostate and breast cancers. Front Biosci 2007; 12:1574–85. [CrossRef] [PubMed]
10. Park SW, Kim HS, Choi MS, Kim JE, Jeong WJ, Heo DS, et al. The influence of cyclooxygenase-1 expression on the efficacy of cyclooxygenase-2 inhibition in head and neck squamous cell carcinoma cell lines. Anticancer Drugs 2011; 22:416-23. [CrossRef] [PubMed]
11. Harris RE. Cyclooxygenase-2 (COX-2) and the inflammogenesis of cancer. In: Harris RE editor. Inflammation in the pathogenesis of chronic diseases. Netherlands: Springer; 2007. p. 93-126. [CrossRef] [PubMed]
12. Liao Z, Raju U, Mason KA, Milas L. Cyclo-oxygenase-2 enzyme and its inhibition in tumor growth and therapy. In: LaRochelle WJ, Shimkets RA, editors. The oncogenomics handbook. New York: Humana Press; 2005. p. 571-96. [CrossRef]
13. Liu JF. Non-steroidal anti-inflammatory drugs and cancer, with an especial focus on esophageal cancer. Asian Pac J Cancer Prev 2011; 12:3159-68. [PubMed]
14. Damnjanovic I, Najman S, Stojanovic S, Stojanovic D, Veljkovic A, Kocic H, et al. Crosstalk between possible cytostatic and antiinflammatory potential of ketoprofen in the treatment of culture of colon and cervix cancer cell lines. Bratisl Lek Listy 2015; 116:227-32. [CrossRef] [PubMed]
15. Kashfi K, Rigas B. Non-COX-2 targets and cancer: expanding the molecular target repertoire of chemoprevention. Biochem Pharmacol 2005; 70:969-86. [CrossRef] [PubMed]
16. Zhang X, Morham S, Langenbach R, Younga D. Malignant transformation and antineoplastic actions of nonsteroidal antiinflammatory drugs (NSAIDs) on cyclooxygenase-null embryo fibroblasts. J Exp Med 1999; 190:451-9. [CrossRef] [PubMed]
17. Vaish V, Sanyal SN. Chemopreventive effects of NSAIDs on cytokines and transcription factors during the early stages of colorectal cancer. Pharmacol Rep 2011; 63(5):1210-21. [CrossRef] [PubMed]

18. Haie-Meder C, Morice P, Castiglione M. Cervical cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010; 21:37-40. [CrossRef] [PubMed]
19. Labianca R, Nordlinger B, Beretta GD, Brouquet A, Cervantes A. Primary colon cancer: ESMO Clinical Practice Guidelines for diagnosis, adjuvant treatment and follow-up. Ann Oncol 2010; 21:70-7. [CrossRef] [PubMed]
20. Damnjanovic I, Kocic G, Najman S, Stojanovic S, Stojanovic D, Veljkovic A, et al. Chemopreventive potential of alpha-lipoic acid in the treatment of colon and cervix cancer cell lines. Bratisl Lek Listy 2014; 115:611-6. [CrossRef] [PubMed]
21. Kocic G, Pavlovic R, Najman S, Nikolic G, Sokolovic D, Jevtovic-Stoimenov T, et al. Circulating ribonucleic acids and metabolic stress parameters may reflect progression of autoimmune or inflammatory conditions in juvenile type 1 diabetes. Scientific World Journal 2011; 11:1496-508. [CrossRef] [PubMed]
22. Yao M, Lam EC, Kelly CR, Zhou W, Wolfe MM. Cyclooxygenase-2 selective inhibition with NS-398 suppresses proliferation and invasiveness and delays liver metastasis in colorectal cancer. Br J Cancer 2004; 90: 712-9. [CrossRef] [PubMed]
23. Liu B, Qu L, Yang Z, Tao H. Celecoxib, a cyclooxygenase-2 inhibitor, induces apoptosis in human osteosarcoma cell line MG-63 via down-regulation of PI3K /Akt. Cell Biol Int 2008; 32:494-501. [CrossRef] [PubMed]
24. Hsu AL, Ching TT, Wang DS, Song X, Rangnekar VM, Chen CS. The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking Akt activation in human prostate cancer cells independently of Bcl-2. J Biol Chem 2000; 275:1397-403. [CrossRef] [PubMed]
25. Soh JW, Weinstein IB. Role of COX-independent targets of NSAIDs and related compounds in cancer prevention and treatment. Prog Exp Tumor Res 2003; 37:261-85. [CrossRef] [PubMed]
26. Elder DJ, Halton DE, Crew TE, Paraskeva C. Apoptosis induction and cyclooxygenase-2 regulation in human colorectal adenoma and carcinoma cell lines by the cyclooxygenase-2-selective non-steroidal anti-inflammatory drug NS398. Int J Cancer 2000; 86:553-60. [CrossRef] [PubMed]
27. Paik JH, Ju JH, Lee JY, Boudreau MD, Hwang DH. Two opposing effects of non-steroidal anti-inflammatory drugs on the expression of the inducible cyclooxygenase. J Biol Chem 2000; 275:28173-9. [PubMed]
28. Hussain SP, Harris CC. Inflammation and cancer: an ancient link with novel potentials. Int J Cancer 2007; 121:2373-80. [CrossRef] [PubMed]
29. Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. Int J Cell Biol 2010; 2010: 215158. [CrossRef] [PubMed]
30. Setia S, Vaish V, Sanyal SN. Chemopreventive effects of NSAIDs as inhibitors of cyclooxygenase-2 and inducers of apoptosis in experimental lung carcinogenesis. Mol Cell Biochem 2012; 366:89-99. [CrossRef] [PubMed]
31. Ruegg C, Zaric J, Stupp R. Non steroidal anti-inflammatory drugs and COX-2 inhibitors as anti-cancer therapeutics: hypes, hopes and reality. Ann Med 2003; 35:476–87. [CrossRef] [PubMed]
32. Marjanović M, Zorc B, Pejnović L, Zovko M, Kralj M. Fenoprofen and ketoprofen amides as potential antitumor agents. Chem Biol Drug Des 2007; 69:222-6. [CrossRef] [PubMed]
33. Matos P, Jordan P. Beyond COX-inhibition: 'side-effects' of ibuprofen on neoplastic development and progression. Curr Pharm Des 2015; 21:2978-82. [CrossRef] [PubMed]
34. Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, et al. Structural basis for selective inhibition of cyciooxygenase-2 by anti-inflammatory agents. Nature 1996; 384:644-8. [CrossRef] [PubMed]
35. Delbridge AR, Strasser A. The Bcl-2 protein family, BH3-mimetics and cancer therapy. Cell Death Differ 2015; 22:1071-80. [CrossRef] [PubMed]
36. Danial NN, Korsmeyer SJ. Cell death: Critical control points. Cell 2004; 116:205-19. [CrossRef] [PubMed]
37. Gao J, Niwa K, Sun W, Takemura M, Lian Z, Onogi K, et al. Non-steroidal anti-inflammatory drugs inhibit cellular proliferation and upregulate cyclooxygenase-2 protein expression in endometrial cancer cells. Cancer Sci 2004; 95:901-7. [CrossRef] [PubMed]
38. Zhou XM, Wong BC, Fan XM, Zhang HB, Lin MC, Kung HF, et al. Non-steroidal anti-inflammatory drugs induce apoptosis in gastric cancer cells through up-regulation of bax and bak. Carcinogenesis 2001; 22: 1393-7. [CrossRef] [PubMed]
39. Naruse T, Nishida Y, Ishiguro N. Synergistic effects of meloxicam and conventional cytotoxic drugs in human MG-63 osteosarcoma cells. Biomed Pharmacother 2007; 61:338-46. [CrossRef] [PubMed]
40. Dong X, Li R, Xiu P, Dong X, Xu Z, Zhai B, et al. Meloxicam executes its antitumor effects against hepatocellular carcinoma in COX-2 - dependent and -independent pathways. PLoS One 2014; 9:40e92864.

Originalni rad **UDC: 618.146-006.6:615.276/277**
doi:10.5633/amm.2018.0407

MOLEKULARNI MEHANIZMI POTENCIJALNO SINERGISTIČKOG EFEKTA KETOPROFENA I MELOKSIKAMA SA KONVENCIONALNIM CITOSTATICIMA U ĆELIJSKOJ LINIJI HUMANOG KARCINOMA GRLIĆA MATERICE

Ivana Damnjanović[1], Gordana Kocić[2], Stevo Najman[3], Sanja Stojanović[3], Katarina Tomović[1], Budimir Ilić[4], Andrej Veljković[2], Andrija Šmelcerović[1,4]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za farmaciju, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Katedra za biohemiju, Niš, Srbija
[3]Univerzitet u Nišu, Medicinski fakultet, Odeljenje za ćelijsko i tkivno inžinjerstvo, Institut za biologiju i humanu genetiku, Niš, Srbija
[4]Univerzitet u Nišu, Medicinski fakultet, Katedra za hemiju, Niš, Srbija

Kontakt: Ivana Damnjanović
Univerzitet u Nišu, Medicinski fakultet, Katedra za farmaciju
Bulevar dr Zorana Đinđića 81 18000 Niš, Srbija
E-mail: ivanad.ph@gmail.com

Ciklooksigenaze su jasno uključene u proces karcinogeneze. Zabeleženo je da je COX-2 eksprimirana tokom celog evolutivnog procesa razvoja i napredovanja karcinoma. Različiti molekularni mehanizmi mogu biti odgovorni za te procese. Epidemiološke i eksperimentalne studije su pokazale da nesteroidni antiinflamatorni lekovi (NSAIL) i selektivni COX-2 inhibitori mogu smanjiti rizik za nastanak karcinoma. Inhibicija ciklooksigenaza jedno je od prihvatljivih objašnjenja uloge NSAIL i prevencije karcinoma. Međutim, molekularni putevi ovih efekata još uvek nisu u potpunosti poznati, dok njihova kompleksnost verovatno uključuje COX zavisne i nezavisne mehanizme, proapoptotične i antiapoptotične članove Bcl-2 familije.

U sprovedenom istraživanju ispitivan je efekat ketoprofena (KT), kao neselektivnog COX-1/2 i meloksikama (MC) kao selektivnog COX-2 inhibitora. Efekat KT i MC je ispitivan samostalno ili u kombinaciji sa 5-fluorouarcilom (FU) i cisplatinom (CP) na proliferaciju (MTT test) i ekspresiju Bcl-2/Bax u ćelijskoj liniji humanog karcinoma grlića materice (HeLa ćelije).

MC, sam ili u kombinaciji sa konvencijalnim citostaticima FU i CP, pokazuje bolji citotoksični i antiproliferativni efekat u odnosu na KT. MC i KT dovode do dozno-zavisnog smanjenja ekspresije Bcl-2 i povećenja ekspresije Bax proteina. Značajnije povećanje ekspre-sije Bax proteina zabeleženo je u ispitivanim grupama HeLa ćelija tretiranim sa MC.

Primećena sinergija u efektima ketoprofena i meloxicama sa cisplatinom i 5-fluoro-uracilom u liniji humanog karcinoma grlića materice može biti posledica indukcije apoptoze u ćelijama. Stoga, ketoprofen i meloxicam mogu predstavljati nove terapeutske kandidate u cilju poboljšanja hemoprevencije i terapije karcinoma grlića materice.

Acta Medica Medianae 2018;57(4):52-59.

Ključne reči: *molekularni mehanizmi, sinergistički efekat, ketoprofen, meloksikam, HeLa ćelije*

Original article

UDC: 616.333-008.8:579.84
doi:10.5633/amm.2018.0408

EFFECT OF HELICOBACTER PYLORI INFECTION ON THE OCCURRENCE OF ESOPHAGEAL REFLUX DISEASE

Vesna Brzački[1,2], Danijela Benedeto-Stojanov[1,2]

The role of *Helicobacter pylori* (*H. pylori*) in the emergence of gastroesophageal reflux disease (GERD) is quite unclear. It is suggested that *H. pylori* has the protective role in esophageal disease development. The aims of the present study were to analyze the frequency of *H. pylori* infection in patients with different forms of GERD and comparison of incidence between the different groups, relation between the degree of esophagitis and *H. pylori* infection in patients with ERD, histological changes in the esophagus and cardia after eradication therapy in patients of both groups who were positive for *H. pylori*, and to compare ERD and NERD after conducting eradication therapy for *H. pylori* infection. Prospective study conducted in the Clinic of Gastroenterology, Clinical Center in Niš, included 90 patients with symptoms of GERD. Patients were divided into two groups according to whether they have endoscopic signs of gastroesophageal reflux (ERD group), or absence of signs of reflux (NERD group). Two biopsies of the antrum and corpus, and 4 esophageal biopsies within 2 cm of the Z line were performed in all patients during the proximal endoscopy. In the gastric mucosa biopsy, we investigated the presence of *H. pylori* infection, as well as histomorphological changes by hematoxylin eosin and modified Giemsa method. The degree of esophagitis was determined by the Los Angeles classification. In *H. pylori* positive patients, eradication therapy was administered for a period of ten days. After eradication of *H.pylori* and treatment of reflux disease for 8 weeks with proton pump inhibitors, esophagus biopsy was repeated. In the antrum, *H. pylori* was positive in 22 (44.89%) subjects of the ERD group compared to 30 (66.66%) of the NERD group. In the corpus, *H. Pylori* was positive in 18 (40.00%) subjects of the ERD group compared to 24 (53.33%) subjects of the NERD group, and no statistical significance was found. In the ERD group, there was no difference in the presence or absence of *H. pylori* infection and severity of esophagitis. After eradication, in both groups of patients, there was a statistically significant improvement in histological findings (χ^2= 22.26; p = 0.00001). After the treatment in the ERD group, there was statistically significant decrease in endoscopic findings of esophagitis. After the treatment in the group of subjects with NERD, three patients had endoscopic findings of esophagitis. In patients with GERD, a long-term anti secretory therapy should be implemented and *H. pylori* needs to be tested and eradicated. *H. pylori* positive status is rarely seen together with GERD, and if it is, it is of lower degree.

Acta Medica Medianae 2018;57(4):60-66.

Key words: *GERB ERB, NERB, H. pylori*

[1]University of Niš, Medical Faculty, Niš, Serbia
[2]Clinic of gastroenterology and hepatology, Clinical Center Niš, Serbia

Contact: Vesna Brzački
Bul.dr Zoran Djindjić 37/52, 18000 Niš, Serbia
E-mail: brzackiv@gmail.com

Introduction

Helicobacter pylori (*H. pylori*) is one of the leading pathogenic factors in the development of gastroduodenal ulceration, dyspepsia and gastric adenocarcinoma. Its role in the emergence of gastroesophageal reflux disease (GERD) is quite unclear and has been the subject of numerous studies. In developed countries, decreasing prevalence of *H. pylori* infection reduced the peptic ulcer disease and adenocarcinoma of the distal stomach. At the same time, there has been an increase in GERD, Barrett's esophagus, adenocarcinoma of distal esophagus and proximal stomach, suggesting a protective role of *H. pylori* in the development of esophageal disease.

The term GERD is used to describe symptoms and esophageal mucosal changes, as a result of the reflux of gastric contents into the esophagus. Peptic esophagitis, reflux esophagitis and erosive esophagitis, erosive reflux disease (ERD) are synonyms for GERD with histopathological changes of esophageal mucosa that usually correlate with the symptoms of acid content reflux (1). Damages of the esophageal mucosa occur due to reflux of gastric contents into

the esophagus and the harmful effects of hydrochloric acid and pepsin. The severity of epithelial changes is assessed by the presence of symptoms or endoscopic signs. The majority of patients with GERD present with clinical manifestations of mild to mode-rate degree of illness.

In recent years, in gastroenterological literature, there has been the term of non-erosive reflux disease-NERD (2) for patients with symptomatic GERD who have no macroscopic mucosal changes during the proximal endoscopy. It is estimated that 50-70% of patients with GERD have NERD (2,3).

Adequate assessment of reflux disease solely on the basis of endoscopy is difficult due to the existence of normal endoscopic findings of the esophagus in NERD. Some authors described the histological changes in GERD, so it seems reasonable that the diagnosis of NERD can be set by taking a biopsy during endoscopy. In the last three decades, there have been histological criteria for reflux disease: basal cell proliferation, regenerative elongation and increase in the number of epithelial papillae, increase in the number and ectasia of capillaries in the epithelial papillae, glycogenic acanthosis in basal epithelium, the presence of the so-called "Balloon cells" (acid-induced degenerative changes in basal epithelium), dilatation of the intracellular space in the parabasal areas of basal epithelium, neutrophils, eosinophils and T lymphocytes infiltration.

In recent years, in patients with GERD, Lugol solution has been applied during endoscopy (chromoendoscopy). Lugol solution is a mixture of iodine and potassium iodide which shows an affinity for glycogen in non keratinized squamous epithelium. Normal esophageal epithelium is rich in glycogen and it gradually turns black, dark-brown or green-brown shortly after application. Mucosal changes that have a small amount of glycogen, if any, will not be stained after application of Lugol solution (4).

Aims

The aims of the present study were to:

- Analyze the frequency of *H. pylori* infection in patients with different form of GERD and comparison of incidence between the different groups,
- Analyze the relation between the degree of esophagitis and *Helicobacter pylori* infection in patients with ERD,
- Analyze the histological changes of the esophagus and cardia after the eradication therapy in patients of both groups who were positive for *Helicobacter pylori*,
- Compare ERD and NERD after conducting eradication therapy for *H. pylori* infection.

Material and methods

A prospective study conducted in the Clinic of Gastroenterology, Clinical Center Niš, included 90 patients with symptoms of gastroesophageal reflux disease. Patients were divided into two groups according to whether they have endoscopic signs of gastroesophageal reflux (ERD group), or absence of signs of reflux (NERD group). Of all patients, 52 (57.77%) were men and 38 (42.23%) were women. There were 24 men in the ERD group, and 28 in the NERD group. There were 17 women in the ERD group, and 21 in the NERD group. The average age of patients in the ERD group was 62.5 years, and in the NERD group 58.1 years. The majority of patients were in the sixth and seventh decade of life.

In all patients during the proximal endoscopy (esophagogastroduodenoscopy), we macroscopically evaluated esophageal, stomach and duodenum mucosa to the border of the descending and horizontal part of the duodenum. Macroscopic mucosal changes of the distal esophagus were measured on the basis of the distance from the Z line, and mucosal damage was classified according to the Los Angeles classification of reflux esophagitis.

In all patients during the proximal endoscopy two biopsies of the antrum and corpus were taken and four esophageal biopsies within 2 cm of the Z line. In the gastric mucosa biopsy, we investigated the presence of *H. pylori* infection, as well as histomorphological changes by hematoxylin eosin and modified Giemsa method. In *H. pylori* positive patients, eradication therapy was administered for a period of ten days. After eradication of *H.pylori* and treatment of reflux disease for 8 weeks with proton pump inhibitors, esophagus biopsy was repeated.

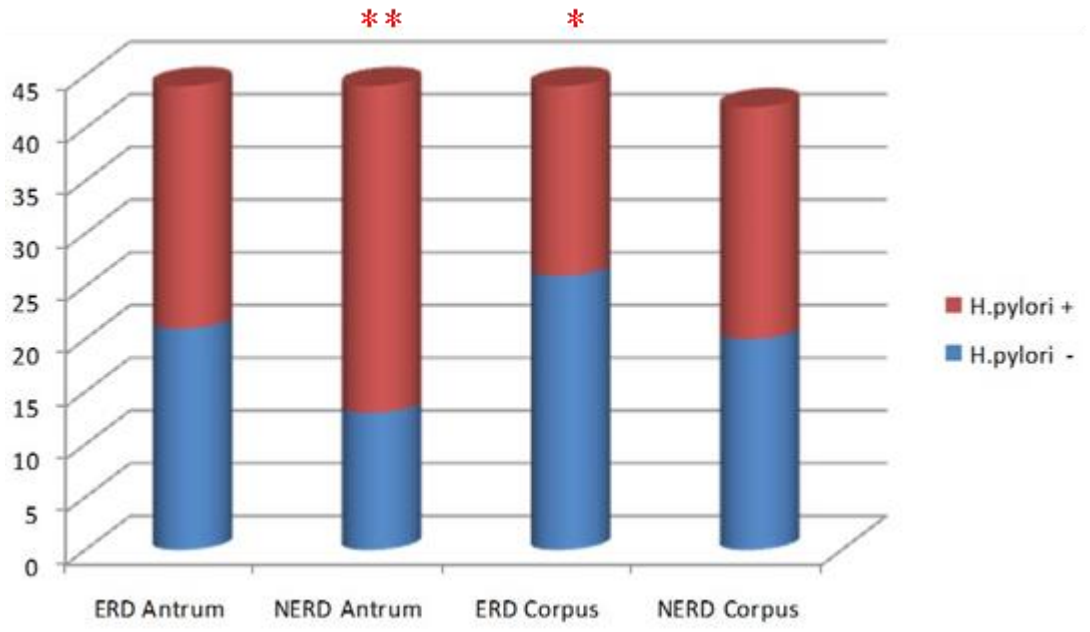
Results

1. The incidence of *H. pylori* infection in patients the ERD and NERD group of the ERD and NERD group.

In the antrum, *H. pylori* was positive in 22 (44.89%) patients of the ERD group, in relation to the 30 (66.66%) subjects of the NERD group. *H. pylori* was negative in 23 (51.11%) patients of the ERD group, in relation to 15 (33.33%) patients of the NERD group. In the corpus, *H. pylori* was positive in 18 (40.00%) patients of the ERD group in relation to 24 (53.33%) patients of the NERD group. *H. pylori was* negative in 27 (60.00%) subjects of the ERD group compared to 21 (46.66%) negative subjects of the NERD group. There was no statistically significant difference in the incidence of *H. pylori* infection between the two groups (Graph 1).

2. The degree of esophagitis and *H. pylori* infection in patients of the ERD group.

In respect of *H. pylori* infection, there was no difference in the presence or absence of *H.pylori* infection and the level of esophagitis (Table 1).



*- p = 0,049 H.p. (+) vs. H.p. (-) **- p = 0,001 vs. H.p. (+) vs. H.p. (-)

Graph 1. *H. pylori* infection of the corpus and antrum in the ERD and NERD groups

Table 1. The degree of esophagitis and *H. pylori* infection in patients of the ERD group.

Group		N	The degree of esophagitis			
			A	B	C	D
ERD (n=45)			12 (26.66%)	29 (64.44%)	4 (8.88%)	0
Corpus	*H. pylori (+)*	18	2	10	5	1
	H. pylori (-)	27	7	11	4	5
Antrum	*H. pylori (+)*	22	3	12	5	2
	H. pylori (-)	23	6	9	4	4

3. Histological changes of the esophagus after eradication therapy in patients of both groups who were *H. pylori* positive

After eradication therapy, in the group of patients with ERD and in the group of patients with NERD, there was a statistically significant decrease of histological findings of multiplied papilla, p = 0.00001. After eradication therapy in the ERD group, there was a statistically significant decrease of elongated papilla findings, p = 0.001. There was also a decrease in the NERD group, but with no statistical significance. There was a statistically significant decrease of basal cells hyperplasia in both the ERD group (p = 0.00001) and the NERD group (p = 0.001). There was a statistically significant decrease in histological findings of vascular dilatation in both the ERD group (p = 0.00001) and the NERD group (p = 0.002). After eradication therapy there was a statistically significant decrease in histological findings of balloon cell in both the ERD group (p = 0.00002), and the NERD group (p = 0.002). There was a statistically significant decrease in histological findings of cells maturation absence in the ERD group (p = 0.03). There was a statistically significant decrease in mitotic activity increase in both the ERD group (p = 0.00003), and the NERD group (p = 0.03). There was a statistically significant decrease of polymorphonuclear leukocytes infiltration in both the ERD group (p = 0.0001), and the NERD group (p = 0.00001) (Table 2).

4. The degree of esophagitis after treatment

After treatment, in the group of subjects with ERD, there was a statistically significant decrease in

endoscopic findings of esophagitis (χ^2 = 22.26; p = 0.00001) (Table 3). After treatment, in the group of subjects with a NERD, three patients had esophagitis.

Table 2. Comparison of histological changes of the esophagus between the ERD and NERD groups before and after therapy.

	ERD (n=23)		**NERD (n=31)**	
	+	**-**	**+**	**-**
multiplied papilla – before therapy	23	0	22	9
multiplied papilla – after therapy	9	14	9	22
χ^2/p	**20.13/0.0001**		**10.9/0.0009**	
elongated papilla - before therapy	22	1	17	14
elongated papilla - after therapy	13	10	8	13
χ^2/p	**9.68/0.001**		-	
basal cells hyperplasia - before therapy	22	1	14	17
basal cells hyperplasia - after therapy	6	17	3	28
χ^2/p	**23.37/0.00001**		**9.8/0.001**	
vascular dilatation - before therapy	14	7	8	23
vascular dilatation - after therapy	0	23	0	31
χ^2/p	**22.5/0.00001**		**9.2/0.002**	
balloon cell - before therapy	13	10	8	23
balloon cell - after therapy	0	23	0	31
χ^2/p	**18.12/0.00002**		**9.19/0.002**	
cells maturation absence - before therapy	4	19	0	31
cells maturation absence - after therapy	0	23	0	31
χ^2/p	**4.38/0.03**		-	
decrease in mitotic activity- before therapy	18	5	4	27
decrease in mitotic activity- after therapy	4	19	0	31
χ^2/p	**17.08/0.00003**		**4.28/0.03**	
polymorphonuclear leukocytes infiltration -before therapy	23	0	27	4
polymorphonuclear leukocytes infiltration - after therapy	12	11	5	26
χ^2/p	**14.46/0.0001**		**31.26/0.0001**	

Table 3. The degree of esophagitis after treatment in patients ERD and NERD groups

	ERD (n=23)		**NERD (n=31)**	
	Esophagitis	Valid findings	**Esophagitis**	Valid findings
Before therapy	23 (100%)	0	0	31 (100%)
After therapy	8 (34.78%)	15 (65.22%)	3 (9.67%)	28 (90.33%)
χ^2/p	**22,26/0,00001**			

Discussion

H. pylori causes chronic gastritis, which can progress in peptic ulcer, gastric atrophy and gastric cancer. The role of *H. pylori* infection in reflux esophagitis and the relationship between reflux esophagitis and atrophic gastritis has not been elucidated yet. GERD with or without reflux esophagitis is the leading cause of discomfort in the proximal part of the digestive tract. The main mechanism of reflux esophagitis includes the reduction in basal pressure of the lower esophageal sphincter (LES), and increase in the frequency of spontaneous transient relaxation of the LES. Gastric acid is also necessary for the development of symptoms and mucosal injury. The assumption that impaction *H. pylori* in the LES or a local acidic secretion may affect the appearance and disappearance of reflux esophagitis is understandable. Patients with duodenal ulcer or ulcer similar symptoms have increased acid secretion and they experienced symptoms similar to reflux esophagitis. On the other hand, reducing the production of gastric acid caused by *H. pylori* infection and the consequent atrophic gastritis may be protective factors in the occurrence of reflux disease (5).

In the group of patients with ERD, there is no significant difference in positive and negative findings of *H. pylori* infection comparing the antrum and corpus. Approximately the same number of patients has the infection both in the antrum and in the corpus. In the group of patients with a NERD there is no significant difference in positive and negative findings of *H. pylori* infection in the antrum and corpus. Approximately the same number of patients has the infection both in the antrum and in the corpus.

In relation to the antrum, there is a statistically significant difference in the occurrence of *H. pylori* infection. Twice as many patients were *H. pylori* positive. In the antrum, *H. pylori* was positive in 22 patients with ERD compared to 30 positive patients in the NERD group. *H. pylori* was negative in the 23 patients with ERD compared to only 15 negative patients of the NERD group. In the corpus, *H. pylori* infection was positive in 18 patients of the ERD group, compared to 24 positive patients of the NERD group. *H. pylori* was negative in 27 patients with ERD, compared to 21 patients of the NERD group. There was no statistically significant difference in the incidence of *H. pylori* infection between the two groups. Our results show a higher *number of H. pylori* infection in the antrum and corpus in patients of the NERD group than in of the ERD group. Many authors suggested that *H. pylori* infection is rarely present in patients with reflux esophagitis, and that the atrophic gastritis has lower level as compared to patients without esophagitis. *H. pylori* infection is the main cause of atrophic gastritis and decreased acid secretion. Koike at al. have shown that the infection with *H. pylori* is significantly lower in patients with reflux esophagitis than in patients without it. There is also a positive correlation between the presence of *H. pylori* and severity of esophagitis. In patients with reflux esophagitis, there is increased acid secretion, indicating a possible connection between *H. pylori* and its protective effect on the occurrence of GERD (6).

Many authors suggest that *H. pylori* infection is negatively correlated with the occurrence of reflux esophagitis, at least in some populations. In other words *H. pylori* infection may protect some groups from developing reflux esophagitis. Many studies have shown that *H. pylori* infection is not associated with the severity of reflux esophagitis (7). After the eradication therapy in the NERD group there were three new cases of reflux esophagitis. Many mechanisms may be involved in the development of reflux esophagitis after eradication of *H. pylori* infection. Eradication of *H. pylori* infection leads to inflammation healing in the corpus of the stomach and normalized function of fundic glands (8). When we eradicate *H. pylori*, the preventive effect of the infection may disappear, allowing peptic gastroesophageal reflux.

Meta analysis summarizes the results of 20 studies and found that *H. pylori* negative status is associated with a significantly increased risk of GERD (9). Some studies suggest that *H. pylori* positive status is rarely seen together with GERD, and if it is present, GERD symptoms are milder in contrast to *H. pylori* negative patients (10).

Conclusion

1. In the NERD subjects, *H. pylori* infection is present more often than in the ERD group, which would support the protective role of *H. pylori* infection in the occurrence of reflux disease.

2. Eradication of *H. pylori* may be associated with transient reflux esophagitis.

3. Eradication of *H. pylori* infection can block the spread of atrophic gastritis and influence the regression of atrophy.

4. In patients with GERD, long-term antisecretory therapy should be implemented, *H. pylori* needs to be tested and eradicated, because long-term gastric acid suppression can accelerate the progression of *Helicobacter pylori* induced corpus atrophic gastritis.

References

1. Jovanović I. Gastroezofagealna refluksna bolest jednjaka. Erozivni ezofagitis i simptomatska refluksna bolest (ERB i NERB). In: Nagorni A, editor. Dijagnostika i terapija bolesti digestivnog trakta povezanih sa pojačanom sekrecijom hlorovodonične kiseline. Niš: Prosveta; 2005. p. 23-30.
2. Nagorni A. Astma i gastroezofagealna refluksna bolest. In: Stanković I, editor. Bronhijalna astma. Niš: Medicinski fakultet Niš; 2005. p. 149-52.
3. Koop H, Schepp W, Mueller-Lissner S, Madisch A, Micklefield G, Messmann H, et al. Consensus conference of the DGSV on gastro-esophageal reflux. Z Gastroenterol 2005; 43:163-93. [CrossRef] [PubMed]
4. Shim CS. Staining in gastrointestinal endoscopy: clinical application and limitations. Endoscopy 1999; 31:487-96. [CrossRef] [PubMed]
5. Ohara S, Sekine H, Iijima K, Moriyama S, Nakayama Y, Kinpara Tet, et al. Gastric mucosal atrophy and prevalence of Helicobacter pylori in reflux esophagitis of the elderly. Jpn J Gastroenterol 1996; 93:235-9. [PubMed]
6. Delaney B, McColl K. Review article: Helicobacter pylori and gastro-oesophageal reflux disease. Aliment Pharmacol Ther 2005; 22 (suppl 1):32-40. [CrossRef] [PubMed]
7. Ribeiro PF, Kubrusly LF, Nassif PA, Ribeiro IC, Bertoldi AS, Batistão VC. Relationship between esophagitis grades and Helicobacter pylori. Arg Bras Cir Dig 2016; 29(3):135-7. [CrossRef] [PubMed]
8. Wang XT, Zhang M, Chen CY, Lyu B. Helicobacter pylori eradication and gastroesophageal reflux disease: a Meta-analysis. Zhonghua Nei Ke Za Zhi 2016; 55(9):710-6. [PubMed]
9. European Helicobacter pylori study group. Current European concepts in the management of Helicobacter pylori infection. The Maastricht Consensus Report. Gut 1997; 41:8-13. [CrossRef] [PubMed]
10. Wu DC,Kuo CH,Tsay FW,Hsu WH,Chen A,Hsu PI. A Pilot Randomized Controlled Study of Dexlansoprazole MR-Based Triple Therapy for Helicobacter Pylori Infection. Baltimor 2016; 95(11):e2698. [PubMed]

Originalni rad **UDC: 616.333-008.8:579.84**
doi:10.5633/amm.2018.0408

UTICAJ HELICOBACTER PYLORI INFEKCIJE NA POJAVU REFLUKSNE EZOFAGEALNE BOLESTI

Vesna Brzački[1,2], Danijela Benedeto-Stojanov[1,2]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[2]Klinika za gastroenterologiju i hepatologiju, Klinički centar Niš, Niš, Srbija

Kontakt: Vesna Brzački
Bul. dr Zoran Đinđić 37/52, 18000 Niš, Srbija
E-mail: brzackiv@gmail.com

Uloga *Helicobacter pylori* (*H. pylori*) u pojavi gastroezofagealne refluksne bolesti (GERB) je prilično nejasna. Smatra se da *H. pylori* infekcija preventivno deluje na pojavu GERB-a. Cilj ove studije bila je analiza učestalosti *H. Pylori* infekcije kod bolesnika sa različitim oblikom GERB-a i poređenje učestalosti između različitih grupa, povezanost stepena ezofagitisa i *H. pylori* infekcije kod bolesnika sa erozivnom refluksnom bolešću (ERB), histoloških promena jednjaka nakon eradikacije bolesnika obe grupe koji su bili pozitivni na *H. pylori* i poređenje ERB i NERB nakon eradikacione terapije za *H. pylori* infekcije. Prospektivna studija, rađena na Klinici za gastroenterologiju Kliničkog centra u Nišu, uključila je 90 bolesnika sa simptomima GERB-a. Bolesnici su podeljeni u dve grupe prema tome da li imaju endoskopske znake refluksne bolesti (ERB grupa) ili su oni odsutni (NERB grupa). Kod svih bolesnika su tokom proksimalne endoskopije uzimane biopsije iz antruma i korpusa za odredjivanje *H. pylori* infekcije i četiri biopsije iz jednjaka na 2 cm od Z linije. U želudačnim biopsijama ispitivano je prisustvo *H. pylori* infekcije, kao i histološke promene, bojene hematoksilinom eozinom i modifikovanom metodom Giemsa. Stepen ezofagitisa je određivan po Los Angeles klasifikaciji. Kod bolesnika sa *H. pylori* pozitivnim nalazom sprovedena je eradikaciona terapija u trajanju od 10 dana. Nakon sprovedene eradikacione terapije i lečenja refluksne bolesti 8 nedelja inhibitorima protonske pumpe, ponovljena je biopsija sluzokože jednjaka na već opisani način.

U antrumu, *H. pylori* je pozitivan kod 22 (44,89%) ispitanika ERB grupe u odnosu na 30 (66,66%) pozitivnih ispitanika NERB grupe. U korpusu, *H. pylori* pozitivan je kod 18 (40,00%) ispitanika ERB grupe u odnosu na 24 (53,33%) ispitanika NERB grupe, bez statističke značajnosti. U grupi ERB, nije bilo razlike u prisustvu ili odsustvu *H. piylori* infekcije i stepena težine ezofagitisa. Nakon eradikacije, u grupi bolesnika sa ERB i NERB postoji statistički značajno poboljšanje histološkog nalaza. Nakon terapije, u grupi ispitanika sa ERB-om je statistički značajno ređi endoskopski nalaz ezofagitisa (χ^2=22,26; p=0,00001). Nakon terapije, u grupi ispitanika sa NERB-om, tri ispitanika su imala endo-skopski nalaz ezofagitisa.

Kod bolesnika sa GERB treba da se sprovede dugoročna antisekretorna terapija. *H. pylori* treba da se testira i eradicira. *H.pylori* pozitivan status retko se sreće zajedno sa GERB-om, a ukoliko je prisutan, lakog je stepena.

Acta Medica Medianae 2018;57(4):60-66.

Ključne reči: *GERB ERB, NERB, H. pylori*

Review article

UDC: 616.14-005.6-092
doi:10.5633/amm.2018.0409

CIRCADIAN PATTERN OF DEEP VEIN THROMBOSIS - TRUE OR FALSE

Zoran Damnjanović

Depending on the pattern length, biological rhythms are divided into three main categories: circadian rhythms (a 24 hour period), ultradian rhythms (a period of less than 24 hours) and infradian rhythms (a period longer than 24 hours). The cardiovascular system is organized on the basis of the weather conditions that oscillate in nature due to which most of the functions follow circadian and seasonal rhythms. The patterns of the maximum and minimum values of the cardiovascular system functions such as blood pressure, heart rate, vascular tone, coagulation and fibrinolysis are well known. Understanding the circadian pattern contributes to the additional clarification of deep vein thrombosis (DVT) pathogenesis and provides the ability to prevent the occurrence of DVT more effectively due to the predictability of critical periods during the day for the origin of an increase in the risk of DVT.

Acta Medica Medianae 2018;57(4):67-70.

Key words: *circadian pattern, deep vein thrombosis, pathogenesis*

Clinic of vascular surgery, Clinical Center of Niš, Serbia

Contact: Zoran Damnjanović
Bulevar Zorana Djindjića 48, 18000 Niš, Serbia
E-mail: damnjanovicz@yahoo.com

Introduction

Depending on the pattern length, biological rhythms are divided into three main categories: circadian rhythms (a 24 hour period), ultradian rhythms (a period of less than 24 hours) and in-fradian rhythms (a period longer than 24 hours). If under certain experimental conditions the biological organism got isolated from the synchronizing environmental sensitivity changes, some of the circadian rhythms would disappear. On the other hand, the endogenous circadian rhythms would continue to exist even under these conditions, but they wouldn't be connected to the day and night change pattern (1).

The cardiovascular system is organized on the basis of the weather conditions that oscillate in nature due to which most of the functions follow circadian and seasonal rhythms. The patterns of the maximum and minimum values of the cardiovascular system functions such as blood pressure, heart rate, vascular tone, coagulation and fibrinolysis are well known. Besides, there is a growing body of evidence supporting the existence of correlation between the time of day, day of the week, months of the year and both morbidity and mortality of certain cardiovascular diseases (2). These patterns exist as a result of the interaction of the rhythms of causal factors of diseases and physiological rhythms of the body. The interactions define the chronorisk concept where the causative factor is probably not powerful enough to bring to the formation of the disease, but in a certain period of time it becomes efficient (3).

Circadian pattern and deep vein thrombosis

The biological mechanisms causing circadian variations are poorly understood. As already known, the circadian rhythm is generated by a central circadian oscillator in the suprachiasmatic nucleus of the hypo-thalamus. Studies in the field of biological rhythms over the last decade have shown that the circadian rhythm in biological, physiological, and behavioral processes has been found in mammals, animalia and plantae organisms (4). The circadian rhythm controls various biological functions in mammals including xenobiotic metabolism and detoxification, immune functions, cell cycle events, apoptosis and angiogenesis. The importance of the circadian rhythm is well known in the chronopharmacology that deals with the biological rhythm dependencies of drugs and examines circadian variations of drug (5). This suggests that chronotherapeutic approaches should be taken for many drugs to enhance their effectiveness. Currently chronotherapeutic approaches are successfully applied in the treatment of peptic ulcer, asthma bronchiale, angina inversa, migraine, allergic rhinitis, rheumatic gout, depressive illness, cardiac infarct, brain infarct, osteoarthropathy and cutaneous hypersensitivity (6, 7). Circadian rhythms are controlled by a cyclical expression of circadian genes. Mutations in these genes lead to the modification and/or disruption of the circadian oscillator. The

investigation of novel genes involved in circadian rhythm - related disease will open up new possibility for therapy and they can be used as markers for improved diagnosis and prognosis (8).

As circadian rhythms modulate vital processes, it is not surprising that several health problems can be associated with the disruption of these rhythms in humans including: gastrointestinal, menstrual irregularities, and sleep disorders (9), but in the past few years, special attention has been paid to the impact of circadian rhythms on the development of deep vein thrombosis (DVT).

The research conducted in Italy by Bilora et al. (10), which included 212 patients diagnosed with deep vein thrombosis and pulmonary embolism (PE) proved the existence of peak for DVT manifestation at 12:26h, while the most cases of PE were recorded at 10:26h. The research conducted by Gallerani et al. (11), which included the analysis of autopsy findings of 507 patients with sudden death in the period from 1983 to 1989, confirmed that the morning hours was the time when the peak for PE occurrence was recorded.

The inflammatory process of DVT pathogenesis involves neutrophils, lymphocytes, platelets, and the integrin subunits β2 and β3, which are connected to their ligands (12). The data found in experimental studies concerning the existence and impact of neutrophil extracellular traps in thrombus formation, have also been confirmed in the researches that involved patients with DVT. It is believed that the role of neutrophil extracellular traps is very important in the formation and maturation thrombus (13, 14). The contribution of erythrocytes in the inflammatory pathogenesis of DVT has not been fully understood yet, despite the well-known fact that large amounts of these blood cells are present at an early stage of DVT (15). Erythrocyte cytoplasm is rich in iron which, due to its oxidative effects, can have highly inflammatory impact on the endothelium if released in the circulation. Hypothetically, it is considered that in the case of thrombus formation, oxidative radicals produced by leukocytes and endothelial cells of blood vessels lead to oxidation of hemoglobin from erythrocytes and the formation of methemoglobin. Methemoglobin contains Fe^{3+} ions that may have an inflammatory effect on the endothelium (16).

The circadian variation of blood coagulation and of the fibrinolytic system suggest a passable state of hypercoagulability in the morning hours. Literature data suggest a tendency to an increased coagulation in the morning hours, which matches the results of this study which show the correlation of the incidence with the time of DVT occurrence in lower limbs. The existence of the morning DVT peaks can be found in the literature data concerning the confirmed endogenous circadian rhythm of blood elements and enzymes. The research conducted by Scheer et al. (17) proved the morning peak activities of platelets (09:00h), with the most expressed aggregation tendency compared to the rest of the day. Recent studies have established that, of the known components of the fibrinolytic system, only tissue-type plasminogen activator and its fast-acting inhibitor, plasminogen activator inhibitor-1, show a marked circadian variation in plasma (18). Studies have shown that the inhibitor of plasminogen activator being an important inhibitor of fibrinolysis, expresses the peak of its activity in the morning (06:30h), which may also explain the increased blood thrombogenicity in the morning (19, 20). Additionally, the morning peak has been identified for the value of coagulation factors VIIa and fibrinogen, as well as for coagulation inhibitors such as protein C, protein S, and antithrombin III (21). The studies that included healthy volunteers have shown decline in endothelial function in the morning, while the blood pressure, heart rate and activity of the renin-angio-tensin-aldosterone system have been increased (17, 19).

Experiments conducted by Pinotte et al. in transgenic mice and in vitro studies demonstrated that variations of coagulation factor VII were controlled at the transcriptional level through the recruitment of circadian transcription factors (20, 22). It was noticed that deletion or mutation of circadian transcription factors in mice changed the time to thrombotic vascular occlusion (23). Conducted studies indicate that blood coagulation is influenced by endogenous biological clocks and daylight, because circadian variations of mRNA expression of coagulation and fibrinolytic factors have been demonstrated in several murine organs (22, 24). Scheer et al. found that other cardiovascular risk factors are under direct circadian control, including platelet activation, count, and aggregability, plasma epinephrine and norepinephrine, plasma cortisol, systolic and diastolic blood pressure, heart rate, and vagal cardiac modulation (19). On the other hand, some investigation showed that melatonin could be important signaling molecule for circadian rhythms of many biological processes, including arterial an venous thromboembolic episodes, indicating an association between blood clotting mechanisms and daylight patterns (25, 26).

Previous investigations on the territory of Southern Serbia have revealed a seasonal pattern of the lower limb DVT appearance, with the highest incidence during the cold period of the year (October-March) (27). The connection between the DVT pathogenesis and the change of external temperature and atmospheric pressure has also been proven. This connection depends on the patient age and the thrombus localization (28, 29).

Conclusion

Further studies should additionally clarify the reasons for circadian DVT rhythm in the lower limbs. Understanding the circadian pattern contributes to the additional clarification of DVT pathogenesis and provides the ability to prevent the occurrence of DVT more effectively due to the predictability of critical periods during the day for the origin of an increase in the risk of DVT.

References

1. Manfredini R, Manfredini F, Malagoni AM, Boari B, Salmi R, Dentali F et al. Chronobiology of Vascular Disorders: a "Seasonal" Link between Arterial and Venous Thrombotic Diseases? JDC 2010; 2(1): 61-7.
2. Takeda N, Maemura K. Circadian clock and cardiovascular disease. J Cardiol 2011; 57(3):249-56. [CrossRef][PubMed]
3. Dentali F, Manfredini R, Ageno W. Seasonal variability of venous thromboembolism. Curr Opin Pulm Med 2009; 15(5):403-7. [CrossRef][PubMed]
4. Baggs JE, Hogenesch JB. Genomics and systems approaches in the mammalian circadian clock. Curr Opin Genet Dev 2010; 20: 581-7. [CrossRef][PubMed]
5. Ozturk N, Ozturk D, Kavakli I H, Okyar A. Molecular Aspects of Circadian Pharmacology and Relevance for Cancer Chronotherapy. Int J Mol Sci 2017; 18(10): 2168. [CrossRef][PubMed]
6. Ando H, Otoda T, Ookami H, Nagai Y, Inano A, Takamura T, et al. Dosing time-dependent effect of raloxifene on plasma plasminogen activator inhibitor-1 concentrations in post-menopausal women with osteoporosis. Clin Exp Pharmacol Physiol 2013; 40: 227-32. [CrossRef][PubMed]
7. Martinod K, Demers M, Fuchs TA, Wong SL, Brill A, Gallant M, et al. Neutrophil histone modification by peptidylarginine deiminase 4 is critical for deep vein thrombosis in mice. Proc Natl Acad Sci U S A 2013; 110: 8674-9. [CrossRef][PubMed]
8. Shanmugam V, Wafi A, Al-TaweelN, Büsselberg D. Disruption of circadian rhythm increases the risk of cancer, metabolic syndrome and cardiovascular disease. Journal of Local and Global Health Science 2013; 3: 2-42.
9. Srinivasan V, Singh J, Pandi-Perumal SR, Brown GM, Spence DW, Cardinali DP. Jet lag, circadian rhythm sleep disturbances, and depression: the role of melatonin and its analogs. Adv Ther 2010; 27(11): 796-813. [CrossRef][PubMed]
10. Bilora F, Manfredini R, Petrobelli F, Vettore G, Boccioletti V, Pomerri F. Chronobiology of nonfatal pulmonary thromboembolism. Panminerva Med 2001; 43 (1): 7-10. [PubMed]
11. Gallerani M, Manfredini R, Ricci L, Grandi E, Cappato R, Calò G, et al. Sudden death from pulmonary thromboembolism: chronobiological aspects. Eur Heart J 1992; 13(5): 661-5. [CrossRef][PubMed]
12. Wang LM, Duan QL, Yang F, Yi XH, Zeng Y, Tian HY, et al. Activation of circulated immune cells and inflammatory immune adherence are involved in the whole process of acute venous thrombosis. Int J Clin Exp Med 2014; 7(3): 566-72. [PubMed]
13. Martinod K, Wagner DD. Thrombosis: tangled up in NETs. Blood 2014; 123(18): 2768-76. [CrossRef][PubMed]
14. Geddings JE, Mackman N. New players in haemostasis and thrombosis. Thromb Haemost 2014; 111(4): 570-4. [CrossRef][PubMed]
15. Turitto VT, Weiss HJ. Red blood cells: their dual role in thrombus formation. Science 1980; 207(4430): 541-3. [CrossRef][PubMed]
16. Woollard KJ, Sturgeon S, Chin-Dusting JP, Salem HH, Jackson SP. Erythrocyte hemolysis and hemoglobin oxidation promote ferric chloride-induced vascular injury. J Biol Chem 2009; 284(19): 13110-8. [CrossRef][PubMed]
17. Scheer FA, Michelson AD, Frelinger AL 3rd, Evoniuk H, Kelly EE, McCarthy M, et al. The human endogenous circadian system causes greatest platelet activation during the biological morning independent of behaviors. PLoS One 2011; 6(9): e24549. [CrossRef][PubMed]
18. Andreotti F, Kluft C. Circadian variation of fibrinolytic activity in blood. Chronobiol Int 1991; 8(5): 336-51. [CrossRef][PubMed]
19. Scheer FA, Shea SA. Human circadian system causes a morning peak in prothrombotic plasminogen activetor inhibitor-1 (PAI-1) independent of the sleep/wake cycle. Blood 2014; 123(4): 590-3. [CrossRef][PubMed]
20. Schoenhard JA, Smith LH, Painter CA, Eren M, Johnson CH, Vaughan DE. Regulation of the PAI-1 promoter by circadian clock components: differential activation by BMAL1 and BMAL2. J Mol Cell Cardiol 2003; 35(5): 473-81. [CrossRef][PubMed]
21. Koukkari WL, Sothern RB. Introducing biological rhythms: a primer on the temporal organization of life, with implications for health, society, reproduction and the natural environment. New York: Springer; 2006.
22. Pinotti M, Bertolucci C, Portaluppi F, Colognesi I, Frigato E, Foa A, et al. Daily and circadian rhythms of tissue factor pathway inhibitor and factor VII activity. Arterioscler Thromb Vasc Res 2005; 25: 646-9. [CrossRef][PubMed]
23. Westgate EJ, Cheng Y, Reilly DF, Price TS, Walisser JA, Bradfield CA et al. Genetic components of the circadian clock regulate thrombogenesis *in vivo*. Circulation 2008; 117: 2087-95. [CrossRef][PubMed]
24. Oishi K, Koyanagi S, Ohkura N. Circadian mRNA expression of coagulation and fibrinolytic factors is organ-dependently disrupted in aged mice. Exp Gerontol 2011; 46: 994-9. [CrossRef][PubMed]
25. Dahm A, Osterud B, Hjeltnes N, Sandset PM, Iversen PO. Opposite circadian rhythms in melatonin and tissue factor pathway inhibitor type 1: does daylight affect coagulation? J Thromb Haemost 2006; 4: 1840-2. [CrossRef][PubMed]
26. Pogan L, Bissonnette P, Parent L, Sauve R. The effects of melatonin on Ca (2+) homeostasis in endothelial cells. J Pineal Res 2002; 33: 37-47. [CrossRef][PubMed]
27. Damnjanovic Z, Jovanovic M, Ilic N, Bogdanovic D, Kudumovic M, Kamenov A, et al. Seasonal variations in the incidence of idiopathic lower extremity deep vein thrombosis on the territory of South Serbia. HealthMED 2012; 6(7): 2477-81.
28. Damnjanović Z, Jovanović M, Bogdanović D, Smiljković I, Ilić N, Damnjanović I. Relationship between the incidence of idiopathic lower extremity deep vein thrombosis and the location of the thrombus with changes of atmospheric pressure. Chirurgia (Bucur) 2012; 107 (4): 483-7. [PubMed]
29. Damnjanović Z, Jovanović M, Stojanović M, Radojković M, Bogdanović D, Potić M, et al. Age Dependent Influence of external temperature on the pathogenesis of idiopathic lower extremity deep vein thrombosis. Chirurgia (Bucur) 2013; 108(4): 530-4. [PubMed]

Revijalni rad

UDC: 616.14-005.6-092
doi:10.5633/amm.2018.0409

CIRKADIJALNI OBRAZAC TROMBOZE DUBOKIH VENA – ISTINA ILI ZABLUDA

Zoran Damnjanović

Klinika za vaskularnu hirurgiju, Klinički centar Niš, Niš, Srbija

Kontakt: Zoran Damnjanovic
Bulevar dr Zorana Đinđića 48, 18000 Niš, Srbija
E-mail: damnjanovicz@yahoo.com

U zavisnosti od dužine ciklusa biološki ritmovi su svrstani u tri glavne vrste: cirkadijalni (period od 24h), ultradialni (period kraći od 24h) i infradijalni (periodi duži od 24h). Kardiovaskularni sistem organizovan je na osnovu vremenskih prilika koje osciliraju u prirodi, usled čega većina funkcija prati cirkadijalni ili sezonski ritam. Obrasci maksimalnih i minimalnih vrednosti funkcija kardiovaskularnog sistema, kao što su arterijski pritisak, srčana frekvencija, vaskularni tonus, koagulacija i fibrinoliza, dobro su poznati. Poznavanje cirkadijalnog obrasca doprinosi dodatnom razjašnjenju patogeneze tromboze dubokih vena (TDV) i pruža mogućnost efikasnije prevencije nastanka TDV usled predvidljivosti kritičnih perioda tokom dana za porast rizika od nastanaka TDV.

Acta Medica Medianae 2018;57(4):67-70.

Ključne reči: *cirkadijalni ritam, tromboza dubokih vena, patogeneza*

Original article **UDC: 616-008:616.127-005.8]:613-056.24**
doi:10.5633/amm.2018.0410

THE INFLUENCE OF METABOLIC SYNDROME ON THE QUALITY OF LIFE IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION WITH ST SEGMENT ELEVATION

Milan Lović[1], Dragan Djordjević[1,2], Ivan S. Tasić[1,2]

Metabolic syndrome (MetS) is a cluster of several risk factors that may indicate worse outcome after myocardial infarction with ST segment elevation (STEMI). The aim of this study was to determine the impact of MetS on quality of life among patients with STEMI.

A prospective study was performed among all STEMI patients who were treated with primary percutaneous coronary intervention in Clinical Centre of Serbia. During the three-year follow up, the occurrence of new cardiovascular events was followed. After 36 months the valid data were collected for 507 patients. At the end of the follow up, all alive and capable patients completed the Short Form 36 (SF-36) questionnaire.

The prevalence of MetS was 42.80%. An increased appearance of new myocardial infarction ($p = 0.004$), new unexpected revascularizations ($p = 0.014$) and the increased number of hospitalizations because of heart failure ($p = 0.050$) were recorded in the group of patients with MetS during a follow-up. Multiple regression analysis revealed that MetS was a predictor for lower scores of: physical functioning (OR 2.684; $p < 0.001$), role physical functioning (OR 2.121; $p = 0.001$), bodily pain (OR 2.559; $p = 0.005$), general health (OR 2.522; $p < 0.001$) and physical component score (OR 2.516; $p < 0.001$). Among mental components, MetS was a predictor of lower scores of vitality (OR 1.999; $p = 0.002$) and mental health (OR 2.142; $p = 0.016$).

Patients with MetS are at high risk for the appearance of new cardiovascular events, and the presence of this syndrome is associated with poorer quality of life after surviving STEMI.

Acta Medica Medianae 2018;57(4):71-78.

Key words: *metabolic syndrome; myocardial infarction with ST segment elevation; quality of life; new cardiovascular events*

[1]Institute for prevention and cardiovascular rehabilitation "Niška Banja", Serbia
[2]University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Milan Lović
Srpskih junaka 7, 18 205 Niška Banja, Serbia
E-mail: milan.lovic@gmail.com

Introduction

Metabolic syndrome (MetS) is defined as a group of interrelated factors that significantly increase the risk of coronary artery disease, other forms of atherosclerotic cardiovascular disease, type 2 diabetes mellitus, cardiovascular mortality, and all-cause mortality. These factors are: hypertrigliceridemia, low level of HDL cholesterol, hypertension, abdominal obesity and insulin resistance (1, 2).

Myocardial infarction with ST-elevation (STEMI) remains to be a significant cause of morbidity and mortality throughout the world among CVD (3, 4). Conventional treatment focuses mainly on functional outcomes, survival and extending life. However, morbidity and mortality rates are incomplete measures of outcome, since they do not reflect all aspects of health. Many patients consider the quality of the additional life years gained equally important as the length of life. Indeed, the goal of today's medicine should be to increase both patients' quantity and quality of life (5). In response, assessment of health-related quality of life (HRQoL) has been increasingly integrated in daily clinical practice. HRQoL is a subjective measure of overall well-being and reflects how a disease and its symptoms are perceived by a patient. Although there is no universal agreement on what constitutes HRQoL, current assessment focuses on the domains of social functioning, physical functioning and psychological functioning (6). It is known that acute cardiovascular events, such as myocardial infarction and stroke, influence on the HRQoL. MetS is a chronic, progressive and multi-complex health

problem that can trigger physical, emotional and psychosocial problems. The impact of MetS on health-related quality of life is yet to be clearly established, although obesity, diabetes and hypertension have obvious consequences of HRQoL (7, 8). However, little is known about how MetS influences the appearance of new cardiovascular events and HRQoL in patients with STEMI treated with primary percutaneous coronary intervention (pPCI). Therefore, our objective was to estimate the influence of MetS to new cardiovascular events and HRQoL among patients with STEMI.

Material and methods

This prospective study included 507 consecutive patients having suffered acute STEMI and treated with primary percutaneous coronary intervention (pPCI) in Clinical Center of Serbia, Belgrade Serbia, between December 2009 and June 2010. The diagnosis of STEMI was established and pPCI performed using guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation of European Society of Cardiology (9). In brief, patients with an episode of chest pain within the last 12 hours and ST-elevation on electrocardiography (ECG) in at least two consecutive leads were included.

After pPCI patients were hospitalized in the Cardiology Department with continuous monitoring including clinical, ECG, laboratory and echocardiography. Echocardiography was performed in the first week of myocardial infarction, on the ultrasonic unit Vivid 4 according to the clinical standard and in accordance with recommendations related to current echocardiography guidelines (10).

Diagnosing of metabolic syndrome

To set up the diagnosis of MetS we used AHA /NHLBI (American Heart Association and the National Heart, Lung and Blood Institute) criteria: central obesity, waist circumference 102/88 cm (M/F); tryglycerides ≥ 1.7 mmol/L; HDL < 1.03/1.3 mmol /L (M/F); systolic blood pressure ≥ 130 mmHg and / or diastolic blood pressure ≥ 85 mmHg or an antihypertensive therapy; fasting blood glucose values greater than 5.6 mmol/L or the use of glucose lowering treatment (11). Parameters according to which the diagnosis was set had been determined in the following way:

Venous blood samples were collected for the biochemical measurements after 48 hours of hospitalization. Serum glucose, HDL cholesterol, triglycerides, LDL cholesterol, total cholesterol and C-reactive protein (CRP) were measured with standard enzymatic colorimetric techniques.

Systolic and diastolic blood pressure was measured with aneroid sphygmomanometer in a sitting position and the average of three consecutive measurements at five minutes intervals was used as final values.

Body weight was measured on a calibrated electronic scale, and height was measured using a standard wall-mounted stadiometer. Body mass index (BMI) was calculated as body weight in kilograms divided by height in squared meters (kg/m^2).

Waist circumferences were obtained using a flexible steel metric tape according to standard procedures. Male patients with waist circumferences more the 102 cm and female more than 88 cm were considered to be obese. All anthropometric variables were measured by the same trained physician, during the hospitalization after the stabilization of vital parameters.

Follow up

During the 3 year follow up period, the authors collected data on mortality, cardiovascular mortality, new myocardial infarction, new revascularization (CABG and PCI), stroke and appearance of heart failure. For patients who died during the follow up, hospital records and necrophy data were reviewed. At the end of 36 month follow up, all alive patient were called for the final examination in order to fill in the questionnaire, the Short Form 36 (SF-36).

Health status

Since we wanted to determine the influence of MetS on HRQoL among the patients with STEMI, we chose to use generic questionnaire, the Short form 36 (SF-36). The reliability, validity and responsiveness of the SF-36 is well documented in patients with coronary artery disease (12). The SF-36 assesses eight health status domains: physical functioning, role physical functioning, role emotional functioning, mental health, vitality, social functioning, bodily pain and general health. Scale scores are obtained by summing the items together within a domain, dividing this outcome by the range of scores and then transforming the raw scores to a scale of 0 to 100 (13). A higher score on the SF-36 sub domains represents a better functioning; a high score on the bodily pain scale indicates freedom from pain. Based on the eight sub domains, physical and mental component summary scores can be calculated according to an algorithm, with the sub domains physical functioning, role physical functioning, bodily pain and general health being the primary contributors to the physical component score and role emotional functioning, vitality, social functioning, and mental health being the primary contributors to the mental component score (14).

Statistical analysis

Statistical analysis was performed with the SPSS 18.0 statistical package. Continuous data were expressed as mean ± SD and categorical data as percentage. Comparisons of prevalence between groups were made using the chi square test or Fisher' s exact test (in case the expected value of the variable was < 5 in at least one group). Mean comparisons were performed using Student's t test. Multivariate analysis model with adjustment for differences at

baseline was used to determine the impact of MetS on HRQoL, and the individual impact of MetS components on HRQoL after 36 month after myocardial infarction. A p value < 0.05 was considered statistically significant.

Ethical considerations

All studies on humans described in the present manuscript were carried out with the approval of the responsible ethics committee and in accordance with national law and the Helsinki Declaration of 1975. (in its current revised form.) Informed consent was obtained from all patients included in the study.

Results

After diagnosing MetS, we formed a group of 217 patients with MetS (Mets+) and another group of 290 patients without the presence of Mets (MetS (-)). Patients with MetS were older, more likely to be women and had a significant prevalence of MetS components. Among the patients with MetS, the most frequent risk factor was hypertension followed by high triglycerides, abnormal fasting glucose level, obesity and low HDL.

In the group of patients without MetS the most frequent risk factor for CVD was smoking, whose presence was significantly higher in this group. In patients with MetS the presence of previous infarction, stroke, PCI and CABG were recorded in a higher percentage but without significant statistical difference (Table 1).

Parameters obtained during hospitalization are shown in Table 2. The parameters according to which the MetS was diagnosed were significantly higher in the group of patients with MetS with the exception of HDL, which was significantly lower in the group of patients with MetS. It can also be observed that marker of inflammation (C-reactive protein) was increased in the group of patients with MetS. Between the examined groups, there was no significant difference in ejection fraction of the left ventricle, as well as extensity of coronary artery disease and localization of infarct-related artery.

The appearance of new adverse events during the three-year follow-up is shown in Table 3. At the end of the follow-up, among the examined groups, a significantly higher percentage of new myocardial infarction was recorded (p = 0.004); and new, unexpected revascularization (p = 0.014). Patients with MetS had a higher percentage of all cause of death, cardiovascular death and strokes but without significant statistical difference. In patients with MetS there was significantly greater percentage of the recorded cases of hospitalization because of heart failure (p = 0.050). Figure 1 shows the mean scale SF-36 in the examined groups. It can be noticed that the middle value scale SF- 36 is significantly lower in the group of patients with metabolic syndrome in all domains.

The results of multivariate logistic regression that we used to examine the impact of MetS on the quality of life, as well as individual influence of MetS components on the quality of life are shown in Tables 4 and 5. The presence of MetS carried a significant risk for low HRQoL; it especially referred to physical components. Among mental components, MetS was a predictor of lower scores of vitality and mental health (Table 4). In the second multivariate model, we analyzed the individual influence of MetS components adjusted for sex, age and smoking. Multivariate logistic regression showed that only elevated blood pressure adversely affected physical functioning, general health and physical component score. Other MetS components individually did not carry a significant risk for low HRQoL (Table 5). There was no association between smoking and HRQoL in both models.

Table 1. Baseline characteristics of analyzed patients

	MetS (+) (N = 217)	MetS (-) (N = 290)	p
Female sex n	59 (27.19%)	54 (18.62%)	0.022
Age, years (± SD)	60.71 ± 11.52	57.50 ± 10.95	0.002
FBG ≥ 5.6mmol/L or th	158 (72.81%)	77 (26.55%)	< 0.001
TG ≥ 1.7 mol/L or th	126 (58.06%)	103 (35.52%)	< 0.001
HDL < 1.03/1.3mol/l (M/F) or th	114 (52.53%)	116 (40.00%)	0.005
BP > 130/85 or th	173 (79.72%)	156 (53.79%)	< 0.001
Obesity*	136 (62.67%)	56 (19.31%)	< 0.001
Family history of CAD	103 (47.47%)	126 (43.44%)	0.368
History of smoking	144 (66.36%)	221 (76.20%)	0.015
Previous M.I.	32 (14.75%)	31 (10.68%)	0.171
Previous stroke	12 (5.53%)	11 (3.79%)	0.073
Previous PCI	13 (5.99%)	9 (3.10%)	0.114
Previous CABG	2 (0.92%)	1 (0.34%)	0.402

FBG –fasting blood glucose; th- therapy; TG tryglicerides; BP- blood pressure *- waist circumference > 102/88 cm (M / F);
M.I. - myocardial infarction; CAD- coronary artery disease; PCI – Percutaneous coronary intervention; CABG-coronary arteries bypass surgery

Table 2. Clinical characteristics of the analyzed patients

		MetS (+) (N = 217)	MetS (-) (N = 290)	p
Systolic blood pressure (mmHg)		138.33 ± 24.30	129.15 ± 21.31	< 0.001
Diastolic blood pressure (mmHg)		88.01± 12.88	82.77± 11.83	< 0.001
Waist circumference (cm)		104.77± 9.98	95.01 ± 14.06	0.001
BMI (kg/m^2)		28.41 ± 3.70	25.61 ± 3.60	< 0.001
Glycemia (mmol/L)		7.46 ± 2.31	6.38 ± 1.96	< 0.001
HDL cholesterol (mmol/L)		1.07 ± 0.25	1.17 ± 0.27	0.001
Triglycerides (mmol/L)		2.20 ± 1.09	1.73 ± 1.00	< 0.001
LDL cholesterol (mmol/L)		3.57 ± 1.06	3.60 ± 1.00	0.180
Total cholesterol (mmol/L)		5.50 ± 1.30	5.69 ± 1.24	0.594
C- reactive protein (mg/L)		5.10 ± 2.02	4.60 ± 1.96	< 0.001
LVEF (%)		49.67 ± 9.55	49.51 ± 10.14	0.865
Localization of M.I.	Anterior	87 (40.09%)	133 (45.86%)	0.163
	Inferior	125 (57.60%)	148 (51.03%)	
	Other	5 (2.30%)	9 (3.10%)	
Infarct vessel	LAD	88 (40. 55%)	134(46. 21%)	0.148
	CX	34 (15.67%)	47 (16.21%)	
	RCA	95 (43.78%)	109 (37.59%)	
One vessel disease		65 (29.95%)	113 (38.97%)	0.089
Two vessel disease		69 (31.80%)	90 (31.03%)	
Three vessel disease		83 (38.25%)	87 (30.00%)	

LVEF - left ventricular ejection fraction; EDD - end diastolic diameter; ESD - end systolic diameter; M.I. - myocardial infarction; LAD - left anterior descending artery; Cx - circumflex artery; RCA - right coronary artery

Table 3. Adverse outcomes during the 36-th month follow up

	MetS (+) n (%)	MetS (-) n (%)	p
All cause of death	35 (16.35)	38 (13.10)	0.336
Cardiovascular death	30 (13.82)	34 (11.72)	0.480
New myocardial infarction	17 (7.83)	7 (2.41)	0.004
New stroke	6 (3.22)	2(0.69)	0.055
New unexpected revascularization (PCI/CABG)	33 (15.20)	24 (8.28)	0.014
Dyspnea	35 (16.13%)	28 (9.66%)	0.028
Hospitalization due to heart failure	9 (4.15)	4 (1.38)	0.050

PCI- percutaneous coronary intervention; CABG-coronary artery bypass surgery

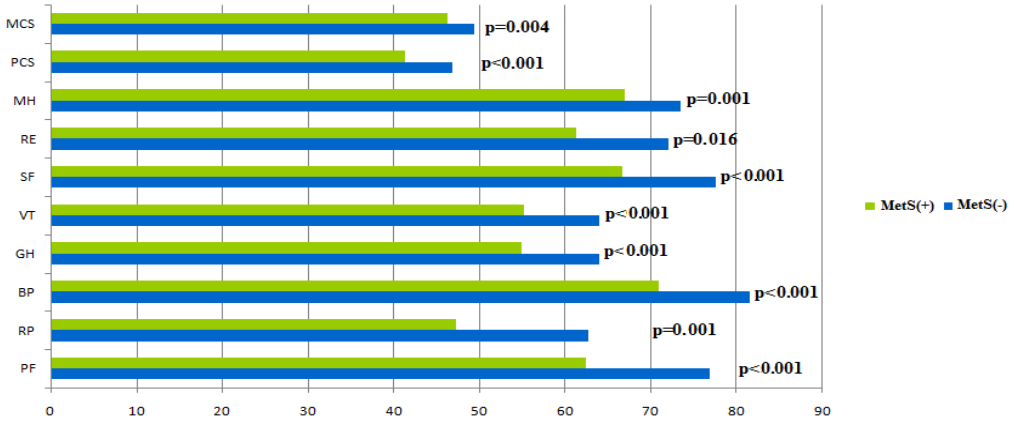


Figure 1. Mean scores (SD) for SF-36 subscales in examined patients at the end the follow-up: mental component summary (MCS); physical component summary (PCS); mental health (MH); role emotional functioning (RE); social functioning (SF); vitality (VT); general health (GH); bodily pain (BP); role physical functioning (RP) and physical functioning (PF)

Table 4. Influence of MetS on HRQoL in multivariate logistic model adjusted for age, sex and smoking

		OR	95%CI	p
PF	Metabolic syndrome	2.684	1.572 - 4.581	< 0.001
	Gender	1.821	1.010 - 3.320	0.050
	Age	1.051	1.021 - 1.081	0.001
RP	Metabolic syndrome	2.121	1.374 - 3.274	0.001
	Age	1.022	1.000 - 1.044	0.050
BP	Metabolic syndrome	2.559	1.318 - 4.966	0.005
GH	Metabolic syndrome	2.522	1.318 - 4.966	< 0.001
PCS	Metabolic syndrome	2.516	1.557 - 4.065	< 0.001
		1.029	1.006 - 1.052	0.012
VT	Metabolic syndrome	1.999	1.284 - 3.113	0.002
	Gender	1.687	1.093 - 2.866	0.043
SF	-	-	-	-
RE	Age	1.021	1.008 - 1.044	0.048
MH	Metabolic syndrome	1.766	1.121 - 2.782	0.016
MCS	Gender	1.955	1.133 - 3.373	0.016

Physical functioning (PF); role physical functioning (RP); bodily pain (BP); general health (GH); physical component summary (PCS); vitality (VT); social functioning (SF); role emotional functioning (RE); mental health (MH) and mental component summary (MCS).

Table 5. Individual influence of MetS components (elevated blood pressure, abnormal fasting glucose level, obesity,elevated triglycerides, low HDL (according to the criteria for MetS)) on HRQoL in multivariate logistic model adjusted for age, sex and smoking

		OR	95%CI	p
PF	Elevated blood pressure	2.418	1.245 - 4.695	0.009
	Gender	2.306	1.220 - 4.358	0.010
	Age	1.047	1.016 - 1.078	0.002
RP	-	-	-	-
BP	-	-	-	-
GH	Elevated blood pressure	1.921	1.141 - 3.233	0.014
PCS	Elevated blood pressure	1.700	1.059 - 2.729	0.028
	Gender	2.040	1.093 - 3.805	0.025
VT	Gender	1.940	1.119 - 3.363	0.018
SF	-	-	-	-
RE	-	-	-	-
MH	-	-	-	-
MCS	Gender	2.066	1.165 -3.665	0.013

Physical functioning (PF); role physical functioning (RP); bodily pain (BP); general health (GH); physical component summary (PCS); vitality (VT); social functioning (SF); role emotional functioning (RE); mental health (MH) and mental component summary (MCS).

Discussion

MetS is a major cause of myocardial infarction, with a rapidly increasing tendency in prevalence (15). This claim was confirmed by the findings of several studies. Namely, Lee et al., Yilmaz et al. and Zeller et al. reported that the prevalence of MetS in patients with STEMI varied from 46% to 59,4% (16-18). Also, a study originating from our region demonstrated a high prevalence of MetS among STEMI patients (19). Our study also confirmed a high prevalence of MetS in these patients; the incidence of MetS in our study was 42.80% which is in accordance with the above mentioned studies. Our study also revealed that female sex was more frequent among MetS patients. Similar to our findings are the findings of two studies that also found higher prevalence of females among MetS in patients with myocardial infarction (18, 19).

During the follow-up period, we found that among patients with MetS, there were a significantly higher number of myocardial infarctions and repeated revascularizations. These findings are in accordance with the results of Mente A. et al. and Takeno

M. et al. (20, 21). Moreover, MetS is connected with the presence of pro-inflammatory condition that is confirmed by the increased presence of inflammatory markers suggesting an increased risk for new cardiovascular events (22, 23). Our study confirms that patients with MetS had significantly increased marker of inflammation (CRP) compared to the group of patients without MetS. In their study, Takeno M. et al. showed that MetS was associated with high CRP level and that it was a significant predictor for the appearance of new cardiovascular events in patients who survived STEMI (21). All these claims suggest that MetS is significantly associated with the development of new coronary stenosis or restenosis during the follow-up period, which increase the need for new coronary revascularization (24) and the occurrence of heart failure (18). Our study confirmed these claims because patients with MetS have a significantly higher percentage of new revascularizations and the hospitalization due to heart failure.

It is well known that a myocardial infarction has a significant impact on HRQoL (25, 26), but little is known about what kind of impact MetS has on HRQoL among patients who survive STEMI. Several studies have shown the adverse impact of MetS on HRQoL. These are mainly studies that have investigated the general population (27). This is the first study that investigated the impact of MetS on HRQoL in a group of patients with STEMI. And we found that MetS was associated with lower HRQoL of the physical components of the SF-36. In addition, participants with MetS scored significantly lower on the PCS score than did persons without the syndrome. By contrast, no association was observed between MetS and the mental component summary score. Our results showed that MetS had an extremely adverse effect on the physical component of SF-36 and physical component score; but it had no impact on mental component score.

These findings can be explained by the fact that MetS can severely inhibit many of the normal physical functions that patient would have. Patients with MetS have increased subjective health complaints of musculoskeletal, neurological, and gastrointestinal pains. An impairment of circulation underlies all of these conditions, and results in pain with physical activity, promoting a sedentary lifestyle and a debilitating cycle ensues (28). In addition, MetS features like obesity and elevated blood pressure increase the work of the heart, while consistently increased insulin levels have macro and micro vascular complications inciting discomfort caused by decreased circulation. Thus, when these conditions are combined in MetS, the consequences can be assumed to be cumulative.

As we already mentioned, the individual components of MetS, such as obesity, elevated blood pressure and abnormal fasting plasma glucose have been shown in several studies to adversely affect the quality of life. Quovadis study, which included 1,822 obese patients, showed that patients with MetS have a poorer quality of life in physical terms, which is in accordance with our study. Individually, obesity, increased blood pressure and fasting plasma glucose had an extremely adverse effect on PCS and no impact on MCS (29). Interestingly, our results indicate that the individual MetS components except high blood pressure had no significant impact on HRQoL in study population. We found that increased blood pressure had a significant adverse impact on physical functioning, general health and physical component scores. These results coincide with the findings of individual studies (30, 31) which indicated that increased blood pressure adversely affected physical functioning. Also, our findings correlate with the findings of Chedraui et al. since they concluded that hypertension had an impact on the physical domains, while hyperglycemia was not found to have any major impact on the physical domain (32). These results may suggest that the metabolic syndrome represented to some degree the cumulative contributions of the individual components.

Conclusion

To our knowledge, the association between MetS and HRQOL has never been addressed among patients who survived STEMI. We conclude that patients with STEMI with MetS have significantly impaired HRQOL even after controlling for confounding variables. Our MetS subjects presented lower QOL in terms of physical health. These findings strongly suggest that HRQOL should be considered in the management of subjects with MetS.

References

1. Huang PL. A comprehensive definition for metabolic syndrome. Dis Model Mech 2009;2:231-7. [CrossRef][PubMed]
2. Kassi E, Pervanidou P, Kaltsas G, Chrosos G. Metabolic syndrome: definitions and controversis BMC MED 2011;9:48. [CrossRef][PubMed]
3. Allender S, Scarborough P, Peto V, Rayner M, Leal J, Luego-Fernandez R et al. European Cardiovascular Disease Statistics 2008.
4. Roger VL, Go AS, Lloyd-Jones DM, Berry JD, Borden WB, Bravata VM et al. Heart Disease and Stroke Statistics Update: A Report from the American Heart. Circulation, 2012.

5. Oldridge N, Saner H, McGee HM. The Euro Cardio-QoL Project. An international study to develop a core heart disease health-related quality of life questionnaire, the HeartQoL. Eur J Cardiovasc Prev Rehabil 2005, 12(2): 87-94. [CrossRef][PubMed]
6. Swenson JR, Clinch JJ. Assessment of quality of life in patients with cardiac disease: the role of psychosomatic medicine. J Psychosom Res 2000, 48 (4-5): 405-15. [CrossRef][PubMed]
7. Huang IC, Frangakis C, Wu AW. The relationship of excess body weight and health-related quality of life: evidence from a population study in Taiwan. Int J Obes (Lond) 2006, 30: 1250-9. [CrossRef][PubMed]
8. Banegas JR, Lopez-Garcia E, Graciani A, Guallar-Castillón P, Gutierrez-Fisac JL, Alonso J et al. Relationship between obesity, hypertension and diabetes, and health-related quality of life among the elderly. Eur J Cardiovasc Prev Rehabil 2007, 14: 456-62. [CrossRef][PubMed]
9. Van de Werf F,Bax J,Betriu A,Blomstrom-Lundqvist C Crea F,Falk V et al. Management of acute myocardial infarction in patients presenting with persistent ST-segment elevation: the Task Force on the Management of ST-Segment Elevation Acute Myocardial Infarction of the European Society of Cardiology. Eur Heart J 2008, 29: 2909-45. [CrossRef][PubMed]
10. Cheitlin MD, Armstrong WF, Aurigemma GP, Beller GA, Bierman FZ, DavisJ. ACC/AHA/ASE Guideline Update for the clinical Application of Echocardiography: Summary article. Circulation, 2003, 108: 1146-62. [CrossRef][PubMed]
11. Grundy SM, Cleeman JI, Daniels SR, Karen AD, Robert HE, Barry AF et al. American Heart Association; National Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation 2005, 112: 2735-2752. [CrossRef][PubMed]
12. Pettersen KI, Reikvam A, Rollag A, Tavem K . Understanding sex differences in health-related quality of life following myocardial infarction. International Journal of Cardiology 2008, 130 (3): 449-456. [CrossRef][PubMed]
13. Ware JE, Sherbourne CD. The MOS 36-item Short-Form Health Survey (SF-36). Med Care 1992, 30: 473-83. [CrossRef][PubMed]
14. Ware JE, Kosinski M. Interpreting SF-36 summary health measures: a response. Qual Life Res 2001, 10: 405-13. [CrossRef][PubMed]
15. Ekmekci A, Gungor B, Uluganyan M, Ozcan KS, Bozbay M, Cicek G et al. Impact of metabolic syndrome on future cardiovascular events in patients with first acute myocardial infarction. Coron Artery Dis 2009; 20: 370-5. [CrossRef][PubMed]
16. Lee MG, Jeong MH, Ahn Y, Chae SC, Hur SH, Hong TJ et al. Impact of the metabolic syndrome on the clinical outcome of patients with acute ST-elevation myocardial infarction. J Korean Med Sci 2010; 25:1456-61. [CrossRef][PubMed]
17. Yilmaz MB, Guray U, Guray Y, Altay H, Demirkan B, Caldir V et al. Metabolic syndrome is associated with extension of coronary artery disease in patients with non-ST elevation acute coronary syndromes. Coronary Artery Dis 2005: 9:287-92.
18. Zeller M, Steg PG, RavisyJ, Laurent Y, Janin-Manificat L, L'Huillier I et al. Observatoire des Infarctus de Cote-d,Or Survey Working group. Prevalence and impact of metabolic syndrome on hospital outcomes in acute myocardial infarction. Arch Intern Med 2005; 165: 1192-8. [CrossRef][PubMed]
19. Jelavic MM, Babic Z, Pintaric H. Metabolic syndrome: influence on clinical severity and prognosis in patients with acute ST-elevation myocardial infarction treated with primary percutaneous coronary intervention. Acta Cardiol. 2015; 70(2):149-56. [CrossRef][PubMed]
20. Mente A, Yusuf S, Islam S, McQueen MJ, Tanomsup S, Onen CL et al. Metabolic Syndrome and Risk of Acute Myocardial Infarction. JACC 2010; 55(21): 2390-8 [CrossRef][PubMed]
21. Takeno M, Yasuda S, Otsuka Y, Morii I, Kawamura A, Yanoet K et al. Impact of Metabolic Syndrome on the Long-Term Survival of Patients With Acute Myocardial Infarction Potential Association With C-Reactive Protein. Circ J, 2008, 72: 415-419. [CrossRef][PubMed]
22. De Pergola G, Pannacciulli N. Coagulatioin and fibrinolysis abnormalitiesin obesity. J Endocrinol Invest 2002; 25: 899-904. [CrossRef][PubMed]
23. Juhan-Vague I, Morange PE, Alessi MC. The insulin resistance syndrome:implications for thrombosis and cardiovascular disease. Pathophysiol Haemost Thromb, 2002; 32: 269-273. [CrossRef][PubMed]
24. Levantesi G, Macchia A, Marfisi R, Franzosi MG, Maggioni AP, Nicolosi GL et al. Metabolic syndrome and risk of cardiovascular events after myocardial infarction. J Am Coll Cardiol, 2005; 46: 277-283. [CrossRef][PubMed]
25. Bengtsson I, Hagman M, Währborg P, Wedel H. Lasting impact on health-related quality of life after a first myocardial infarction. International Journal of Cardiology, 2004; 97: 509-516. [CrossRef][PubMed]
26. Lewis EF, Li Y, Pfeffer MA, Solomon SD, Weinfurt KP, Velazquez EJ et al. Impact of cardiovascular events on change in quality of life and utilities in patients after myocardial infarction: a VALIANT study (valsartan in acute myocardial infarction). JACC Heart Fail, 2014; 2(2): 159-65. [CrossRef][PubMed]
27. Ford ES, Li C. Metabolic Syndrome and Health-Related Quality of Life among U.S. Adults Ann Epidemiol, 2008; 18: 165-171. [CrossRef][PubMed]
28. Hjellset VT, Ihlebæk CM, Bjørge B, EriksenHR, Høstmark AT. Health-Related Quality of Life, Subjective Health Complaints, Psychological Distress and Coping in Pakistani Immigrant Women With and Without the Metabolic Syndrome: The Innva Diab - DEPLAN Study on Pakistani Immigrant Women Living in Oslo, Norway. J Immigr Minor Health; 2010.
29. Corica F, Corsonello A, Apolone G et al and the QUO-VADIS study group. Metabolic syndrome, psychological status and quality of life in obesity: the QUO-VADIS Study. International Journal of Obesity, 2008; 32: 185-191. [CrossRef][PubMed]
30. Lalonde L, O'Connor A, Joseph L, Grover SA. Canadian Collaborative Cardiac Assessment Group Health-related quality of life in cardiac patients with dyslipidemia and hypertension. Qual Life Res, 2004;13(4): 793-804. [CrossRef][PubMed]
31. Bardage C, Isacson DG. Hypertension and health-related quality of life. An epidemiological study in Sweden. J Clin Epidemiol, 2001;54(2): 172-81. [CrossRef][PubMed]
32. Chedraui P, Hidalgo L, Chavez D, Morocho N, Alvarado M, Huc A. Quality of life among postmenopausal Ecuadorian women participating in a metabolic syndrome screening program. Maturitas. 2007;20;56(1):45-53. [PubMed]

Originalni rad **UDC: 616-008:616.127-005.8]:613-056.24**
doi:10.5633/amm.2018.0410

UTICAJ METABOLIČKOG SINDROMA NA KVALITET ŽIVOTA BOLESNIKA SA AKUTNIM INFARKTOM MIOKARDA SA ST SEGMENT ELEVACIJOM

Milan Lović[1], Dragan Đorđević[1,2], Ivan S. Tasić[1,2]

[1]Institut za prevenciju i rehabilitaciju "Niška Banja", Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

Kontakt: Milan Lović
Srpskih junaka 7, 18 205 Niška Banja, Srbija
E-mail: milan.lovic@gmail.com

Metabolički sindrom (MetS) je skup više faktora rizika koji ukazuju na lošu prognozu nakon infarkta miokarda sa elevacijom ST segmenta (STEMI). Cilj ovog istraživanja bio je da se utvrditi uticaj MetS na kvalitet života kod bolesnika sa STEMI.

Ova prospektivna studija je sprovedena među STEMI bolesnicima koji su lečeni primarnom perkutanom koronarnom intervencijom u Kliničkom centru Srbije. Tokom tri godine, praćena je pojava novih velikih kardiovaskularnih događaja. Nakon 36 meseci prikupljeni su podaci za 507 bolesnika. Na kraju praćenja svi živi i sposobni bolesnici pozvani su kako bi popunili upitnik 36 (SF-36).

Prevalencija MetS je bila 42,80%. Tokom praćenja je zabeležen povećan broj novih infarkta miokarda (p = 0,004), novih neplaniranih revaskularizacija (p = 0,014) i povećan broj hospitalizacija zbog srčane isuficijencije (p = 0,050) u grupi bolesnika sa MetS. Višestruka logistička regresijska analiza je pokazala da je MetS prediktor za lošije vrednosti: fizičkog funkcionisanja (OR 2,684; p <0,001), fizičku ulogu (OR 2,121; p = 0,001), telesnu bol (OR 2,559; p = 0,005), opšte zdravlje (OR 2,522; p < 0,001) i skor fizičkih komponenti (OR 2,516; p < 0,001). Među komponentama koje utiču na mentalni status, Mets je prediktor lošije vitalnosti (OR 1,999; p = 0,002) i mentalnog zdravlja (OR 2,142; p = 0,016).

Bolesnici sa Mets su u visokom riziku za pojavu novih kardiovaskularnih događaja, a prisutnost ovog sindroma povezana je s lošijim kvalitetom života onih koji su preživeli STEMI.

Acta Medica Medianae 2018;57(4):71-78.

Ključne reči: *metabolički sindrom; infarkt miokarda sa elevacijom ST segmenta; kvaliteta života; novi kardiovaskularni događaj*

Original article

UDC: 616-006.44:613.8
doi:10.5633/amm.2018.0411

FOLLICULAR LYMPHOMA INCIDENCE AND MORTALITY IN RELATION TO OVERWEIGHT, OBESITY AND PHYSICAL ACTIVITY: A META-ANALYSIS

Ilija Golubović[1,2], Goran Marjanović[1,6], Danijela Radojković[3,6], Dušan Sokolović[4], Aleksandar Karanikolić[2,6], Milan Radojković[2,6], Milorad Pavlović[5]

In the last few years, there has been a growing interest in exploring the association between risk factors such as overweight, obesity and physical activity, and incidence of various cancers.

Meta-analysis was performed to investigate the risk ratio of follicular lymphoma incidence and mortality in overweight and obese individuals, and in individuals with a different physical activity levels using the random-effects model. A literature search through September 2016 was performed. Case-control studies accounted for over 2.100 cases and 12.700 controls, whereas cohort studies accounted for over 2.600 cases in cohort of about 3.000.000 individuals.

In overweight individuals (body mass index between 25 and 29.99 kg/m^2) risk ratio for the development of follicular lymphoma was 1.03 (0.95-1.11; 95% CI; p = 0.51) and in obese (body mass index ≥ 30 kg/m^2) it was 1.15 (1.01-1.31; 95% CI; p = 0.04) when compared to individuals with normal body mass index (< 25 kg/m^2). The risk ratio of specific follicular lymphoma mortality in overweight was 0.59 (0.38-0.91; 95% CI; p = 0.02), while in obese patients it was 1.08 (0.68-1.71; 95% CI; p = 0.75). In patients with the highest physical activity levels, the risk ratio for follicular lymphoma occurrence was 0.95 (0.75-1.21; 95% CI; p = 0.68) when compared to patients that had the lowest physical activity levels.

In summary, our meta-analysis has shown statistically significant direct association between obesity and follicular lymphoma incidence.

Acta Medica Medianae 2018;57(4):79-90.

Key words: *follicular lymphoma, meta-analysis, obesity, overweight, physical activity*

[1]Hematology and Immunology Clinic, Clinical Center Niš, Niš, Serbia
[2]General Surgery Clinic, Clinical Center Niš, Niš, Serbia
[3]Endocrinology Clinic, Clinical Center Niš, Niš, Serbia
[4]University of Niš, Faculty of Medicine, Department of Biochemistry, Niš, Serbia
[5]Thoracic surgery Clinic, Clinical Centre Niš, Serbia
[6]University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Ilija Golubović
Clinical Center Niš
Blvd. Dr Zoran Djindjić 48, 18000 Niš, Serbia
E-mail: golubovicilija@yahoo.com

Introduction

Follicular lymphoma (FL) is the second most common subtype of non-Hodgkin lymphoma (NHL) in Europe and North America, and accounts for about 20-30 percent of all NHL cases (1). Although it is known that the risk factors, such as alcohol (2), cytogenetic abnormality (3), immunosuppression, and some autoimmune diseases (4), may influence FL incidence, indubitable risk factor for its occurrence is not yet known.

In the last few years there has been a growing interest in exploring an association between risk factors as obesity (OB) and physical activity (PA), and incidence of various cancers (5, 6). Obesity, the prevalence of which has lately increased in many countries (7), is associated with a chronic state of inflammation. Thus, as the principal source of inflammatory cytokines, adipose tissue may be linked with the development of cancer (8, 9). For many malignancies over-weight (OW) and OB are noted as risk factors (10, 11). However, the authors' findings regarding FL are controversial. While some claim that there is an association between these risk factors and FL incidence (12-15), others deny it (16, 17). Furthermore, current research on reduction of PA associated with OB is focused on their link with various cancers (6). So far, there has been no meta-analysis of the association be-tween OW and OB and

a relative risk of FL mortality, while the influence of PA on FL incidence emphasized only in one published meta-analysis (18).

The aim of this study was to evaluate the association of OW and OB with FL incidence and mortality, as well as association of PA with FL incidence, using the both cohort and case-control studies. Also, we investigated the influence of OW, OB and PA on FL incidence depending on the type of study, gender and region in which examinees lived.

Methods

Study selection

Using PubMed and searching the reference list of retrieved articles, the literature search through September 2016 was performed. The key words searched were: follicular lymphoma, overweight, obesity, body mass index, meta-analysis, physical activity, mortality. No language restrictions were imposed. Also, references cited in the corresponding articles were incorporated in the search. Both prospective and case-controls studies were included in the meta-analysis. Abstracts of articles, narrative studies, letters to the authors and cross-sectional studies were not included in this meta-analysis.

A study was relevant if it contained data reporting on the association between body mass index (BMI) and incidence and mortality of FL as well as the association between PA and incidence of FL. We identified a total of 39 articles with data that were potentially eligible for inclusion in this meta-analysis, of which 36 were related to FL incidence (31 were included in the analysis of influence of OW and OB, and 9 were included in the analysis of the influence of PA on FL incidence, with 4 studies overlapping, that is containing both topics), while 3 studies, all with a prospective design, were related to FL mortality. A total of 19 studies related to FL incidence were excluded because these articles did not include data on BMI values in relation to FL incidence. From the studies of FL mortality, one was excluded because it lacked data on mortality in relation to BMI. At the end of the identification process there were 17 applicable studies left with the data on FL incidence (14 for analysis of the influence of OW and OB (12-26), and 7 for analysis of the influence of PA, from which 4 were previously included (13, 15, 17, 25, 27-29) as well as 2 studies with data on association between OW and OB and FL mortality (30, 31).

Data analysis

For both cohort and case-control studies, we extracted the following data: authors last names; publication year; study type; source (region); gender and age; case - total cohort or case-control size; time of follow-up; method of BMI determining; type and assessment of PA; the highest and the lowest PA level; adjustments (for cohort studies) or matching (for case-control studies); the risk ratio (RR) and hazard ratio (HR) for cohort studies and the odds ratio (OR) for case-control studies, with 95% confidence interval for all (95% CI).

Statistical analysis

OW was defined as BMI values between 25 and 29.99 kg/m^2 (pre-obese), OB as BMI values over 30 kg/m^2 (obese class I), while the high OB was defined as BMI values over 35 kg/m^2 (obese class II) according to the WHO criteria (32). Normal BMI (<25 kg/m^2) values were the reference category for all computing related to BMI. For analysis of the influence of PA, we compared the highest to the lowest PA level. The lowest level was the reference category for all comparisons. For studies with the highest PA level as the reference category, instead of the lowest level, the inverse value was used. If risk estimates for PA were reported for more than one period, the most recent data were used. For studies that reported recreational and occupational activity separately the former was preferred, and if a publication included data on total activity, these data were used. For all analysies, if study reported risk estimates for men and women separately, values for both were analyzed.

The summary RR with 95% CI was computed to assess the risk for FL incidence and mortality in overweight and obese examinees in relation to examinees with normal BMI, or the risk for the development of FL in examinees with the highest versus the lowest PA level. In the analysis of FL incidence (influence of OW and OB, and PA), we performed subgroup analysis depending on the type of study, gender and region in which the examinees lived. While analyzing the mortality depending on the BMI index, it was calculated as a specific/univariate or nonspecific/multivariate mortality.

DerSimonian-Laird method for the random-effects model was used to compute the outcome of study-specific RR (33). To assess statistical heterogeneity among studies, the Q and I2 statistics were used (34). Heterogeneity was defined as mild (I2 of 25%), moderate (I2 of 50%) and severe (I2 of 75%). Publication bias was noticed on funnel plots and was assessed by using Egger's test (35). Statistical significance was defined for p-value < 0.05. All statistical analyses and graphics were performed with Comprehensive Meta-Analysis (Version 3; Biostat, Inc, Englewood, NJ).

Results

For studies observing the influence of OW and OB on FL incidence, a total number of cohort studies were 7 and studies included 1,979 cases in cohort of 2,712,088 individuals; a total number of case-control studies were 7 and these studies included 1,959 cases and 11,725 controls.

For studies observing the influence of OW and OB on FL mortality, a total number of cohort studies were 2 and studies included 493 cases in a cohort of over 215,000 individuals.

For studies observing the influence of PA on FL incidence, a total number of cohort studies were 4 (two of which overlapped with previous included 7 cohort studies related to FL incidence) and studies included 490 cases in a cohort of 661,878 individuals; a total number of case control studies was 3 (two of which overlapped with previous included 7 case-control studies related to FL incidence) and these studies included 583 cases and 4,583 controls (Tables 1 and 2).

Table 1. Characteristics of studies observing follicular lymphoma incidence or mortality in relation to overweight and obesity

Author (Year)	Type of studies	Source (Region)	Gender and age (years)	Case - total cohort (No.-No.) or case-control (No.-No.) size	Enrollment (Years)	BMI determining	Adjustments (cohort)/matching (case-control)
INCIDENCE							
Skibola C.F. (2004)[14]	Case-control	United States	M and W; 21-74	351-2,400	1988-1995	Self-reported	Age, gender, country of residence
Willett E.V. (2005)[19]	Case-control	Europe	M and W; 18-59	227-911	1998-2001	Self-reported	Gender, date of birth
Chang E.T. (2005)[16]	Case-control	Europe	M and W; 18-74	582-3,158	1999-2002	Self-reported	Age, gender
Cerhan J.R. (2005)[17]	Case-control	United States	M and W; 20-74	289-977	1998-2000	Self-reported	Age, gender, race, study center
Pan S.Y. (2005)[15]	Case-control	Canada	M and W; 20-76	239-3,027	1994-1997	Self-reported	Age, province, gender, education, smoking, alcohol, chemicals, occupational exposure
Chiu B.C.H. (2007)[20]	Case-control	United States	M and W; 20-75	121-527	1999-2002	Self-reported	Age, gender
Lim U. (2007)[12]	Cohort	United States	M and W; 50-71	257-473,984	1995-2000	Self-reported	Age, gender, ethnicity, education, alcohol, smoking, height, physical activity
Britton J.A. (2008)[21]	Cohort	Europe	M and W; 25-70	131-371,983	1993-1998	Measured	Age, study center
Maskarinec G. (2008)[22]	Cohort	United States	M and W; 45-75	129-193,051	1993-1996	Self-reported	Age, ethnicity, education, alcohol
Lu Y. (2009)[13]	Cohort	United States	W; 22-84	121-121,216	1995-2007	Self-reported	Height, age at menarche, long-term strenuous plus moderate physical activity
Troy J.D. (2010)[23]	Cohort	United States	M and W; 55-74	162-142,982	1993-2001	Self-reported	Age, race/ethnicity, gender, education
Kanda J. (2010)[24]	Case-control	Japan	M and W; 18-80	149-725	1988-2005	Self-reported	Age, gender
Kabat G.C. (2012)[25]	Cohort	United States	W; 50-79	214-158,975	1993-1998	Self-reported	Age, alcohol, smoking, caloric intake, education, ethnicity, enrollment in the OS, treatment arm assignment in the clinical trials
Murphy F. (2013)[26]	Cohort	Europe	W; 56.6 (The mean age)	965-1,249,897	1996-2009	Self-reported	Height, alcohol, smoking, socioeconomic status
MORTALITY							
Leo Q.J.N. (2014)[30]	Cohort	United States	M and W; 45-75	214-215,000	1993-2007	Self-reported	Age, gender, SEER stage, education, NHL type, therapy, smoking, alcohol, comorbidity, age at diagnosis
Hong F. (2014)[31]	Cohort	United States	M and W; 56 (The median age)	279- Not reported	1998-2003	Not reported	Age, gender, B-symptom, FLIPI score, treatment

Abbreviations: BMI - Body mass index, M - Men, W - Women, SEER - the National Cancer Institute's Surveillance, Epidemiology, and End Results, FLIPI - Follicular lymphoma International Prognostic Index

Table 2. Characteristics of studies observing physical activity in relation to follicular lymphoma incidence

Author (Year)	Type of studies	Source (Region)	Gender and age (years)	Case - total cohort (No.-No.) or case-control (No.-No.) size	Enrollment (Years)	Type and assessment of physical activity	The highest vs. the lowest physical activity level	Adjustments (cohort)/matching (case-control)
Cerhan J.R. (2002)[27]	Cohort	United States	W; 55-69	57-37,931	1986-1998	Recreational; Self-reported questionnaire	High vs. low (Responses created a level activity score)	Age
Cerhan J.R. (2005)* [17]	Case-control	United States	M and W; 20-74	119-406	1998-2000	Recreational; Self-administered questions	>1.080 METs/week vs. <30 METs/week	Age, gender, race, study center
Pan S.Y. (2005)* [15]	Case-control	Canada	M and W; 20-76	242-3,106	1994-1997	Recreational; The questionnaire elicited information	>34.4 MET-h/week vs. <6.3 MET-h/week	Age, province, gender, education, smoking, alcohol, chemicals, occupational exposure
Lu Y. (2009)* [13]	Cohort	United States	W; 22-84	121-121,216	1995-2007	Recreational; Self-administered baseline questionnaire	≥4 h/week/year vs. 0-0.50 h/week/year	Weight, height, age at menarche, long-term strenuous plus moderate physical activity
V Veldhoven C.M. (2011)[28]	Cohort	Europe	M and W; 57.9 ± 8.3 (mean ± SD)	98-343,756	1992-2000	Total; Self-administered or interview-based questionnaires	Standing and manual/heavy manual work + recreational PA (≥45.75 MET-h/week) vs. Sedentary and unwilling + recreational PA (<14.25 MET-h/week)	Education, smoking, alcohol, hypertension, hyperlipidaemia, diabetes, BMI, weight, height, waist and hip circumference, waist-to-hip ratio
Kabat G.C. (2012)* [25]	Cohort	United States	W; 50-79	214-158,975	1993-1998	Recreational; Self-administered questionnaires	≥17.5 MET-h/week vs. <1.6 MET-h/week	Age, alcohol, smoking, caloric intake, education, ethnicity, BMI, enrollment in the OS, treatment arm assignment in the clinical trials
Kelly J.L. (2012)[29]	Case-control	United States	M and W; <40->70	222-1,071	2002-2008	Total; Self-administered risk-factor questionnaire	>2.701 METs/week vs. <615 METs/week	Age, gender, country of residence

*Previously included studies in Table 1. Abbreviations: BMI - Body mass index, M - Men, W - Women, PA - Physical activity, MET - Metabolic equivalent task, SD - Standard deviation

OW and FL incidence and mortality

In overweight examinees, RR for the development of FL was 1.03 (0.95-1.11; 95% CI; p = 0.51) when compared to examinees that had a normal BMI. The RR in cohort studies was 1.00 (0.90-1.11; 95% CI; p = 0.97) and in case-control studies was 1.06 (0.95-1.19; 95% CI; p = 0.31). The RR in men was 1.15 (0.87-1.53; 95% CI; p = 0.33), in women was 0.99 (0.88-1.12; 95% CI; p = 0.87), while in both men and women the RR was 1.05 (0.94-1.17; 95% CI; p = 0.40). Americans had the RR of 1.12 (0.99-1.27; 95% CI; p = 0.06), while Europeans had the RR of 0.97 (0.87-1.08; 95% CI; p = 0.54). In overweight examinees, the statistically significant RR for specific mortality was 0.59 (0.38-0.91; 95% CI; p = 0.02), while for non-specific was 0.72 (0.46-1.12; 95% CI; p = 0.14). There was no statistically significant heterogeneity among studies. There was no statistically significant publication bias (Figure 1 and Table 3).

OB and high OB and FL incidence and mortality

Obese examinees had a significantly increased RR of 1.15 (1.01-1.31; 95% CI; p = 0.04) for the development of FL when compared to examinees that had a normal BMI. The RR in cohort studies was 1.14 (0.97-1.34; 95% CI; p = 0.13) and in case-control studies was 1.17 (0.92-1.48; 95% CI; p = 0.21). The RR in men was 1.42 (0.95-2.12; 95% CI; p = 0.09), in women was 1.24 (0.95-1.63; 95% CI; p = 0.12), while in both men and women the RR was 1.08 (089-1.30; 95% CI; p = 0.45). Americans had the RR of 1.25 (1.00-1.58; 95% CI; p = 0.06), while Europeans had the RR of 1.03 (0.89-1.18; 95% CI; p = 0.72). Obese examinees had the RR of 1.08 (0.68-1.71; 95% CI; p = 0.75) for specific mortality and 1.56 for non-specific mortality (0.96-2.55; 95% CI; p = 0.08). There was no statistically significant heterogeneity among studies. There was only one statistically significant publication bias (Fig. 2 and Table 4).

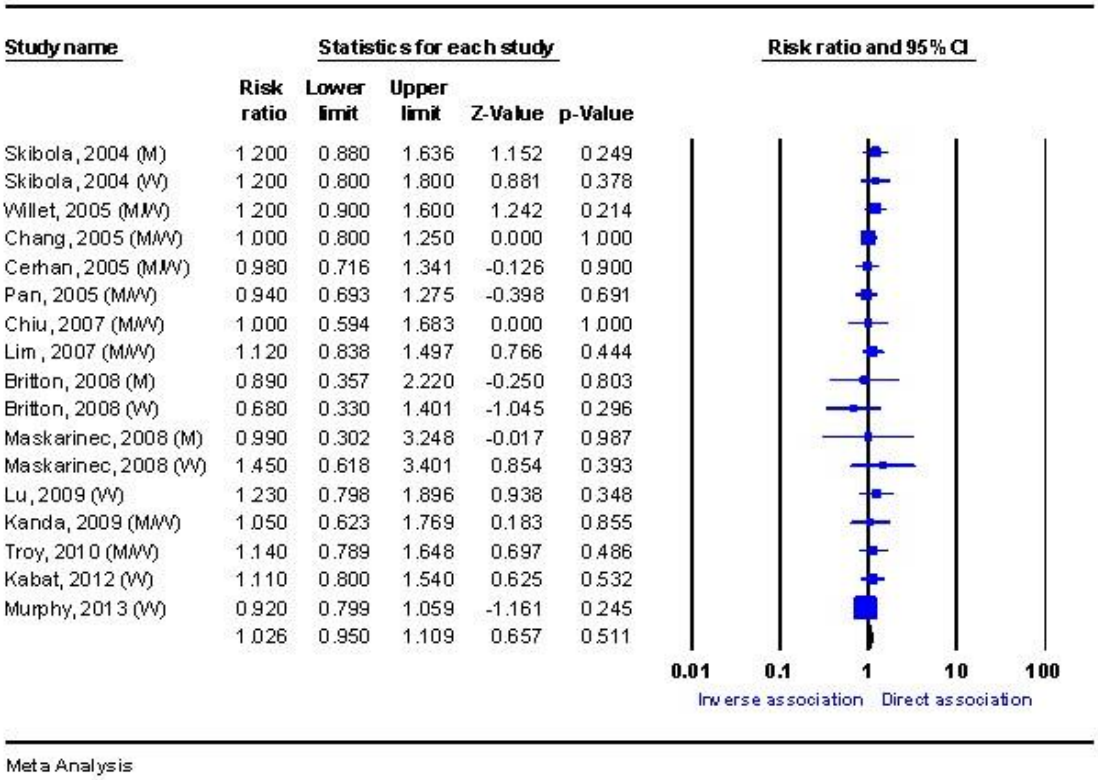


Figure 1. Relative risks of follicular lymphoma incidence associated with overweight.
Abbreviations: M – men, W – women, M/W – men and women.
Heterogeneity: Q = 9.00, p = 0.91, I^2 = 0%.
Publication bias assessed by Egger's test: p = 0.18

Table 3. Relative risks of follicular lymphoma incidence and mortality associated with overweight

	Studies (No.)	RR (95% CI)	*p* - Value	Heterogeneity		Egger's test
				p – Value	I^2 (%)	(*p* – Value)
INCIDENCE						
All studies	14	1.03 (0.95-1.11)	0.51	0.91	0	0.18
Type of studies						
Cohort	7	1.00 (0.90-1.11)	0.97	0.69	0	0.33
Case-control	7	1.06 (0.95-1.19)	0.31	0.90	0	0.73
Gender						
Men	3	1.15 (0.87-1.53)	0.33	0.80	0	0.26
Women	6	0.99 (0.88-1.12)	0.87	0.40	2.70	0.27
Men and women	8	1.05 (0.94-1.17)	0.40	0.95	0	0.88
Source (Region)						
United States	8	1.12 (0.99-1.27)	0.06	0.99	0	0.63
Europe	4	0.97 (0.87-1.08)	0.54	0.45	0	0.97
MORTALITY						
Specific	2	0.59 (0.38-0.91)	0.02	0.83	0	-
Non-specific	2	0.72 (0.46-1.12)	0.14	0.98	0	-

In high obese examinees, the RR for the development of FL was 0.94 (0.72-1.24; 95% CI; p = 0.66) when compared to examinees that had a normal weight. The RR in cohort studies was 1.09 (0.74-1.59; 95% CI; p = 0.67) and in case-control studies was 0.81 (0.55-1.20; 95% CI; p = 0.29). The RR in both men and women was 0.93 (0.68-1.28; 95% CI; p = 0.66). There was no statistically significant heterogeneity among studies. There was no statistically significant publication bias (Figure 2 and Table 4).

In both obese and high obese examinees, an RR of FL incidence was 1.11 (0.98-1.25; 95% CI; p = 0.10). There was no statistically significant heterogeneity among overall studies (Figure 2).

PA and FL incidence

In examinees with the highest PA level, the RR for the development of FL was 0.91 (0.72-1.15; 95% CI; p=0.42) when compared to examinees that had the lowest PA level. The RR in cohort studies was 1.07 (0.81-1.42; 95% CI; p=0.62) and in case-control studies was 0.77 (0.59-1.02; 95% CI; p=0.06). The RR in men was 1.23 (0.51-2.91; 95% CI; p=0.66), in women was 1.02 (0.78-1.32; 95% CI; p=0.89), while in both men and women the statistically significant RR was 0.68 (0.48-0.97; 95% CI; p=0.03). Americans had the RR of 0.96 (0.77-1.21; 95% CI; p=0.74). There was no statistically significant heterogeneity among studies. There was only one statistically significant publication bias (Fig. 3 and Table 5).

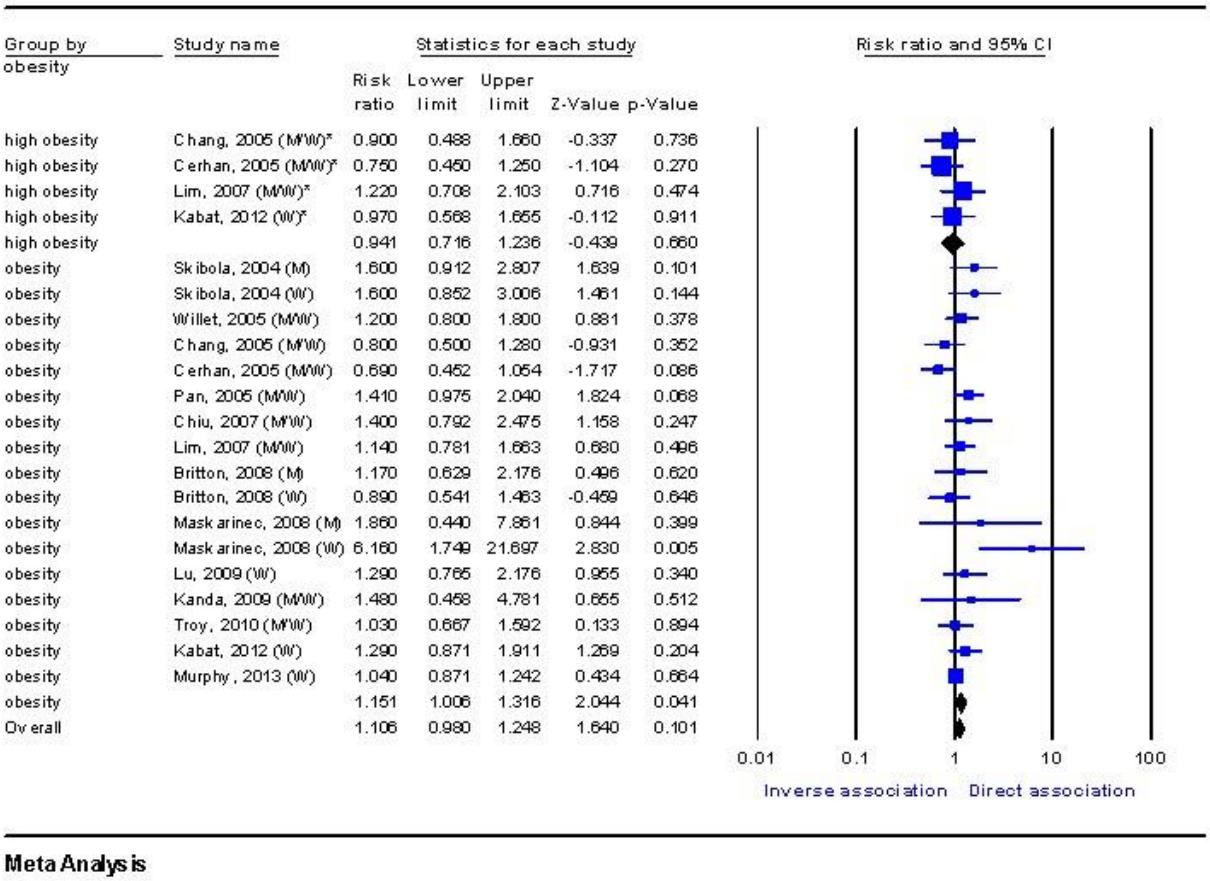


Figure 2. Relative risks of follicular lymphoma incidence associated with obesity and high obesity.
Abbreviations: M – men, W – women, M/W – men and women.
Heterogeneity for obesity: Q = 22.14, p = 0.14, I^2 = 27,72%;
for high obesity: Q = 1.66, p = 0,64, I^2 = 0%; for overall Q = 25.18, p = 0,195, I2 = 20.58.
Publication bias assessed by Egger's test for obesity: p = 0.06; for high obesity: p = 0.76

Table 4. Relative risks of follicular lymphoma incidence and mortality associated with obesity and high obesity

	Studies (No.)	RR (95% CI)	*P* value	Heterogeneity		Egger's test
				p – Value	I^2 (%)	(*p* – Value)
INCIDENCE (Obesity)						
All studies	14	1.15 (1.01-1.31)	0.04	0.14	27.72	0.06
Type of studies						
Cohort	7	1.14 (0.97-1.34)	0.13	0.27	19.38	0.06
Case-control	7	1.17 (0.92-1.48)	0.21	0.097	42.17	0.50
Gender						
Men	3	1.42 (0.95-2.12)	0.09	0.71	0	0.77
Women	6	1.24 (0.95-1.63)	0.12	0.061	52.56	0.08
Men and women	8	1.08 (0.89-1.30)	0.45	0.23	24.91	0.85
Source (Region)						
United States	8	1.25 (1.00-1.58)	0.06	0.062	44.53	0.03
Europe	4	1.03 (0.89-1.18)	0.72	0.71	0	0.76
MORTALITY (Obesity)						
Specific	2	1.08 (0.68-1.71)	0.75	0.30	4.87	-
Non-specific	2	1.56 (0.96-2.55)	0.08	0.49	0	-
INCIDENCE (High obesity)						
All studies	4	0.94 (0.72-1.24)	0.66	0.64	0	0.76
Type of studies						
Cohort	2	1.09 (0.74-1.59)	0.67	0.56	0	-
Case-control	2	0.81 (0.55-1.20)	0.29	0.65	0	-
Gender						
Men and women	3	0.93 (0.68-1.28)	0.66	0.44	0	0.83

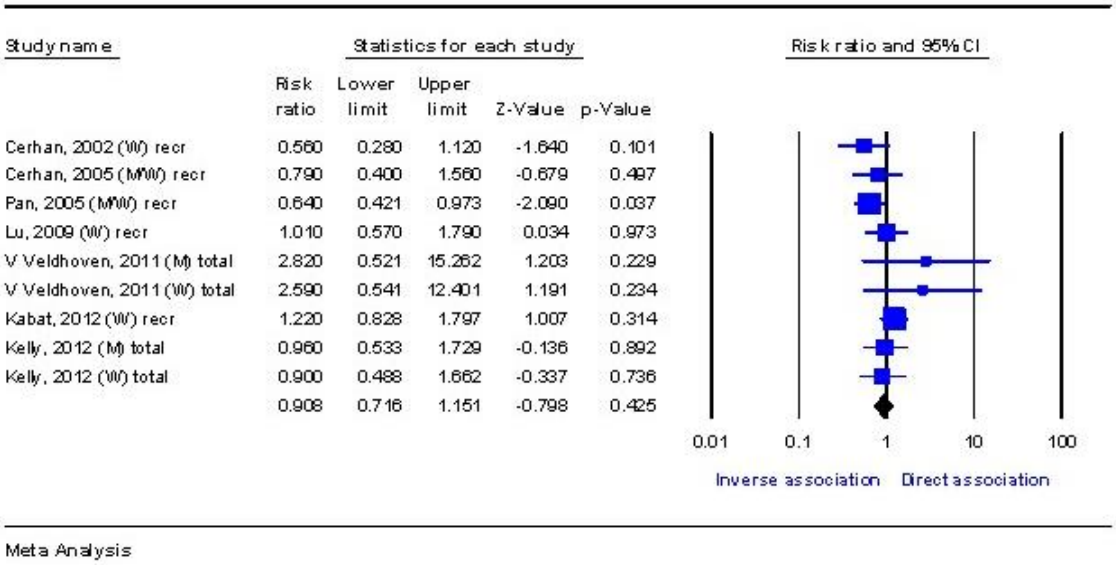


Figure 3. Relative risks of follicular lymphoma incidence associated with physical activity.
Abbreviations: M – men, W – women, M/W – men and women.
Heterogeneity: Q = 10.57, p = 0.28, I^2 = 24.29%.
Publication bias assessed by Egger's test: p = 0.35

Table 5. Relative risks of follicular lymphoma incidence associated with physical activity

	Studies (No.)	RR (95% CI)	*P* value	Heterogeneity		Egger's test
				p – Value	I^2 (%)	(*p* – Value)
All studies	7	0.91 (0.72-1.15)	0.42	0.28	24.29	0.35
Type of studies						
Cohort	4	1.07 (0.81-1.42)	0.62	0.18	36.71	0.61
Case-control	3	0.77 (0.59-1.02)	0.06	0.67	0	0.19
Gender						
Men	2	1.23 (0.51-2.91)	0.66	0.24	28.31	-
Women	5	1.02 (0.78-1.32)	0.89	0.27	23.34	0.99
Men and women	2	0.68 (0.48-0.97)	0.03	0.60	0	-
Source (Region)						
United States	5	0.96 (0.77-1.21)	0.74	0.52	0	0.02
Europe	-	-	-	-	-	-

Discussion

This research was set with the aim of assessing the importance of BMI and PA for the development of FL. Our meta-analysis of cohort and case-control studies pointed out that overweight individuals did not have increased risk for the development of FL in comparison to individuals with normal weight, while obese individuals had 15% higher risk compared to non-obese. Overweight individuals had a higher risk of FL specific mortality than those with normal weight. However, a note of caution is mandatory since the number of included publication with data on mortality is small. Results of cohort and case-control studies related to the association between BMI and FL incidence did not show statistically significant difference.

In previous meta-analyses the association between BMI and FL incidence was investigated and statistically non-significant positive association between BMI and the relative risk for the development of FL was found. Thus, in a meta-analysis by Larsson and Wolk (2011) relative risk of FL and 5kg/m^2 BMI increase were positively associated (1.03; 0.93 to 1.13; 95% CI), but statistically non-significant (36). In addition, the second meta-analysis from the same authors (2007) demonstrated statistically non-significant association between obese individuals and the relative risk for the development of FL (37). However, this meta-analysis did not include data on overweight individuals (37). Nonetheless, the findings of the current study do not support the previous research. Moreover, the results of this study indicated that OB individuals had increased risk for the development of FL.

In terms of smaller body fat percentage in men, it may be assumed that the females would be more susceptible for the development of FL. Also, considering that subcutaneous adipose tissue is predominate in women, it may be assumed that visceral adipose tissue has a much more essential role for the development of many cancers, including FL (38, 39). This type of body fat is associated with obesity-related diseases (40), because it is more harmful and inflammatory active than subcutaneous fat which is generally thought to be responsible for OB. However, based on the results of our meta-analysis, there was no increased risk of FL associated with either overweight and obese men or women.

Finally, a direct association between OW and OB, and FL incidence is more pronounced among Americans as compared to Europeans. But, none of these differences were statistically significant. For both overweight and obese American individuals, increased risk of FL was formally not significant (p = 0.06). These results seem to be consistent with other research which has not found the association between BMI and NHL incidence across strata of geographic region (36). In the study of Castillo et al. (2014) for both overweight and obese American individuals the RR for the development of diffuse large B-cell lymphoma was significantly increased, but not in Europeans (41). Although different life habits and harmful effects of the environment may cause the differences between these two groups of individuals, no association was identified in this meta-analysis. In addition, more research on this topic is necessary before the association between geographic area and the risk of FL is more clearly understood.

The prevalence of OB is increasing and is associated with other risk factors such as increased intake of high-fat food and decreased PA (7, 42). McTiernan (6) proposed that excessive weight and a sedentary lifestyle are linked with about 25% of cancers, and that PA may decrease risk for various cancers by several mechanisms. In this meta-analysis only, for both men and women statistically inverse association of PA with the risk for the development of FL was found. However, this subgroup

analysis could have been affected by small sample size. Thus, this finding cannot be extrapolated to all the patients. Thus, the summary risk estimate derived from all studies showed no significant influence of physical activity on the risk of FL. The findings of this study are consistent with the data obtained in other meta-analyses which focused not only to FL but also generally to NHL (18) or NHL and Hodgkin lymphoma (43).

There are numerous studies that were designed to explore the association between OB and cancer (5, 44), but the mechanisms of this causal link are still unclear. Basically, the focus is on three hormonal systems that include insulin and insulin-like growth factor (IGF) axis, sex steroids and adipokines. Obesity-related inflammatory cytokines, genetic background, obesity-related hypoxia, and oxidative stress were also noted as possible causes, and more other etiological factors which may have a role in the occurrence and development of cancer (45).

This meta-analysis has some limitations. First, other obesity-related factors, such as change of weight during the time, diet and caloric intake, may also influence the risk for the development of FL. Second, the sample size may be a source of erroneous conclusions. For example, statistically significant inverse association between BMI and FL mortality was found in overweight individuals, while non-significant direct association was presented in obese. Furthermore, these results are not conclusive because of the dependence on FL subtype, treatment, comorbidity and SEER stage. Third, determining of BMIs in all included studies was based on the principle of personal declaration. Any discrepancies, such as false weight loss in obese, may have an unquestionable impact on the final results. Fourth, the BMI indicates total body fat (combined subcutaneous adipose tissue and visceral adipose tissue) (45), and therefore it cannot assess what type of body fat has a greater impact - visceral adipose tissue (VAT) or subcutaneous adipose tissue. And finally, all associations either direct when the effects of excessive weight or OB were analyzed, or inverse when the influence of PA was analyzed, may be influenced by publication bias. In this meta-analysis, there was not evidence of publication bias in most of the studies.

In summary, this meta-analysis of cohort and case-control studies has identified the direct association between obesity and FL incidence. The research has also shown statistically significant association between BMI and FL mortality in overweight individuals, as well as association between PA and the risk of FL incidence in both male and female individuals. It could be argued that these last two results could have been affected by small sample size. The current data highlights the importance of obesity for the risk of FL developments. It would be interesting to assess the impact of other risk factors such as insulin and IGF, sex steroids and adipokines involved in the association between OB and malignant lymphomas. Future meta-analyses on the current topic with a larger number of included studies are recommended to confirm these results.

Acknowledgment

This work was supported by the Ministry of Education, Science and Technological Development of Serbia (Project 43012).

References

1. Morton L, Wang S, Devesa S, Hartge P, Weisenburger DD, Linet MS. Lymphoma incidence patterns by WHO subtype in the United States, 1992–2001. Blood. 2006; 107(1): 265-76. [CrossRef][PubMed]
2. Casey R, Piazzon-Fevre K, Raverdy N, Forzy ML, Tretare B, Carli PM, et al. Case-control study of lymphoid neoplasm in three French areas: description, alcohol and tobacco consumption. Eur J Cancer Prev. 2007; 16(2): 142-50. [CrossRef][PubMed]
3. Bende R, Smit L, van Noesel C. Molecular pathways in follicular lymphoma. Leukemia. 2007; 2(1): 18-29. [CrossRef][PubMed]
4. Ekstrom Smedby K, Vajdic C, Falster M, Engels EA, Martínez-Maza O, Turner J, et al. Autoimmune disorders and risk of non-Hodgkin lymphoma subtypes: a pooled analysis within the InterLymph Consortium. Blood. 2008; 111(8): 4029-38. [CrossRef][PubMed]
5. Roberts D, Dive C, Renehan A. Biological Mechanisms Linking Obesity and Cancer Risk: New Perspectives. Ann Rev Med. 2010; 61: 301-16. [CrossRef][PubMed]
6. McTiernan A. Mechanisms linking physical activity with cancer. Nat Rev Cancer. 2008; 8(3): 205-11. [CrossRef][PubMed]
7. Hossain P, Kawar B, El Nahas M. Obesity and Diabetes in the Developing World - A Growing Challenge. N Engl J Med 2007; 356: 213-215. [CrossRef][PubMed]
8. Ambinder A, Shenoy P, Malik N, Maggioncalda A, Nastoupil LJ, Flowers CR. Exploring Risk Factors for Follicular Lymphoma. Adv Hematol. 2012; 2012: 626035. [CrossRef][PubMed]
9. Fenton J, Hursting S, Perkins S, Hord N. Interleukin-6 production induced by leptin treatment promotes cell proliferation in an Apc (Min/+) colon epithelial cell line. Carcinogenesis. 2006; 27(7): 1507-15. [CrossRef][PubMed]
10. Rapp K, Schroeder J, Klenk J, Stoehr S, Ulmer H, Concin H, et al. Obesity and incidence of cancer: a large cohort study of over 145 000 adults in Austria. Br J Cancer. 2005; 93(9): 1062-7. [CrossRef][PubMed]
11. Bianchini F, Kaaks R, Vainio H. Overweight, obesity, and cancer risk. Lancet Oncol. 2002; 3(9): 565-74. [CrossRef][PubMed]
12. Lim U, Morton L, Subar A, Baris D, Stolzenberg-Solomon R, Leitzmann M, et al. Alcohol, Smoking, and Body Size in Relation to Incident Hodgkin's and Non-Hodgkin's Lymphoma Risk. Am J Epidemiol. 2007; 166(6): 697-708. [CrossRef][PubMed]
13. Lu Y, Prescott J, Sullivan-Halley J, Henderson KD, Ma H, Chang ET, et al. Body Size, Recreational Physical Activity, and B-Cell Non-Hodgkin Lymphoma Risk Among Women in the California Teachers Study. Am J Epidemiol. 2009; 170(10): 1231-40. [CrossRef][PubMed]
14. Skibola CF, Holly EA, Forrest MS, Hubbard A, Bracci PM, Skibola DR, et al. Body mass index, leptin and leptin receptor polymorphisms, and non-hodgkin lymphoma. Cancer Epidemiol Biomarkers Prev. 2004; 13 (5): 779-86. [PubMed]
15. Pan S, Mao Y, Ugnat A; Canadian Cancer Registries Epidemiology Research Group. Physical Activity, Obesity, Energy Intake, and the Risk of Non-Hodgkin's Lymphoma: A Population-based Case-Control Study. Am J Epidemiol. 2005; 162(12): 1162-73. [CrossRef][PubMed]
16. Chang E, Hjalgrim H, Smedby K, Akerman M, Tani E, Johnsen HE, et al. Body Mass Index and Risk of Malignant Lymphoma in Scandinavian Men and Women. J Natl Cancer Inst. 2005; 97(3): 210-8. [CrossRef][PubMed]
17. Cerhan J, Bernstein L, Severson R, Davis S, Colt JS, Blair A, et al. Anthropometrics, Physical Activity, Related Medical Conditions, and the Risk of Non-Hodgkin Lymphoma. Cancer Causes Control. 2005; 16(10): 1203-14. [CrossRef][PubMed]
18. Jochem C, Leitzmann M, Keimling M, Schmid D, Behrens G. Physical Activity in Relation to Risk of Hematologic Cancers: A Systematic Review and Meta-analysis. Cancer Epidemiol Biomarkers Prev. 2014; 23 (5): 833-46. [CrossRef][PubMed]
19. Willett E, Skibola C, Adamson P, Skibola DR, Morgan GJ, Smith MT, et al. Non-Hodgkin's lymphoma, obesity and energy homeostasis polymorphisms. Br J Cancer. 2005; 93(7): 811-6. [CrossRef][PubMed]
20. Chiu B, Soni L, Gapstur S, Fought AJ, Evens AM, Weisenburger DD. Obesity and risk of non-Hodgkin lymphoma (United States). Cancer Causes Control. 2007; 18(6): 677-85. [CrossRef][PubMed]
21. Britton J, Khan A, Rohrmann S, Becker N, Linseisen J, Nieters A, et al. Anthropometric characteristics and non-Hodgkin's lymphoma and multiple myeloma risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). Haematologica. 2008; 93(11): 1666-77. [CrossRef][PubMed]
22. Maskarinec G, Erber E, Gill J, Cozen W,Kolonel LN. Overweight and Obesity at Different Times in Life as Risk Factors for Non-Hodgkin's Lymphoma: The Multiethnic Cohort. Cancer Epidemiol Biomarkers Prev. 2008; 17(1): 196-203. [CrossRef][PubMed]
23. Troy J, Hartge P, Weissfeld J, Oken MM, Colditz GA, Mechanic LE, et al. Associations between anthropometry, cigarette smoking, alcohol consumption, and non-Hodgkin lymphoma in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening trial. Am J Epidemiol. 2010; 171(12): 1270-81. [CrossRef][PubMed]
24. Kanda J, Matsuo K, Inoue M, Iwasaki M,Sawada N, Shimazu T, et al. Association of Anthropometric Characteristics with the Risk of Malignant Lymphoma and Plasma Cell Myeloma in a Japanese Population: A Population-Based Cohort Study. Cancer Epidemiol Biomarkers Prev. 2010; 19(6): 1623-31. [CrossRef][PubMed]
25. Kabat G, Kim M, Jean-Wactawski-Wende, Bea JW, Edlefsen KL, Adams-Campbell LL, et al. Anthropometric factors, physical activity, and risk of Non-Hodgkin's lymphoma in the Women's Health Initiative. Cancer Epidemiol. 2012; 36(1): 52-9. [CrossRef][PubMed]
26. Murphy F, Kroll M, Pirie K, Reeves G, Green J, Beral V. Body size in relation to incidence of subtypes of haematological malignancy in the prospective Million Women Study. Br J Cancer. 2013; 108(11): 2390-8. [CrossRef][PubMed]
27. Cerhan J, Janney C, Vachon C, Habermann TM, Kay NE, Potter JD, et al. Anthropometric characteristics, physical activity, and risk of non-Hodgkin's lymphoma subtypes and B-cell chronic lymphocytic leukemia: a prospective study. Am J Epidemiol. 2002; 156(6): 527-35. [CrossRef][PubMed]

28. van Veldhoven C, Khan A, Teucher B, Rohrmann S, Raaschou-Nielsen O, Tjønneland A, et al. Physical activity and lymphoid neoplasms in the European Prospective Investigation into Cancer and nutrition (EPIC). Eur J Cancer. 2011; 47(5): 748-60. [CrossRef][PubMed]
29. Kelly J, Fredericksen Z, Liebow M, Shanafelt TD, Thompson CA, Call TG, et al. The association between early life and adult body mass index and physical activity with risk of non-Hodgkin lymphoma: impact of gender. Ann Epidemiol. 2012; 22(12): 855-62. [CrossRef][PubMed]
30. Leo Q, Ollberding N, Wilkens L, Kolonel LN, Henderson BE, Le Marchand L, et al. Obesity and non-Hodgkin lymphoma survival in an ethnically diverse population: the Multiethnic Cohort study. Cancer Causes Control. 2014; 25(11): 1449-59. [CrossRef][PubMed]
31. Hong F, Habermann T, Gordon L, Hochster H, Gascoyne RD, Morrison VA, et al. The role of body mass index in survival outcome for lymphoma patients: US intergroup experience. Ann Oncol. 2014; 25(3): 669-74. [CrossRef][PubMed]
32. World Health Organisation: BMI classification. Avalaible at: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html. Accessed: March 4, 2017.
33. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986; 7(3): 177-88. [CrossRef][PubMed]
34. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002; 21(11): 1539-58. [CrossRef][PubMed]
35. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997(7109); 315: 629-34. [CrossRef][PubMed]
36. Larsson, S, Wolk A. Body mass index and risk of non-Hodgkin's and Hodgkin's lymphoma: a meta-analysis of prospective studies. Eur J Cancer. 2011; 47(16): 2422-30. [CrossRef][PubMed]
37. Larsson S, Wolk A. Obesity and risk of non-Hodgkin's lymphoma: A meta-analysis. Int J Cancer. 2007; 121(7): 1564-70. [CrossRef][PubMed]
38. Tchkonia T, Morbeck D, Von Zglinicki T, Van Deursen J, Lustgarten J, Scrable H, et al. Fat tissue, aging, and cellular senescence. Aging Cell. 2010; 9(5): 667-84. [CrossRef][PubMed]
39. Blaak E. Gender differences in fat metabolism. Curr Opin Clin Nutr Metab Care. 2001; 4(6): 499-502. [CrossRef][PubMed]
40. Carey D, Cowin G, Galloway G, Jones NP, Richards JC, Biswas N, et al. Effect of Rosiglitazone on Insulin Sensitivity and Body Composition in Type 2 Diabetic Patients. Obes Res. 2002; 10(10): 1008-15. [CrossRef][PubMed]
41. Castillo JJ, Ingham RR, Reagan JL, Furman M, Dalia S, Mitri J. Obesity is associated with increased relative risk of diffuse large B-cell lymphoma: a meta-analysis of observational studies. Clin Lymphoma Myeloma Leuk. 2014; 14(2): 122-30. [CrossRef][PubMed]
42. World Health Organization. Obesity: preventing and managing the global epidemic. World Health Organization, 2000.
43. Vermaete N, Wolter P, Verhoef G, Kollen BJ, Kwakkel G, Schepers L, et al. Physical Activity and Risk of Lymphoma: A Meta-Analysis. Cancer Epidem Biomar Prev. 2013; 22(7): 1173-84. [CrossRef][PubMed]
44. Renehan A, Roberts D, Dive C. Obesity and cancer: Pathophysiological and biological mechanisms. Arch Physiol Biochem. 2008; 114(1): 71-83. [CrossRef][PubMed]
45. Renehan A. Epidemiology of overweight/obesity and cancer risk. In: McTiernan A, ed.: Physical Activity, Dietary Calorie Restriction, and Cancer. Springer, New York, 2011, pp 5-23. [CrossRef]

Originalni rad **UDC: 616-006.44:613.8**
doi:10.5633/amm.2018.0411

INCIDENCIJA I MORTALITET FOLIKULARNOG LIMFOMA U ODNOSU NA PREKOMERNU TEŽINU, GOJAZNOST I FIZIČKU AKTIVNOST: METAANALIZA

Ilija Golubović[1,2], Goran Marjanović[1,6], Danijela Radojković[3,6], Dušan Sokolović[4], Aleksandar Karanikolić[2,6], Milan Radojković[2,6], Milorad Pavlović[5]

[1]Klinika za hematologiju i imunologiju, Klinički centar Niš, Niš, Srbija
[2]Klinika za opštu hirurgiju, Klinički centar Niš, Niš, Srbija
[3]Univeritet u Nišu, Medicinski fakultet, Institut za biohemiju, Niš, Srbija
[4]Klinika za endokrinologiju, Klinički centar Niš, Srbija
[5]Klinika za grudnu hirurgiju, Klinički centar Niš, Srbija
[6]Univeritet u Nišu, Medicinski fakultet, Niš, Srbija

Kontakt: Ilija Golubović
Klinički centar Niš
Bulevar dr Zorana Đinđića 48, 18000 Niš, Srbija
E-mail: golubovicilija@yahoo.com

U poslednjih nekoliko godina postoji veće ineteresovanje za istraživanje povezanosti između faktora rizika kao što su prekomerna težina, gojaznost i fizička aktivnost i incidencije brojnih karcinoma.

Ova metaanaliza je sprovedena da bi se istražio rizik za incidenciju i mortalitet folikularnog limfoma kod osoba sa prekomernom težinom i gojaznih, kao i kod osoba sa različitim stepenom fizičke aktivnosti, korišćenjem modela slučajnog efekta (random-effects model). Pretraživanje literature bilo je sprovedeno tokom septembra 2016. godine. Preko 2.100 slučajeva i 12.700 kontrola bili su obuhvaćeni studijama po tipu slučaj-kontrola, dok su kohortne studije obuhvatale preko 2.600 slučajeva u kohorti od oko 3.000.000 subjekata.

Kod osoba sa prekomernom težinom (body mass index-BMI između 25 i 29,99 kg/m^2) rizik za pojavu folikularnog limfoma bio je 1,03 (0,95-1,11; 95% CI; p = 0,51), a kod gojaznih osoba (BMI ≥ 30 kg/m^2) 1,15 (1,01-1,31; 95% CI; p = 0,04), u poređenju sa osobama sa normalnim BMI (< 25 kg/m^2). Rizik za specifični mortalitet folikularnog limfoma kod osoba sa prekomernom težinom bio je 0,59 (0,38-0,91; 95% CI; p = 0,02), dok je kod gojaznih bio 1,08 (0,68-1,71; 95% CI; p = 0,75). Među bolesnicima sa visokim stepenom fizičke aktivnosti, rizik za pojavu folikularnog limfoma bio je 0,95 (0,75-1,21; 95% CI; p = 0,68) u poređenju sa bolesnicima koji sui mali nizak stepen fizičke aktivnosti.

Naša metaanaliza je pokazala direktnu ststistički značajnu povezanost između gojaznosti i incidencije folikularnog limfoma.

Acta Medica Medianae 2018;57(4):79-90.

Ključne reči: *folikularni limfom, metaanaliza, gojaznost, prekomerna težina, fizička aktivnost*

Review article **UDC: 618.11-006.6:616.381-003.217-08**
doi:10.5633/amm.2018.0412

TREATMENT MODALITIES FOR THE MANAGEMENT OF ASCITES IN OVARIAN CANCER PATIENTS

Radomir Živadinović[1,2], Dane Krtinić[3,4], Biljana Živadinović[5,6], Aleksandra Petrić[1,2], Aleksandar Živadinović[7], Sonja Pop Trajković-Dinić[1,2], Milan Trenkić[1,2]

Ascites involve the presence of a higher amount of free fluid accumulated in the abdominal cavity. Pathophysiology of malignant ascites is multifactorial and represents a combination of two basic pathogenetic mechanisms, increased vascular permeability and obstruction of lymphatic drainage. Ascites is the most common symptom of patients with ovarian cancer reporting to a doctor. The primary therapeutic option in the treatment of ovarian cancer is cytoreductive surgery and platinum therapy. Intraperitoneal chemotherapy aims to increase the concentration of the drug at the target site by avoiding a resorptive toxic effect. Of the surgical methods used in palliative treatment of ascites, the creation of peritoneal shunts should be mentioned. A modern innovative approach in the treatment of ascites involves the use of specific monoclonal antibodies that focus on one of the basic etiological factors of ascites – neoangiogenesis. In treatment, a multidisciplinary approach is needed not only for gynecologists but also for anaesthesiologists, gastroenterologists, surgeons, palliative doctors, and a medical oncologist.

Acta Medica Medianae 2018;57(4):91-95.

Key words: *ascites, ovarian cancer, chemotherapy, monoclonal antibodies*

[1]University of Niš, Faculty of Medicine, Department of Obstetrics and Gynecology, Niš, Serbia
[2]Obstetrics and Gynecology Clinic, Clinical Center Niš, Niš, Serbia
[3]University of Niš, Faculty of Medicine, Department of Pharmacology and Toxicology, Niš, Serbia
[4]Oncology Clinic, Clinical Center Niš, Niš, Serbia
[5]University of Niš, Faculty of Medicine, Department of Neurology, Niš, Serbia
[6]Neurology Clinic, Clinical Center Niš, Niš, Serbia
[7]University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Dane Krtinić
3 Svetozara Markovića Street, 18000 Niš, Serbia
E-mail: kdane86@gmail.com,
dane.krtinic@medfak.ni.ac.rs

Epidemiology and pathophysiology of malignant ascites

The term 'ascites' is defined as the presence of large volumes of fluid accumulated in the abdominal cavity. Under normal conditions, several liters of peritoneal fluid are produced daily and it is not accumulated, but effectively absorbed. This fluid continuously circulates in a clockwise direction helping in the lubrication of intestines for their normal movement. Ascites may be of malignant and nonmalignant etiology. Malignant ascites occurs less frequently and accounts for about 10% of all cases of ascites (1).

The pathophysiology of malignant ascites is multifactorial and is related to a combination of two basic pathogenic mechanism, increased vascular permeability and obstructed lymphatic drainage.

Vascular endothelial growth factor VEGF is the most important factor that stimulates increased vascular permeability and the formation of new blood vessels, neoangiogenesis, but other cytokines, such as basic fibroblast growth factor (bFGF), angiogenin, transforming growth factors (TGF α and β) and interleukin - 8 play important roles as well. Along with an increase of peritoneal blood vessels in size and number, neoangiogenesis results not only in increased permeability, but also in increased overall surface area for filtration.

The next pathogenic mechanism of malignant ascites is increased hydrostatic pressure difference as a result of minor elevation of portal venous pressure in patients with ovarian cancer (portal veins compression by tumour mass and metastases). On the other hand, the oncotic pressure difference is reduced since the albumins that are responsible for osmotic intravascular pressure (allows fluid to leak out from the interstitial space) exit blood vessels or degrade into smaller peptides or amino acids (2).

Ascites and ovarian cancer prognosis

Ascites is the most common symptom that prompts cancer patients to visit the doctor's office. In 54 % of patients with peritoneal carcinomatosis, ascites was the first detectable sign of malignancy (3).

Unfortunately, the presence of ascites most commonly reveals an advanced stage of the disease, since ascites are produced in only 7% of cases in stages I and II of the disease, and in 89% of cases in stages III and IV. The amount of ascitic fluid is in correlation with the stage of the disease, for stages I and II its presence is < 0.5 liters, but in more than 66% of cases with stage III and IV its presence is > 0.5 liters.

More than 2/3 of patients report to their doctors in stages III and IV when ascites is indirectly noticed by increased abdominal size and abdominal distension, dyspnea, weight gain, lower extremity edema, nausea and vomiting, the phenomenon of fluid wave and shifting dullness. Survival rate in advanced stages of the disease (III and IV) is 5-20% (4).

Malignant ascites is not only a sign or a symptom associated with malignant disease. The presence of ascitic fluid in ovarian cancer has an important, almost key role in further progression of malignant disease. The spread of ovarian cancer and the development of abdominal and peritoneal metastases, as well as peritoneal carcinomatosis, depend on ascitic fluid.

Specific cellular and acellular components of ascites form a tumour-friendly environment that may promote the spread and growth of tumour cells, but they may also inhibit the positive response to chemotherapy in tumour cells, thus directly stimulating tumour chemoresistance (5, 6).

Chemoresistance and poor response to chemotherapy, often caused by the presence of ascitic fluid, directly correlates with the survival rate and the recurrence of the disease. In chemoresistant tumours, a five-year survival rate is less than 27% (7). In this way ascites may indirectly affect a malignant disease prognosis, not only by forming a specific microenvironment for stimulating tumour growth, but also by developing chemoresistance.

Therapeutic approach in ovarian carcinoma patients

Primary treatment option in treating ovarian cancer is cytoreductive surgery and platinum-based therapy with an expected positive treatment response rate of 70 %. However, in 12-18 months many of these women will have ascites and recurrence of the disease refractory to standard platinum treatment.

Successful management of ascites is limited by the fact that the complete pathogenic mechanism is still poorly understood, and on the other hand the advanced stage of the disease limits the successful management of the disease and quality of life.

Standard therapy of ascites mainly includes palliative repeated paracentesis in more than 98% of cases. Paracentesis is performed by inserting a 14 - gauge needle with a 16 - gauge catheter. This method is effective in rapid relief of distressing symptoms, primarily including dyspnea, orthopnea, pain and peritoneal reaction in 78% of cases (8).

However, this method has its limitations, since the risk of paracentesis rises with more than 5L of ascitic fluid removal that may affect plasma volume and renal function. For these reasons, 5% dextrose infused simultaneously with paracentesis has been widely recommended. Other possible risks and complications of this method also include hypoproteinemia, hypotension, secondary peritonitis, perforation, and pulmonary embolism (9).

In order to prevent possible complications and homeostatic imbalance it is necessary to perform blood tests control, focusing on protein and electrolyte levels, and the catheter needle should not be left in situ for longer than 1 day. In order to reduce the risk of infection, antibiotic therapy is sometimes used during the first week of treatment after paracentesis is performed (10).

Diuretic therapy in the management of the ascites is rarely performed (61% of all ascites) and is less effective than paracentesis (45%) (11). Unlike benign ascites (liver cirrhosis and congestive heart failure), malignant ascites respond poorly to the therapy including fluid and salt restrictions and diuretics that may cause complications such as a decrease in volume, electrolytic imbalance, and renal dysfunction.

It is reported that good control of ascites is achieved with spironolactone at a dose of 150 - 400 mg in patients who showed sodium retention and elevated plasma renin, without serious problems of electrolyte imbalance (12). Patients with malignant ascites and hepatic metastases benefit most from diuretic therapy. Reduction of the blood volume causes the renin-angiotensin - aldosterone system activation, leading to salt retention. Spironolactone is an antagonist to aldosterone, thus the reabsorption of water and salt is decreased.

Pockros proved in his paper that renin levels were elevated in patients with hepatic metastases, while normal renin levels were confirmed in carcinomatosis without hepatic metastases (13). Patients without hepatic metastases and with diuretic use had 1kg/d in weight loss without hypotension, and those without metastases and in carcinomatosis group had 0.5 kg/d in weight loss with hypotension and renal dysfunction.

Apart from aforementioned Spironolactone used at a dose of 100 - 200 mg daily, Furosemide at initial dose of 40 - 80 mg daily is also used in the management of ascites (14). Due to already mentioned numerous harmful effects, the usage of these drugs is allowed, but for a limited period of time only. Contraindications are hyponatremia < 125 mmol/l, hepato-renal related decrease of sodium excretion to < 30 mmol/day, renal insufficiency with serum creatinine > 1.5 mg/dl, acute encephalopathy and acute bacterial infection (15). The use of diuretics also increases the risk of thromboembolic complications due to chemotherapy drug concentrations, and pos-

sible additional symptoms include gynecomastia, renal tubular acidosis, and hyperkalemia.

Another palliative treatment of ascites in ovarian cancer is the application of chemotherapeutics into the peritoneal cavity. This treatment aims at delivering higher concentrations of drugs to the target site, while avoiding resorption toxic effects. The most common cytostatic drugs used for the intraperitoneal treatment are cisplatin and paclitaxel. Complications of this method include infections and pain. Limiting factors are short-term effects and a maximum of 5 mm penetration into a tumour deposit with limiting effects to existing adhesions. Other side-effects include ileus, peritonitis, abscess, and necrosis.

The attempts to potentiate the cytotoxicity of cisplatin and paclitaxel in intraperitoneal application have resulted in utilization of hyperthermic medium (40.5 - 43°C). This procedure is called hyperthermic intraperitoneal chemotherapy (HIPEC). The results of HIPEC treatment regarding overall survival rate are better in comparison to reduction of ascitic fluid, but without statistically significant difference (12).

Hyperthermia (over 39 degrees) increases local cytotoxic effects by inhibiting replication and repair. The best results are achieved directly after the surgery (complete cytoreduction) since fibrin depositions and adhesion formations are thin at that time. Combined-modality treatment of surgical procedure and intraperitoneal chemotherapy using cisplatin, bleomycin, and mitomycin C prevents recurrence of ascites in 75% of patients (14).

Besides intraperitoneal application of cytostatics, other drugs can be used intraperitoneally, such as intraperitoneal tumor necrosis factor (TNF), interferon, and other immunomodulators (15).

TNF is used at a dose of 0.08 - 0.014 mg/m^2 diluted in 5% human albumin, applied into the abdomen for 24 - 48 hours, and the procedure is repeated on the 8th day (16).

Improvements regarding reduction of ascitic fluid can be seen after three doses, but improvements in mucinous ovarian cancer have not been reported (17).

Intraperitonel interferon α (IFN) 2b application was described in the studies by Sartori et al. (18). Complete response was achieved in 29.3%, a partial response in 36.6% and no response in 34.1% of patients.

One of the surgical methods used in palliative treatment of ascites is peritoneovenous shunting. The first data on peritoneovenous shunts date back to 1974. A modified Denver shunt was developed later. The benefits of this method in comparison to paracentesis include reduced need for repeated paracentesis and maintenance of normal serum albumin concentrations. In malignant ascites, reduction and control of ascites by application of this method was achieved in 75% of shunts (19). Patients selected for shunt placement should undergo cardiac and respiratory evaluations.

By this method, surgical peritoneovenous shunt is formed, connecting the peritoneum to the vena cava. At a specific pressure, a valve opens and leads the fluid into the vein. There are three different forms of shunts named after their authors: the Hyde, Denver and LeVeen shunts.

Faught et al. evaluated some possible complications of this method, such as fever, coagulopathy, infection and tumor embolization (20). Contraindications are portal hypertension, coagulation disorders, elevated bilirubin levels, cardiac or renal failure, hemorrhagic ascites or fluid protein > 4.5 g/l. Increased probability of disseminating malignant cells by this treatment modality has not been proved in this study. What is important is that the application of this shunt showed better clinical results for ascites in ovarian cancer patients than in gastrointestinal cancer patients, in relation 50 : 15 % respectively. However, the application of shunts is indicated only for patients in whom other treatments have failed and who can derive benefits if their life expectancy is long enough.

Finally, among other surgical therapeutic procedures, radical peritonectomy is worth mentioning. It is an extensive surgical intervention involving complete removal of the peritoneum, combined with intraperitoneal chemotherapy.

A modern, innovative approach in treating malignant ascites includes administration of monoclonal antibody-based therapy, directed at one of the basic etiological factors of ascites – neoangiogenesis. In that respect, the drugs used, such as anti-vascular endothelial vascular factor (VEGF), may have potential tumour-suppressive effects.

Bevacizumab (Avastin®; Genentech, Inc., South San Francisco, CA) is a recombinant humanized monoclonal antibody to VEGF composed of human IgG_1 framework regions and antigen-binding complementarity-determining regions from a murine antibody that blocks the binding of human VEGF to its receptors (21).

Bevacizumab is a humanized monoclonal antibody directed against VEGF-A as target therapy (21). After its initial approval by the Food and Drug Administration (FDA) in 2004 for unresectable colorectal cancer, its indication for the treatment of different cancers has been investigated (22, 23). Some investigations report benefits of this therapy combined with platinum therapy in patients with ovarian cancer. The AURELIA trial studied bevacizumab in combination with non-platinum chemotherapy and proved its success in platinum-resistant ovarian cancer (24). Later bevacizumab was approved for use only in recurrent, platinum-resistant ovarian cancer, and today it is approved for platinum-sensitive recurrent ovarian cancer (25).

Therapeutic application of Bevacizumab has also demonstrated significant benefits in patients with recurrent disease and accompanying ascites. Most common side effects are neutropenia and thrombocytopenia, rarely gastrointestinal bleeding, thromboembolic events, hypertension and proteinuria.

The studies analyzing quality of life and the recurrence of the disease in patients with ascites treated with repeated paracentesis and monoclonal anti-vascular drugs have shown that palliative treatment of malignant ascites using paracentesis or combined paracentesis and intraperitoneal chemo-

therapy negatively impacts patients' health-related quality of life (HRQL) and shortens the disease-free interval. Monoclonal antibody treatment results in better quality of life and in a longer disease-free interval. The median puncture free survival with catumaxomab was 46 days compared with 11 days in the paracentesis group (26).

Conclusion

Management of patients with ascites and ovarian carcinoma is complex, with additional recurrences, and it is often directed to palliative procedures that necessitates hospital environment.

The treatment requires a multidisciplinary approach and includes not only a gyneacologist, but also an anesthesiologist, gastroenterologist, surgeon, palliative care doctor, as well as medical oncologist.

In order to improve overall quality of life and survival of these patients, further investigations of new drugs, monoclonal antibodies, and immunomodulators are needed aiming at prolonged periods between relapses.

References

1. Runyon BA. Care of patients with ascites. N Eng J Med. 1994; 330(5): 337-42. [CrossRef][PubMed]
2. Stanojević Z, Rančić G, Radić S, Potić-Zećević N, Djordjević B, Marković M et al. Pathogenesis of malignant ascites in ovarian cancer patients. Archives of Oncology. 2004; 12(2): 115-18. [CrossRef]
3. Garrison RN, Kaelin LD, Galloway RH, Heuser LS. Malignant ascites. Clinical and experimental observations. Ann Surg. 1986; 203(6): 644-51. [CrossRef][PubMed]
4. Shen-Gunther J, Mannel RS. Ascites as a predictor of ovarian malignancy. Gynecol Oncol. 2002; 87(1): 77-83. [CrossRef][PubMed]
5. Wels J, Kaplan RN, Rafii S, Lyden D. Migratory neighbors and distant invaders: Tumor associated niche cells. Genes Dev. 2008; 22(5): 559-74. [CrossRef][PubMed]
6. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. Nature. 2004; 432(7015): 332-7. [CrossRef][PubMed]
7. Kipps E, Tan D, Kaye S. Meeting the challenge of ascites in ovarian cancer: New avenues for therapy and research. Nat Rev Cancer. 2013; 13: 273-82. [CrossRef][PubMed]
8. Fischer DS. Abdominal paracentesis for malignant ascites. Arch Inter Med. 1979; 139(2): 235. [CrossRef][PubMed]
9. Stukan M. Drainage of malignant ascites: Patient selection and perspectives. Cancer Management and Research. 2017; 9: 115-30. [CrossRef]
10. Kim S, Kim B, Song YS. Ascites modulates cancer cell behavior, contributing to tumor heterogeneity in ovarian cancer. Cancer Sci. 2016; 107(9): 1173-8. [CrossRef][PubMed]
11. Gamblin V, Da Silva A, Villet S, El Hajbi F. Supportive care for malignant ascites in palliative phase: Place of paracentesis and diuretics. Bull Cancer. 2015; 102 (11): 940- 5. [CrossRef][PubMed]
12. Chung M, Kozuch P. Treatment of malignant ascites. Curr Treat Options Oncol. 2008; 9(2-3): 215-33. [CrossRef][PubMed]
13. Pockros PJ, Esrason KT, Nguyen C, Duque J, Woods S. Mobilization of malignant ascites with diuretics is dependent on ascitic fluid characteristics. Gastroenterology. 1992; 103(4): 1302-6. [CrossRef][PubMed]
14. Sharma S, Walsh D. Management of symptomatic malignant ascites with diuretics: Two case reports and a review of the literature. J Pain Symptom Manage. 1995; 10(3): 237-42. [CrossRef][PubMed]
15. Hulin-Curtis SL, Uusi-Kerttula H, Jones R, Hanna L, Chester JD, Parker AL. Evaluation of CD46 re-targeted adenoviral vectors for clinical ovarian cancer intraperitoneal therapy. Cancer Gene Ther. 2016; 23(7): 229-34. [CrossRef][PubMed]
16. Kaufmann M, Schmid H, Raeth U, Grischke EM, Kempeni J, Schlick E et al. Therapy of ascites with tumor necrosis factor in ovarian cancer. Geburtshilfe Frauenheilkd. 1990; 50(9): 678-82. [CrossRef][PubMed]
17. Loggie BW, Perini M, Fleming RA, Russell GB, Geisinger K. Treatment and prevention of malignant ascites associated with disseminated intraperitoneal malignancies by aggressive combined-modality therapy. Am Surg. 1997; 63: 137-43. [PubMed]
18. Sartori S, Nielsen I, Tassinari D, Trevisani L, Abbasciano V, Malacarne P. Evaluation of a standardized protocol of intracavitary recombinant interferon α-2b in the palliative treatment of malignant peritoneal effusions. A prospective pilot study. Oncology. 2001; 61(3): 192-6. [CrossRef][PubMed]
19. Smith EM, Jayson GC. The current and future management of malignant ascites. Clin Oncol (R Coll Radiol). 2003; 15(2): 59-72. [PubMed]
20. Faught W, Kirkpatrick JR, Krepart GV, Heywood MS, Lotocki RJ. Peritoneovenous shunt for palliation of gynecologic malignant ascites. J Am Coll Surg. 1995; 180(4): 472-4. [PubMed]
21. Kobold S, Hegewisch-Becker S, Oechsle K, Jordan K, Bokemeyer C, Atanackovic D. Intraperitoneal VEGF inhibition using Bevacizumab: A potential approach for the symptomatic treatment of malignant ascites? Oncologist. 2009; 14(12): 1242-51. [CrossRef][PubMed]
22. Ferrara N, Hillan KJ, Novotny W. Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy. Biochem Biophys Res Commun. 2005; 333(2): 328-35. [CrossRef][PubMed]
23. NC Institute. FDA Approval for Bevacizumab. 2016.
24. Pujade-Lauraine E, Hilpert F, Weber B, Reuss A, Poveda A, Kristensen G et al. Bevacizumab combined

with chemotherapy for platinum-resistant recurrent ovarian cancer: The AURELIA open-label randomized phase III trial. J Clin Oncol. 2014; 32: 1302-8. [CrossRef][PubMed]
25. Jayson GC, Kerbel R, Ellis LM, Harris AL. Antiangiogenic therapy in oncology: Current status and future directions. Lancet. 2016; 388: 518-29. [CrossRef][PubMed]
26. Wimberger P, Gilet H, Gonschior AK, Heiss MM, Moehler M, Oskay-Oezcelik G et al. Deterioration in quality of life (QoL) in patients with malignant ascites: results from a phase II/III study comparing paracentesis plus catumaxomab with paracentesis alone. Ann Oncol. 2012; 23(8): 1979-85. [CrossRef][PubMed]

Revijalni rad

UDC: 618.11-006.6:616.381-003.217-08
doi: 10.5633/amm.2018.0412

TERAPIJSKI MODALITETI U LEČENJU ASCITA KOD KARCINOMA JAJNIKA

Radomir Živadinović[1,2], Dane Krtinić[3,4], Biljana Živadinović[5,6], Aleksandra Petrić[1,2], Aleksandar Živadinović[7], Sonja Pop Trajković-Dinić[1,2], Milan Trenkić[1,2]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za ginekologiju i akušerstvo, Niš, Srbija
[2]Klinika za ginekologiju i akušerstvo, Klinički centar Niš, Niš, Srbija
[3]Univerzitet u Nišu, Medicinski fakultet, Katedra za farmakologiju sa toksikologijom, Niš, Srbija
[4]Klinika za onkologiju, Klinički centar Niš, Niš, Srbija
[5]Univerzitet u Nišu, Medicinski fakultet, Katedra za neurologiju, Niš, Srbija
[6]Klinika za neurologiju, Klinički centar Niš, Niš, Srbija
[7]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

Kontakt: Dane Krtinić
Ul. Svetozara Markovića br.3, 18000 Niš, Srbija
E-mail: kdane86@gmail.com,
dane.krtinic@medfak.ni.ac.rs

Ascites podrazumeva prisustvo veće količine slobodne tečnosti akumulirane u trbušnoj duplji. Patofiziologija malignog ascita je multifaktorijalna i predstavlja kombinaciju dva osnovna patogenetska mehanizma, povećanu vaskularnu propustljivost i opstrukciju limfatične drenaže. Ascites predstavlja najčešći simptom zbog koga se bolesnice sa karcinomom jajnika javljaju lekaru. Primarna terapijska opcija u lečenju ovarijalnog karcinoma je citoreduktivna hirurgija i terapija platinum. Intraperitonelna hemioterapija ima za cilj da se povećana koncetracija leka nađe na ciljnom mestu, uz izbegavanje resorptivnog toksičnog efekta. Od hirurških metoda koje se koriste u palijativnom tretmanu ascita treba spomenuti stvaranje peritovenskih šantova. Savremeni inovativni pristup u lečenju ascita podrazumeva korišćenje specifičnih monoklonskih antitela koja su usmerena na jedan od osnovnih etioloških faktora ascita – neoangiogenezu. U lečenju je neophodan multidisciplinaran pristup ne samo ginekologa, već i anesteziologa, gastroeneterologa, hirurga, palijativnog doktora, kao i medikalnog onkologa.

Acta Medica Medianae 2018;57(4):91-95.

Ključne reči: *ascites, karcinom jajnika, hemioterapija, monoklonska antitela*

Case report

UDC: 611.714:616-001.5-089
doi:10.5633/amm.2018.0413

OPEN COMMINUTED EXPRESSED-DEPRESSED SKULL FRACTURE

Boban Jelenković[1], Vesna Nikolov[1,2], Slavko Živković[1], Luka Berilažić[1], Predrag Milošević[1]

Skull fractures occur as the result of the effect of kinetic forces and represent discontinuity of the bones of the skull. They can be opened and closed affecting tissues; linear, diastatic, comminuted affecting cranial level; or depressed ones often leading to injuries of meninx, brain tissue with different types of intracranial bleeding. The paper presents a 56-year old male patient who suffered severe craniocerebral injury of the frontal region including orbit while operating the wood processing machine. The injury manifested as scalp damage, expressed-depressed open fracture of frontal-orbital region with cerebrospinal fluid leak. Computerized tomography of the brain showed the presence of epidural, subdural, and intracerebral hematoma with mass effect. The injuries were surgically treated, hematomas evacuated, and skull defect was reconstructed by previous plasticizing the dura in order to stop cerebrospinal fluid leak In the reconstruction of the multifragmentary fracture, a star titanium implant was used, but significant implantation of artificial material was not performed due to already contaminated wound and the possibility of a subsequent infection.

Acta Medica Medianae 2018;57(4):96-100.

Key words: *expressed-depressed fracture, frontal-orbital region, intracranial bleeding, defect reconstruction, cerebrospinal fluid leak*

[1]Neurosurgery Clinic, Clinical Center Niš, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Boban Jelenković
Radoja Dakića 47a/3, 18000 Niš, Serbia
E-mail: bobanjelenko@gmail.com

Introduction

The size of the contact surface of the mechanical force impact on the skull larger or smaller than 5 cm^2 determines the type of fracture and often leads to different intracranial lesions (1, 2). The incidence peak is between 4^{th} and 6^{th} decade of life (3), with global incidence of mortality relating to head injury 91-546 out of 100,000 in the given population (4). Fractures are most frequent in parietal region (38.6%,), in frontal region (28.9%), and in the base (20%) (5). They are classified as fractures of the epicranium including linear, diastatic fractures (characteristic for children), cominuted and limultilinear, as well as depressed skull base fractures. Depending on the intactness of the epicranium integrity fractures can be open and closed.

Open fractures almost always require surgical treatment, as they often lead to different types of intracranial hemorrhage (epidural, subdural, intracerebral) and in order to prevent infections in leaking of liquor (6).

In clinical practice, even if there is no intracranial hemorrhage, surgical treatment is undertaken if the bone fragment is depressed below the tabula interna.

The goal of the surgical treatment as the primary treatment modality is not only to manage the injury but to prevent communication of liquor space with the external environment in order to prevent the possibility of infection. Satisfying the aesthetic principles of the head and bones of the skull is always the ultimate goal in the management of such injuries (7).

Case report

A 56-year old man was admitted to the Emergency Centre of the Clinical Centre in Niš due to serious head injury caused by the rotary blade. The frontal parietal part of the skull was affected as the result of the impact of tangential force of the sharp object on the epicranium.

The patient's state of consciousness was changed, GCS was 10, and vital parameters were stable. There was no pyramidal motor deficiency in the neurological state. During the primary and temporary treatment of the wound with sutures and ligation of injured superficial temporal artery, severe skull injuries manifested as bone fragments were

found. The largest bone fragment was on the right frontal part with the destruction of a larger part of the orbital roof, and was above the level of the rest of the cranium, while the smaller ones were depressed into the cranium covering lacerations of the meninx and parenchyma. Diagnostic procedures included computer tomography (CT) of the brain with bone windows and cross sections of 1-1.5 mm and sagittal reconstruction important in the assessment of the injury.

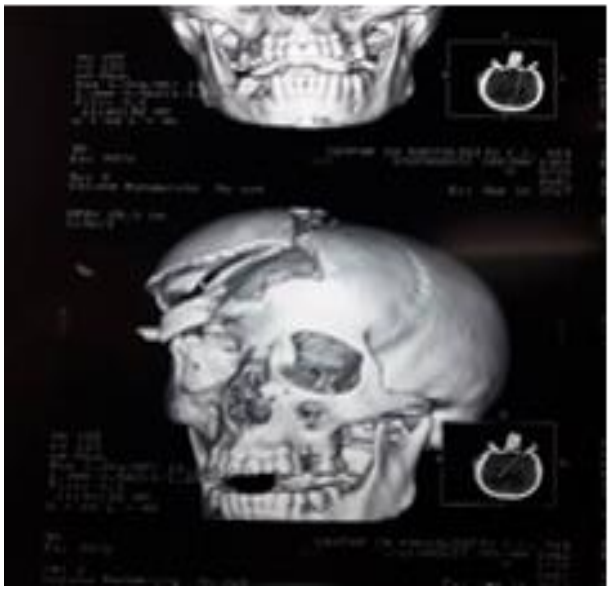


Figure 1. Expressed fracture - CT of the brain with bone window showed bone fragments out of which larger part of the right half of frontal squama descending to orbital roof was significantly above in relation to other depressed bone fragments

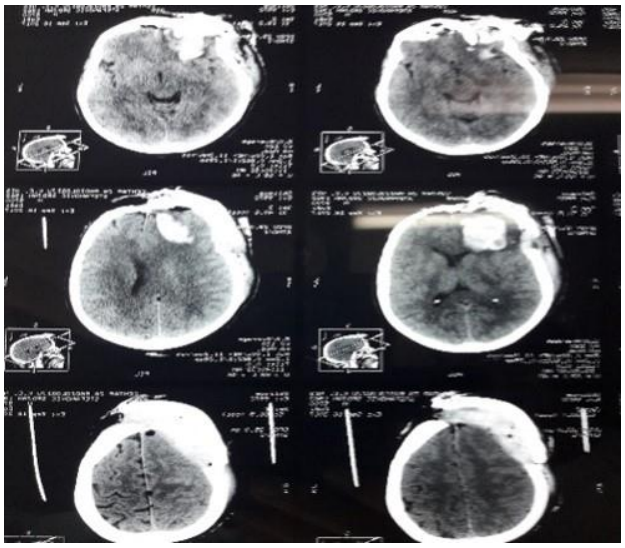


Figure 2. Intracranial hematomas - signs of brain edema as well as of epidural, subdural and intracerebral hematoma

The finding was subsequently confirmed by CT of the brain (Figures 1 and 2), and showed the presence of deeper contusion hemorrhagic zones. After short preparation that included analysis of biohumoral status (Glu 4.5 mmol/L, urea 4.2 mmol/L, Crea 90.1 mmol/L, WBC 7.5/L, RBC 5.48/L, PLT 300.0/L), coagulation factor screening (prothrombin time 70-140%, INR = 1, aPTT 25-35s) and the blood group, surgical treatment was performed.

During the operation, soft epicranial tissues, periosteum in particular, were treated; smaller, contaminated parts of the skull were eliminated, and larger ones were removed and preserved for further reconstruction of the skull. Laceration of the dura mater was expanded for intradural surgery, but only after removal of epidural hematoma. After the wide opening of the dura, we removed subdural hematoma, as well as significant intracerebral hematoma, with careful hemostasis. The defect of the meninx was plasticized, periosteum was strengthened by synthetic derivative of fibrin (Surgicel, absorbable hemostat, ETHICON, GELITA-CEL Standard Medical, hemostat, GELITA MEDICAL GmbH). After that bone fragments were restored and fixed with osteosynthetic material (Flap Fix Cranial Clamp size 5, a product of Johnson & Johnson).

The epicranium was treated with suture material (Polypropilene blue monofilament 3.0 HS 27, Vicryl Plus 3-0, ETHICON).

After postoperative stabilization of the patient, brain CT was performed on the third postoperative day, (Figures 3, 4 and 5), showing a favorable intracranial finding with stable reconstruction of bone elements of the skull.

Further treatment course included administration of II generation group of Cephalosporin and analgetics (Novalgetol every 12 hours for three days

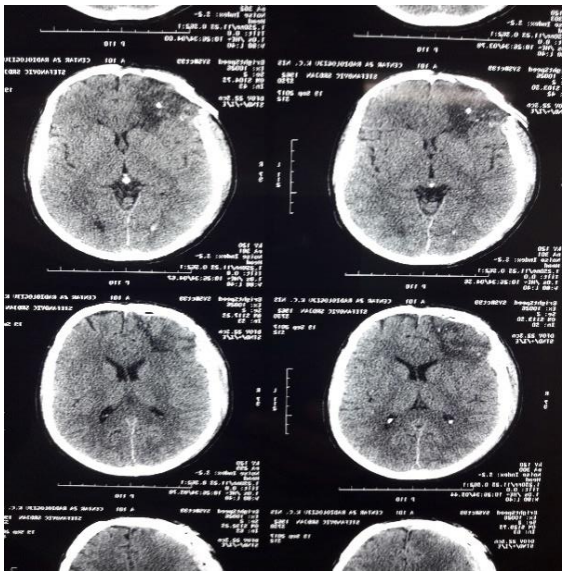


Figure 3. Control CT of the brain

and then as required), and rehydration therapy (Ringer's Solution 1000.0 for 12 hours, sol. Glucose 5% 500.0 every 12 hours, sol. Aminosol 250ml every 12 hours), as well as anti-edematous therapy (sol. Manitol 20%, 125 every 6 hours for three days) with accompanying antiepileptic protection (1 tablet of Phenobarbitone in the evening). The patient was discharged from clinic after ten days of treatment with the wound completely healed. His GCS was 15, and vegetative state stable without neurological deficit.

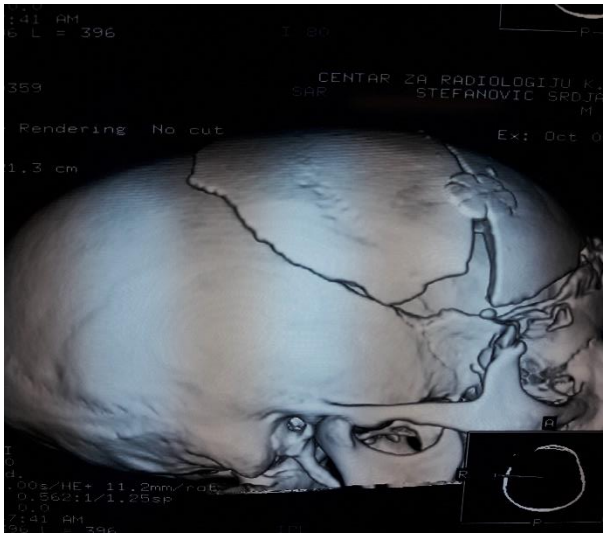


Figure 4. Reconstruction of bone fragments

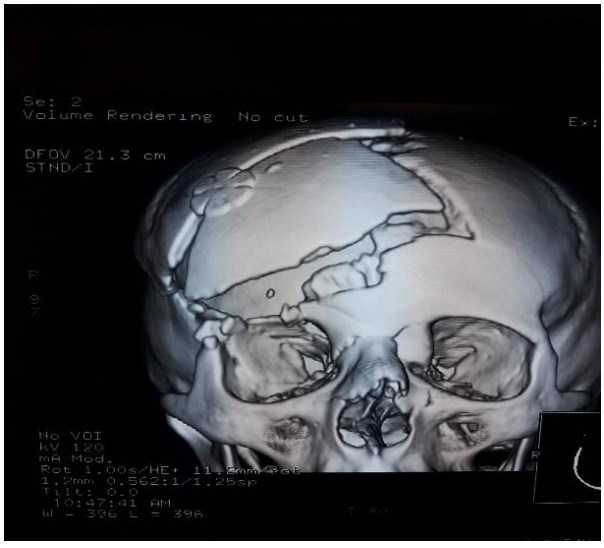


Figure 5. Fixed bone fragment

Discussion

Skull fractures belong to the group of craniocerebral injuries the incidence of which ranges from 1-2 ‰ (4). Epicranium fractures account for 80% of all skull fractures and 5 to 10% are depressed fractures. Half of the depressed fractures are open and may lead to damage of the dura or focal brain damage (6). According to a metaanalysis from literature out of 22,058 traumatized patients, 3.4% of them had fracture, and 0.6% of them had intracranial injury. Of this number, 91% of patients with fracture had no intracranial injury (5). The incidence of depressed fractures is 20 per 1 million, 85% are working men between 4th and 6th decade of life. The mortality rate is relatively low, about 11%, and intracranial hematomas are present between 5 and 7% (7).

Our case report presents a 56-year old working man with head injury. Such craniocerebral injury is a combination of all of the aforementioned types of fractures with the expression of a part of squama of the frontal bone with the destruction of a larger part of the orbital roof, thus increasing the degree of mutilation of the injury with intracranial bleeding. The injury was caused by mechanical force of high kinetic energy at a higher contact area in the frontal region (7). The consequence of the effect of such a force was extensive injury with the elevation of broken bone fragments in relation to the depressed fragments belonging to expressed-depressed fracture type. According to the literature data, within the first 30 minutes after the injury, the concentration of bacteria is 30.000 per gram of injured tissue on average (8), so primary closing of the wound is recommended within 6 hours from the moment of injury. In addition, care should be taken of the preservation of scalp vascularization during the surgical preparation. By closing the dura with plasticization by means of prepared periosteum, potential development of meningitis, cerebral abscess and pseudomeningocele formation is prevented, which is mentioned as the main goal of surgery in literature (9). Then, the planned reconstruction of the cranial defect was performed. In addition to closing the intracranial space, aesthetic correction of defects was taken into account as well. Free fragments were used for the partial reconstruction of the frontal squama and the superciliary arch. According to literature data (9), reconstruction by means of cranial bone grafts, the so-called 'titanium mesh', especially for frontal-orbital region, remains the method of choice. The advantage of using synthetic materials such as Polyhydroxyethyl methacrylate is the ade quate correction of frontal orbital defects (10), and main disadvantages include the possibility of infection, potential development of fistula, graft migration, as well as formation of granuloma and erosion. Considering these facts, and taking into account the fact that the tissue tolerates preserved bone fragments and represents reliable reconstructive material, we decided to postpone further reconstruction of a larger portion of the superciliary arch at this stage. As there were no functional defects including immobility of the eyeballs, enophtalmus, hypoglobus and upper lateral fissure syndrome, indexes of eye nerve compression, this decision turned out to be correct. However, early reconstruction is recommended in literature due to good condition of bone fragments, absence of contractures and fibrous changes in the soft tissues. Antibiotic parenteral therapy reduces

the risk of infection (11). In the later course after the recovery of the patient, the possibility of remediation of the defect would be considered, thus completing aesthetic correction using planned titanium mesh. The titanium mesh in the reconstitution may significantly shorten the time of intervention with a simple placement technique with a minimum incidence of complications of about 10% according to the literature data and excellent esthetic recovery in 90% of cases (11). Surgical closing of the scalp involved preservation of the hair line, follicular hair orientation, scar remediation and prevention of alopecia (12).

Conclusion

Prompt and adequate surgery of open skull fractures with evident intracranial complications is of vital importance to the patient. Appropriate and moderate use of synthetic material may represent a compromise between the infection risk, stability of damaged soft or bone tissue, and management of functional aesthetic problems. The extent of this injury was also reflected in elevated bone fragments that we defined as expressed-depressed fracture.

References

1. Yogandan N, Pintar FA, Sances A Jr, Walsh PR, Ewing CL, Thomas DJ, et al. Biomechanics of skull fracture. J Neurotraum 1995; 12(4):659- 68. [CrossRef] [PubMed]
2. Hsiao KY, Hsiao CT, Weng HH, Chen KH, Lin LJ, Huang YM. Factors predicting mortality in victims of blunt trauma brain injury in emergency department settings. Emerg Med J 2008; 25(10):670-3. [CrossRef] [PubMed]
3. Vuleković P, Cigić T, Kojadinović Ž. Osnovi neurohirurgije. Novi Sad: Medicinski fakultet; 2012.
4. Bullock MR, Chesnut R, Ghajar J, Gordon D, Hartl R, Newell DW, et al. Surgical management of depressed cranial fractures. Neurosurgery 2006; 58 (3Suppl): S56-60. [CrossRef] [PubMed]
5. Muhammad G, Aurangzeb A, Khan SA, Hussain I, Alam S, Khan Afridi EA, et al. Dural tears in patients with depressed skull fractures. J Ayub Med Coll Abbottabad 2017; 29(2):311-5. [PubMed]
6. Samardžić M, editor. Neurohirurški problemi u ortopediji i traumatologiji. Beograd: Zavod za udžbenike i nastavna sredstva; 2000.
7. Manolidis S, Weeks BH, Kirby M, Scarlett M, Hollier L. Classification and surgical management of orbital fractures: experience with 111 orbital reconstructions. Journal Craniofac Surg 2002; 13(6):726-37. [CrossRef] [PubMed]
8. Tindall GT, Cooper PR, Barrow DL, editors. The practice of neurosurgery. Volume II. Philadelphia: Lippincott Williams & Wilkins; 1995.
9. Nayak PK, Mahapatra K. Primary reconstruction of depressed skull fracture-The Changing scenario. Indian J Neurotrauma 2007; 5(1):35-8. [CrossRef]
10. Dajashankara R, Malhorta V, Ravi S, Abishek K. Esthetic correction of depressed frontal bone fracture. Natl J Maxillofac Surg 2011; 2(1):69-72. [CrossRef] [PubMed]
11. Ghaareb FM, Elbarah AM, Elsheikh YM, Nassar ATh, Ebied OM, Nohc HZ. Fronto-orbital bone fracture: management and outcome. Menoufia Med J 2014; 27(2):379-85. [CrossRef]
12. Blackwel KE, Ranswley JD. Aesthetic considerations in of scalp reconstruction. Facial Plast Surg 2008; 24 (1): 11-21. [CrossRef] [PubMed]

Prikaz bolesnika

UDC: 611.714:616-001.5-089
doi:10.5633/amm.2018.0413

OTVORENA KOMINUTIVNA EKSPRESIONO-DEPRESIVNA FRAKTURA KRANIJUMA

Boban Jelenković[1], Vesna Nikolov[1,2], Slavko Živković[1], Luka Berilažić[1], Predrag Milošević[1]

[1]Klinika za neurohirurgiju, Klinički centar Niš, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

Kontakt: Boban Jelenković
Radoja Dakića 47a/3, 18000 Niš, Srbija
E-mail: bobanjelenko@gmail.com

Prelomi lobanje nastaju pod dejstvom kinetičkih sila i podrazumevaju prekid kontinuiteta kostiju lobanje. Mogu biti otvoreni i zatvoreni u odnosu na tkiva, u nivou kranijuma (linerani, dijastatski, kominutivni) ili depresivni, često dovodeći do povrede moždanih ovojnica, moždanog tkiva, sa različitim tipovima intrakranijalnog krvarenja. U radu je prikazan bolesnik star 56 godina, muškog pola, koji je u toku rada mašinom za obradu drveta zadobio tešku kraniocerebralnu povredu frontalne regije sa zahvatanjem orbite. Povreda se manifestovala oštećenjem skalpa, *ekspresiono*-depresivnim otvorenim prelomom fronto-orbitalne regije i likvorejom. Kompjuterizovana tomografija (CT) mozga pokazala je postojanje epiduralnog, subduralnog i intracerebralnog hematoma sa prisutnim mas efektom. Operativnim tretmanom povrede su bile zbrinute, hematomi evakuisani, rekonstruisan defekt lobanje uz prethodno plastifikovanje dure u cilju zaustavljanja likvoreje. U rekonstrukciji multifragmentarne frakture koristili smo zvezdasti titanijumski implant, ali nismo pristupili značajnoj ugradnji veštačkog materijala, zbog primarno kontaminirane rane i mogućnosti kasnije infekcije.

Acta Medica Medianae 2018;57(4):96-100.

Ključne reči: *ekspresiono-depresivna fraktura, fronto-orbitalna regija, intrakranijalno krvarenje, rekonstrukcija defekta, likvoreja*

Original article

UDC: 616.34-002-089
doi:10.5633/amm.2018.0414

ENDOSCOPIC CHANGES IN THE GASTRIC STUMP MUCOSA AFTER SURGICAL TREATMENT FOR ULCER DISEASE

Biljana Radovanović-Dinić[1,2], Snežana Tešić-Rajković[1,2]

Surgical treatment is today restricted mostly to the patients with ulcer disease complications or the small portion of patients with ulcers refractory to conventional therapy. Gastric 'two-thirds' resection is one of the surgical interventions in surgical treatment of ulcer disease. Our prospective study involved 67 patients with two-thirds gastric resection and reconstruction by Billroth I or Billroth II method. In all the examinees, proximal endoscopy was performed. During the endoscopy, esophagus, gastric stump, anastomosis and afferent limbs were observed. There were more males in the group of patients with Billroth I resection (χ^2 = 1.90; p = 0.1676). The average age of all the examinees was 64.27 ±1 0.07 years. In 39 patients (58.21%), the resection was performed for gastric ulcer or its complications, and in 28 patients (41.79%) for ulcer at the duodenal bulb or its complications (χ^2 = 8.75; p = 0.0678). In patients with ulcer of the stomach, Billroth II resection was statistically significantly (p < 0.05) more commonly performed. The average time from resection to endoscopy was slightly longer in those with Billroth I resection (p value being close to the 0.05 significance cut-off value). Duodenogastric reflux was more commonly encountered in patients with Billroth II gastric resection. Ulceration in the gastric stump was present in 7 (10.44%) examinees. Carcinoma was found in 2 patients (2.98%) in gastric stump, and in 3 patients (4.95%) at the anastomosis site. Although in our clinical practice it is increasingly rare to encounter the patients with resected stomach, we should not overlook the possibility of damage to the remnant of the stomach.

Acta Medica Medianae 2018;57(4):101-109.

Key words: *gastric stump, endoscopy, peptic ulcer*

[1]University of Niš, Faculty of Medicine, Niš, Serbia
[2]Clinical Centre Niš, Clinic for Gastroenterology and Hepatology, Niš, Serbia

Contact: Biljana Radovanović-Dinić
Elektronska 1/1, 18 000 Niš, Serbia
E-mail: bikius@yahoo.com

Introduction

Peptic ulcer disease is a defect in gastrointestinal mucosa as the result of inability of epithelial cells to resist the caustic action of HCl and pepsin present in the organ lumen. By their localization, ulcers can be divided into duodenal, distal gastric (antrum, prepyloric region), proximal gastric (angular portion, most of the corpus), and cardial ulcers (cardia and immediate subcardial portion) (1, 2).

Ulcer disease is always confirmed morphologically, nowadays most commonly endoscopically. For more than a century, surgery played the most prominent role in the treatment of ulcer disease and its complications. Based on new insights in the field, discovery of powerful antisecretory drugs, significant advances in endoscopic diagnosis and hemostasis, discovery of the role of Helicobacter infection in the pathogenesis of ulcer, the use of surgery is significantly reduced and modified. Surgical treatment is today restricted mostly to the patients with ulcer disease complications or the small portion of patients with ulcers refractory to conventional therapy (3).

Gastric resection is one of the surgical interventions in surgical pathology of the stomach. Resections can be elective (planned) or emergency surgical interventions (ulcer bleeding or perforation). Gastric 'two thirds' resection (subtotal gastrectomy) involves ulcer removal together with gastrin-producing portion of the stomach and most of the body of the stomach with parietal cells. After resection of the part of the stomach, the organ is reconstructed by the methods Billroth I or Billroth II, i.e. by their modifications (4, 5).

A postresection gastric stump undergoes not only some significant anatomical-physiological changes, but in the new situation the conditions are created for the stomach remnant to be affected by certain diseases as well. Removal of the antropyloric portion of the stomach reduces its motor function.

Gastrin cells are located in the antrum and their removal disturbes the endocrine stimulation of gastric secretion; moreover, physiological trophic impact of gastrin on the gastrointestinal mucosa will be absent concomitantly. Removal of a part of the body of the stomach involves the removal of a part of parietal cells, and this will be an additional component in the reduced secretory function of the stomach. By way of the afferent limb, duodenal biliopancreatic juice comes into contact with the gastric stump and splashing the gastric mucosa creates a nonphysiological medium in which harmful effects of bile salts on gastric mucosa become evident. In such conditions, gastric mucosa of the gastric stump suffers significant dystrophic-inflammatory-metaplastic changes, representing in fact a precancerosis (2, 4).

The aim of our study was to assess endoscopic changes in the gastric stump after gastric resection undertaken as the therapy of ulcer disease or its complications.

Material and methods

Our prospective study involved 67 patients with two-thirds gastric resection and reconstruction by Billroth I or Billroth II method. Indications for the resection were recurrent ulcer disease, duodenal perforations and bleeding gastric ulcers.

In all the examinees, proximal endoscopy was performed using an Olympus or Pentax esophagogastroduodenoscope (EGDS). The examination was indicated for the symptoms of dyspepsia or as a regular control after gastric resection. During the endoscopy, esophagus, gastric stump, anastomosis and afferent limbs were observed. From each of the examinees biopsy samples were taken from the gastric stump mucosa, mucosa tissue 1-3 cm away from the anastomosis, and from all endoscopically suspicious sites. All the biopsy samples were histopathologically analyzed after adequate processing.

Statistical analysis of data was performed using R 2.15.3 software (R Foundation for Statistical Computing, Vienna, Austria) (6).

Results

Sixty seven patients were enrolled to the study with two-thirds gastric resection; there were 24 patients (35.82%) with Billroth I gastric reconstruction (Figure 1) and 43 (62.18%) with Billroth II gastric reconstruction (Figure 2).

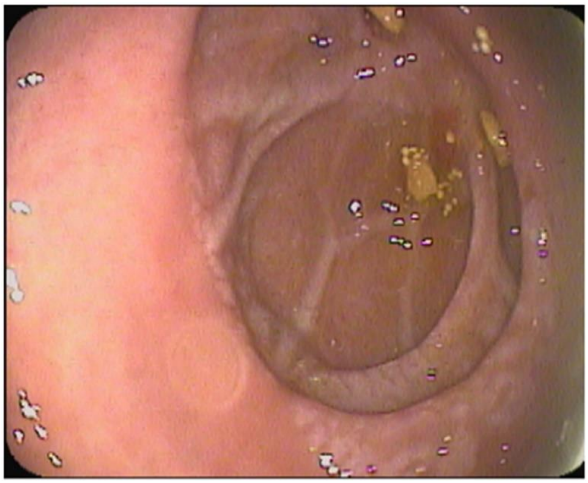


Figure 1. Two-thirds gastric resection with gastroduodenostomy (Billroth I)

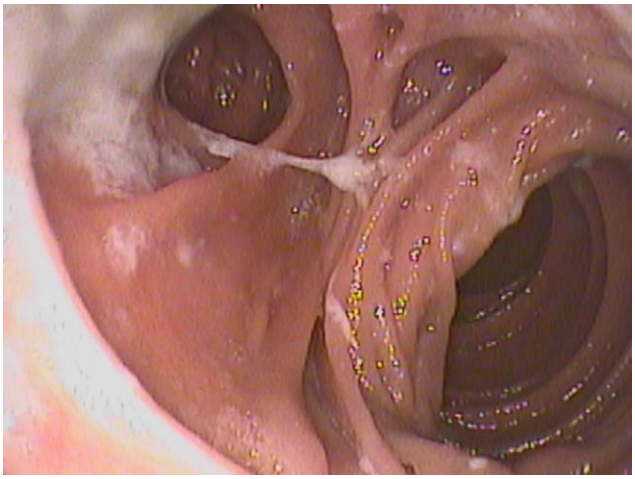


Figure 2. Two-thirds gastric resection with gastrojejunostomy (Billroth II)

There were more males (although not statistically significantly) in the group of patients with Billroth I resection (χ^2 = 1.90; p = 0.1676). The average age of all the examinees was 64.27 ± 10.07 years. Using the Student's t-test, we were not able to establish any statistically significant difference in the mean age between the patients surgically treated by Billroth I (66.42 ± 8.61) and those treated by Billroth II resection (63.07 ± 10.70) (t = 1.31 p = 0.1942). In 39 patients (58.21%) the resection was performed for gastric ulcer or its complications, and in 28 patients (41.79%) for ulcer at the duodenal bulb or its complications (χ^2 = 8.75; p = 0.0678). In patients with ulcer of the stomach, Billroth II resection was statistically significantly (p < 0.05) more commonly performed, in contrast to those with ulcers at the duodenal bulb in whom both surgical resections were equally common (Table 1).

We could not establish a statistically significant difference in the period of time from resection to endoscopy between Billroth I patients (22.67 ± 9.27) and Billroth II patients (14.22 ± 11.48) (t =

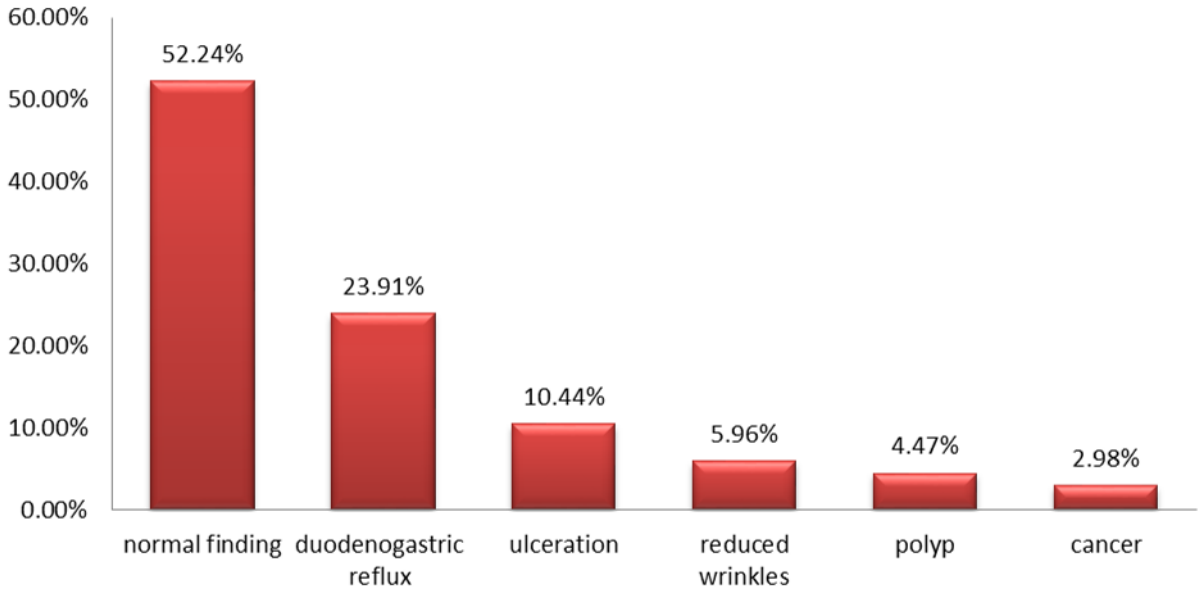
1.91 p = 0.0675). However, the average time from resection to endoscopy was slightly longer in those with Billroth I resection (p value being close to the 0.05 significance cut-off value) (Table 2). Graph 1 presents an endoscopic finding verified on a gastric stump. Duodenogastric reflux was more commonly encountered in patients with Billroth II gastric resection (χ^2 = 8.75; p = 0.0678) (Figure 3). Duodenogastric reflux was more commonly seen in patients with longer postoperative period (Table 3).

Table 1. Demographic characteristics of patients with gastric resection

Characteristics	N = 67	Billroth I	Billroth II	p-value
Gender(male/female)	43/24	18/6	25/18	
age (years)	64.27 ± 10.07	66.42 ± 8.61	63.07 ± 10.70	0.1676
gastric ulcer	39 (58.21%)	10 (25.64%)	29 (74.36%)	0.1942
duodenal ulcer	28 (41.79%)	14 (50.00%)	14 (50.00%)	

Table 2. Period of time from resection to endoscopy (in years)

Groups	N	X	SD	SG	95%	CI	Min.	Max.	CV
Billroth I	24	22.67	9.27	3.09	15.54	29.80	11	43	40.91
BillrothII	43	14.22	11.48	2.71	8.52	19.93	1	38	80.69
Total	67	17.04	11.36	2.19	12.54	21.53	1	43	66.68



Graph 1. Endoscopic findings in the gastric stump

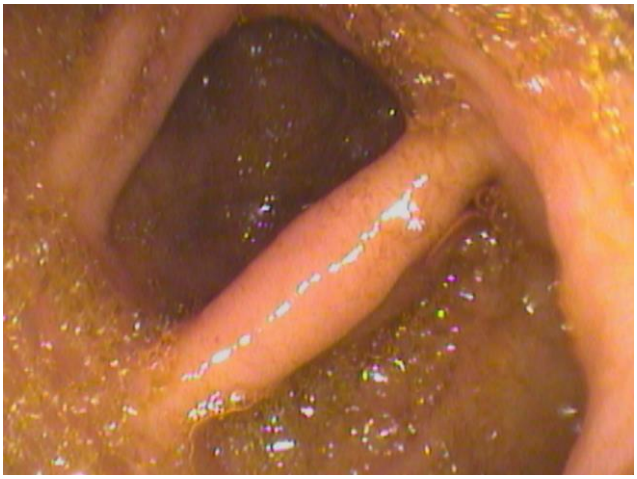


Figure 3. Duodenogastric reflux in the gastric stump after Billroth II resection

Table 3. Postoperative period (years) and presence of duodenogastrix reflux in the gastric stump

Biliogastric reflux	N	X	SD	SE	95%	CI	Min	Max	CV
Yes	15	18.55	10.03	2.14	14.10	22.99	2	43	54.07
No	52	10.40	15.60	6.98	-8.97	29.77	1	38	
Total	67	17.04	11.36	2.19	12.54	21.53	1	43	66.68

Ulceration in the gastric stump was present in 7 (10.44%) examinees (Figure 4). The period of time from resection to confirmed ulcerations was 10.4 years. In 4 (57.14%) examinees, ulcerations appeared within 2 years after their resections. Carcinoma of the gastric stump was found in 2 cases (2.98%) out of the total number of examinees (Figure 5). During a proximal endoscopic examination, changes in the gastroenteroanastomosis could be seen (Graph 2).

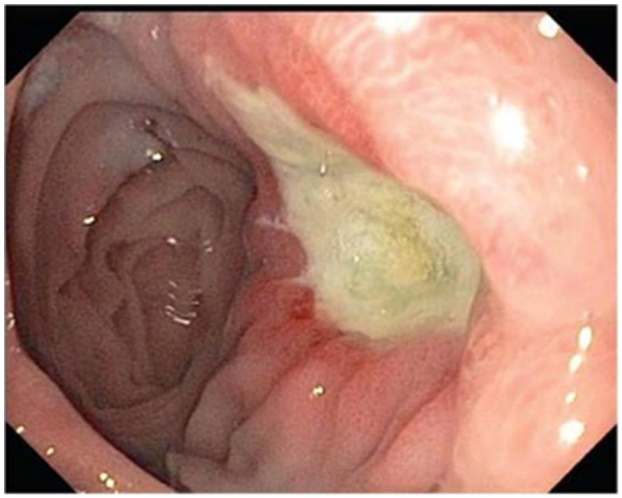


Figure 4. Ulceration in the gastric stump

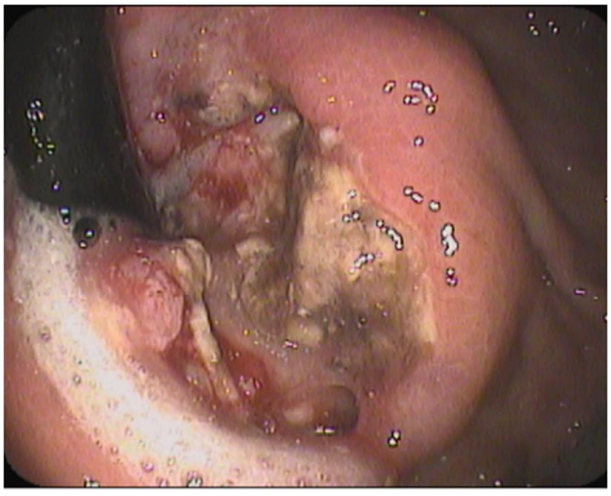
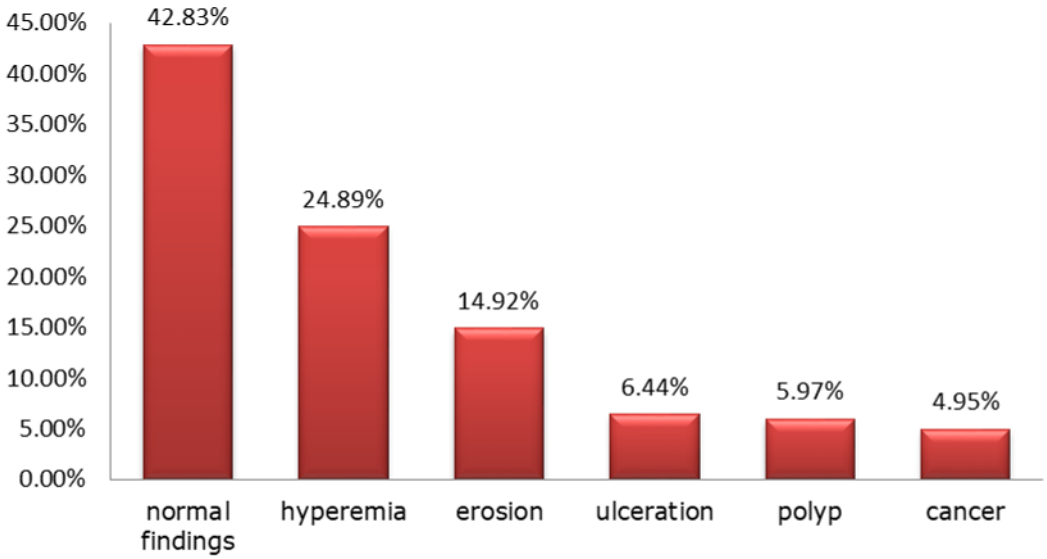


Figure 5. Carcinoma in the gastric stump



Graph 2. Endoscopic findings in gastroenteroanastomosis

Carcinoma of the gastroenteroanastomosis was found in 4.95% out of the total number of examinees. In both patient groups, regardless of the type of resection, morphological transformation of the gastric folds was present in the immediate vicinity of gastroenteroanastomosis (Figure 6).

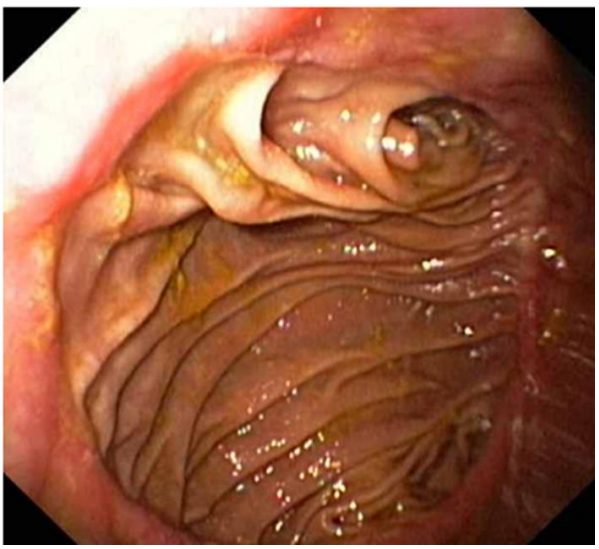


Figure 6. Transformation of gastric folds in the vicinity of gastroenteroanastomosis

Discussion

Partial resection is one of the surgical methods of choice in the treatment of recurrent ulcers of the stomach or duodenal bulb and their respective complications. Recurrent gastric or duodenal ulcers have become a rare clinical entity as the consequence of ever increasing availability of drugs for long-term reduction of stomach acid and nowadays common consideration of Helicobacter pylori infection and expanding possibilities of its eradication. Furthermore, in clinical practice, the awareness has considerably grown of the significance of gastroprotection when the use of NSAIDs is concerned (as well as with other drugs which increase the risk for ulcer disease).

The decision about the type of reconstruction (Billroth I or Billroth II) during gastroresection is made by the surgeon. In our examinees, Billroth II resections were more common, but the difference was without statistical significance. In the study by Vera et al. Billroth I gastric resections were statistically significantly more frequently performed (52.6%) than Billroth II surgeries (7).

The study aimed to evaluate which type of resection was a better surgical treatment for gastric ulcer favored Billroth I resection over Billroth II. The results indicated better prognosis in the patients who underwent Billroth I resection, while quality of life was comparable in both groups of examinees.

Analyzing the factor of gender, we found that men were more commonly affected, as expected, since statistical information indicated that peptic ulcer disease was more prevalent in men compared to women. In 58.2% of patients, resections of the stomach were done for recurrent ulcer or its complications, and in 41.8% for ulcer at the duodenal bulb or its complications. The obtained results agreed well with the results obtained in other studies (8-10).

In normal conditions, endoscopic examination can be performed even as early as seven days after the resection. Esophagogastroduodenoscopy of the resected stomach is associated with some specific elements and difficulties compared to the examination of an unresected stomach. Post-resection morphology of the gastric stump varies considerably depending on the surgical approach employed and dimensions of the remnant stomach. In more extensive resections and with short gastric stumps, the

insufflated air can escape through the stoma into the bowels, the endoscope is leaned onto the mucosa and a clear field of vision is more difficult to obtain. Further, respiratory movements are transferred via the diaphragm to the gastric stump, which also may disturb the inspection of the targeted portion of the stump. Moreover, occasional reflux from the afferent limb may splush the anastomosis and stump mucosa and disrupt the examination. Nevertheless, in most of the cases a clear field of vision can be obtained and an adequate endoscopic examination can be performed.

In most of our examinees endoscopic findings in the gastric stump were in order. Bilio-gastric refluxate was confirmed in 25.49% of all our patients, which was a lower proportion than that in other similar studies, where the percentage ranged from 60% to 90% (7).

The average age of our examinees with reflux (64.07 ± 7.45) was higher compared to the examinees without bilio-gastric reflux. These findings agree with the observations in a study by Vera et al. in 2005. Some authors believe that bilio-gastric reflux is more common in older examinees as the consequence of a higher prevalence of partial gastric resection in older populations (7). In our study, we found that duodenogastric reflux was more common after Billroth II resection, especially when the intervention was undertaken for gastric ulcer or its complications. Duodenogastric reflux was more often seen in patients with longer postoperative period of time. The average time from the surgery to endoscopically confirmed reflux was 16.91 years, approximating the results of Vera et al. (7). Several clinical and experimental studies have shown that bile acids and pancreatic proteolytic enzymes can damage gastric mucosa, and reflux of bile and duodenal contents also has a possible pathogenetic role in gastritis, gastric ulcer, chronic gastritis, reflux esophagitis, and esophageal and gastric cancer (11, 12). The exact mechanisms of gastric mucosal damage caused by duodenogastric reflux are still unknown. Duodenogastric reflux is probably an independent etiological factor and might play a synergistic role in the pathogenesis of gastric mucosal lesions along with gastric acid and Helicobacter pylori infection (12). Bechi et al. established in their study that with Billroth II resections the reflux was more abundant and concentrated. These authors recommended that in gastric ulcer or its complications partial gastrectomy with Billroth I reconstruction should be done. The principal endoscopic signs in patients with duodenogastric reflux are erythema of the gastric mucosa, presence of bile in the stomach, altered gastric folds and erosions (13, 14).

In the period of 24 hours, around 800 ml of bile and around 1000 ml of pancreatic juice flow into the stomach via the afferent limb. A scanty and occasional reflux indicates a small amount of bile that splashes only the edges of the anastomosis. A medium or moderately abundant reflux is characterized by a larger amount of bile splashing the anastomosis or its immediate vicinity. An abundant reflux denotes a large amount of bile splashing a broad area of gastric mucosa and accumulating in the gastric grooves (2, 15). The presence of duodenogastric reflux depends as well on the type of resection (16).

In our study, ulcers were confirmed in 7 examinees (10.44%) in the gastric stump, and in 4 cases (6.44%) at the site of anastomosis. In the literature, the prevalence of ulcer recurrence is around 3%. It most commonly occurs within two years of surgery. Bleeding and perforation are here more common than with primary ulcers. Recurrences are more common after surgery for duo denal than for gastric ulcers. They are most commonly the consequence if an inadequate surgical intervention, failing to suppress sufficiently the acidosecretory activity of the gastric juice. Recurrences are usually situated immediately below the suture line at the anastomosis site, while they are extremely rare in the gastric stump. One of the causes of ulcer recurrence is Zollinger-Ellison syndrome (ZES), with its ulcer diathesis due to gastrinomas. Since they are prone to complications, ulcer recurrences represent an absolute indication for surgical intervention, excluding in that regard Zollinger-Ellison syndrome, since it requires a different therapeutic approach (gastrinoma removal). For ulcer recurrences a resection (reduction of the acidosecretory area) or vagotomy should be performed (2, 4).

The results of our study indicate that carcinoma of the gastric stump is more common after resection for bulbar ulcer complications. Most authors attach importance to the period of time that has elapsed since the surgery and age of the patient at the time of surgical intervention (17, 18).

Studies have shown that in 22% of patients subjected to surgery carcinoma of the stomach occurs within 26-30 years of surgery, while in 1.9-4.7% it occurs within 5-15 years of surgery. If the prevalence of carcinoma after resection is compared with that after medicamentous treatment of ulcer disease, a higher prevalence in the latter group has created the suspicion whether gastric resection may in fact have a protective effect (4, 17).

Histopathological preoperative finding and patient age at the time of surgery have an impact on the time of possible development of carcinoma of the resected stomach. Various studies have demonstrated that carcinoma of the stomach, after a longer time interval after the resection, is eight times more common in the gastric stump in comparison to control examinees with intact stomach. The same authors found that the period of time after the surgery, patient age at the time of surgery and histological changes in the stomach before the surgery had an impact on the occurrence of this carcinoma (7, 9). The postoperative interval is the most important determinant of cancer risk following gastrectomy (19). In the study by Vera et al. Carcinomas were diagnosed in 2.24% of examinees (7). Carcinoma of the gastric stump was most frequently situated at the site of anastomosis, in the vicinity of the afferent limb, in the stump and cardia. The disease prognosis in patients with carcinoma of the gastric stump is worse than that in patients with primary gastric carcinoma. The treatment is exclusively surgical, with subsequent oncological therapy. The prevention of carcinoma of the gastric stump consists of regular endoscopic and histologic controls

of the surgically treated patients, following the surveillance protocol for precancerous conditions (2, 4).

In our study, various changes at the site of anastomosis were seen as well, differing depending on the type of performed resection. In the case of gastrojejunal anastomosis, the stoma was wide and the delineation between afferent and efferent limbs was not conspicuous. The inflammation process almost regularly involved the gastric mucosa at the site of anastomosis. The process of inflammation very rarely represented localized anastomositis, and was often a part of the general inflammatory process involving the whole gastric stump mucosa. In some cases the mucosa of the anastomosis was inflamed as a whole, shiny, and red as if lacquered. At such a changed anastomosis, erosions and ulcerations can be seen in varying numbers, and contact bleedings are not rare as well due to increased fragility of the inflamed mucosa (2, 15).

In patients resected by Billroth II method, the gastric stump opens into a so called 'anastomotic chamber', created by the jejunal lumen more or less dilated as the consequence of surgical juncture with the stomach. In this 'anastomotic chamber' openings of the afferent and efferent limbs can be seen. Folds of the afferent limb mucosa are circular and mucosa is light pink in color. The inflammation is characterized by redness, fold alterations, and occasional presence of erosions and ulcerations. A peptic ulcer in the afferent limb is a rare finding. It is sometimes very difficult or even impossible to enter the afferent limb. Endoscopic examination of the efferent limb reveals the jejunal mucosa with circular folds and characteristic color, different than the color of gastric mucosa. In the immediate vicinity of the anastomosis, mucosa of the efferent limb is pink red, with thickened or thin and divergent sometimes unconspicuous folds.

Conclusion

After gastric resection and reconstruction by Billroth I or II method in around half of the patients endoscopic changes in the remnant stomach are present. Ulcerations in the gastric stump or at the anastomosis site are more common in patients with Billroth II resection. Further, bilio-gastric refluxate is more common after Billroth II resection, especially in those with longer postoperative period of time. Although in our clinical practice it is increasingly rare to encounter the patients with resected stomach, we should not overlook the possibility of damage to the remnant of the stomach. This especially relates to an increased risk of carcinoma developing in the gastric stump after a period of time after the resection. In that regard, timely preventive endoscopic control examinations should be introduced.

References

1. Pulimood BM, Knudsen A, Coghill NF. Gastric mucosa after partial gastrectomy. Gut 1976; 17:463-70. [CrossRef] [PubMed]
2. Vucelić B. Ulkusna bolest. In: Gastroenterologija i hepatologija. Zagreb: Medicinska naklada; 2002. p. 480-500.
3. Laušević D, Peško PM, Krstic SN, Sijacki A, Gvozdenović MS, Bumbasirević V, et al. Perspektive hirurškog lečenja krvarećeg peptičkog ulkusa. Acta Chir Iugosl 2007; 54(1):157-64. [PubMed]
4. Yamada T, Alpers DH, Kalloo AN, Kaplowitz N, Owyang C, Powell DW. Textbook of gastroenterology. 5th ed. Hoboken (NJ): Wiley-Blackwell; 2009. [CrossRef] [PubMed]
5. Greene FL. Neoplastic changes in the stomach after gastrectomy. Surg Gynecol Obstet 1990; 171(6):477-80. [PubMed]
6. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical

Computing, Vienna, Austria, 2014. Available at: http://www.R-project.org/. Accessed March 3, 2015.
7. Vere CC, Cazacu S, Comănescu V, Mogoantă L, Rogoveanu I, Ciurea T. Endoscopical and histological features in bile reflux gastritis. Rom J Morphol Embryol 2005; 46(4):269-74. [PubMed]
8. Pulimood BM, Knudsen A, Coghill NF. Gastric mucosa after partial gastrectomy. Gut 1976; 17:463-70. [CrossRef] [PubMed]
9. Lin JK, Hu PJ, Li CJ, Zeng ZR, Zhang XG. A study of diagnosis of primary biliary reflux gastritis, Zhonghua Nei Ke Za Zhi 2003; 42(2):81-83. [PubMed]
10. Fukuhara K, Osugi H, Takada N, Takemura M, Lee S, Taguchi S, et al. Correlation between duodenogastric reflux and remnant gastritis after distal gastrectomy. Hepatogastroenterol 2004; 51(58):1241-4. [PubMed]
11. Lee Y, Tokunaga A, Tajiri T, Masuda G, Okuda T, Fujita I, et al. Inflammation of the gastric remnant after gastrectomy: mucosal erythema is associated with bile reflux and inflammatory cellular infiltration is associated with Helicobacter pylori infection. J Gastroenterol 2004; 39(6):520–6. [CrossRef] [PubMed]
12. Ma M, Chen J, Zhang YY, Li ZY, Jiang MZ, Yu JD. Pathogenic effects of primary duodenogastric reflux on gastric mucosa of children. Zhonghua Er Ke Za Zhi. 2008; 46(4):257-62. [PubMed]
13. Bechi P, Amorosi A, Mazzanti R, Romagnoli P, Tonelli L. Gastric histology and fasting bile reflux after partial gastrectomy. Gastroenterology 1987; 93(2):335-43. [CrossRef] [PubMed]
14. Tan SY, Davis JD, Davis CA. Theodor Billroth (1829-1894): pioneer of modern surgery. Singapore Med J 2008; 49 (1):72-75.
15. Katičić M. Peptička ulkusna bolest. Medicus 2006; 15(1):39-52.
16. Zlatić A, Stojanović M, Mihailović D, Radovanović-Dinić, Protić M, Veljković R. The role of duodenogastric reflux in formation of precarcinogenic gastric lesions: An experimental study. Med Pregl 2013; 66(7-8):285-29. [CrossRef] [PubMed]
17. Whiting JL, Sigurdsson A, Rowlands DC, Hallissey MT, Fielding JW. The long term results of endoscopic surveillance of premalignant gastric lesions. Gut 2002; 50:378–81. [CrossRef] [PubMed]
18. Dinis-Ribeiro M, Lopes C, da Costa-Pereira A, Guilherme M, Barbosa J, Lomba-Viana H, et al. A follow up model for patients with atrophic chronic gastritis and intestinal metaplasia. J Clin Pathol 2004; 57:177–82. [CrossRef] [PubMed]
19. Safatle-Ribeiro AV, Ribeiro Júnior U, Sakai P, Iriya K, Ishioka S, Gama-Rodrigues J. Gastric stump mucosa: is there a risk for carcinoma? Arq Gastroenterol 2001; 38(4):227-31. [CrossRef] [PubMed]

Originalni rad **UDC: 616.34-002-089**
doi:10.5633/amm.2018.0414

ENDOSKOPSKE PROMENE SLUZNICE ŽELUDAČNOG PATRLJKA NAKON HIRUŠKE TERAPIJE ULKUSNE BOLESTI

Biljana Radovanović-Dinić[1,2], Snežana Tešić-Rajković[1,2]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[2]Klinički centar Niš, Klinika za gastroenterologiju i hepatologiju, Niš, Srbija

Kontakt: Biljana Radovanović-Dinić
Elektronska 1/1, 18000 Niš, Srbija
E-mail: bikius@yahoo.com

Danas je hirurška terapija uglavnom rezervisana samo za bolesnike s komplikacijama ulkusne bolesti ili one malobrojne bolesnike čiji su ulkusi refrakterni na konvencionalnu terapiju. Dvotrećinska resekcija želuca je jedna od hiruških intervencija u hirurškom tretmanu ulkusne bolesti. Naša prospektivna studija analizirala je 67 bolesnika sa dvotrećinskom resekcijom želuca i rekonstrukcijom po metodi Billroth I ili Billroth II. Kod svih ispitanika uradjena je proksimalna endoskopija. Tokom endoskopije opservirani su jednjak, želudačni patrljak, anastomoza i priključne vijuge. Prednost muškaraca je bila izraženija u grupi bolesnika sa Billroth I resekcijom (χ^2 = 1,90; p = 0,1676). Prosečno starosno doba ispitanika bila je 64,27 ± 10,07 godina. Kod 39 (58,21%) bolesnika uzrok resekcije je bio ulkus želuca ili komplikacije istog, a kod 28 (41.79%) ulkus bulbusa duodenuma ili komplikacije istog (χ^2 = 8,75; p = 0,0678). Kod bolesnika sa ulkusom želuca statistički značajno češće je urađena resekcija želuca po metodi Billroth II (p < 0,05). Prosečno vreme od resekcije do nalaza je nešto duže kod bolesnika sa resekcijom po metodi Billroth I (vrednost p bliska nivou značajnosti od 0,05). Duodenogastrični refluks je češće verifikovan kod bolesnika sa resekovanim želucem po metodi Billroth II (χ^2 = 8,75; p = 0,0678). Ulceracija u želudačnom patrljku je bila prisutna kod 7 (10.44%) ispitanika. Karcinom je bio prisutan kod dva pacijenta (2,98%) u želudačnom patrljku, odnosno kod tri (4,95%) na anastomozi. I pored toga što se u kliničkoj praksi sve ređe susrećemo sa bolesnicima koji imaju resekovan želudac, nikako ne treba izgubiti iz vida mogućnost nastanka oštećenja preostalog dela želuca.

Acta Medica Medianae 2018;57(4):101-109.

Ključne reči: *želudačni patrljak, endoskopija, ulkus želuca*

Original article

UDC: 577:616.831-005.1
doi:10.5633/amm.2018.0415

THE INFLUENCE OF CAT-262 C/T POLYMORPHISM ON CATALASE ACTIVITY IN PATIENTS WITH ISCHEMIC STROKE

Jelena Bašić[1], Vuk Milošević[2], Milena Despotović[1], Tatjana Jevtović-Stoimenov[1], Tatjana Cvetković[1], Milica Živanović[1], Miroslava Živković[2,3], Dušica Pavlović[1]

Although there is a disturbance of oxidative stress markers in acute ischemic stroke (AIS), genetic contribution of -262C/T polymorphism of catalase (CAT) gene on plasma CAT activity in this disease is not yet established.

The aim of this study was to investigate the distribution of CAT-262C/T polymorphism in AIS patients compared to controls, as well as to evaluate whether this polymorphism can influence plasma CAT activity.

A total of 34 patients with AIS and 32 healthy volunteers were screened for the CAT-262C/T gene polymorphism using the polymerase chain reaction–restriction fragment length polymorphism method (PCR-RFLP). Plasma CAT activity was determined using spectrophotometric method according to Goth.

Although the patients with the diagnosis of AIS had a higher frequency of polymorphic -262T allele in comparison to the group of healthy subjects, the difference was not statistically significant ($p = 0.117$). CAT activity was significantly lower in the patients (12.95 ± 2.86 kU/L) compared to the controls (25.58 ± 13.50 kU/L, $p < 0.001$). The patients carriers of the -262T allele, showed significant decrease of plasma CAT activity (11.93 ± 2.82 kU/L) compared to the patients with genotype -262CC (13.99 ± 2.59 kU/L, $p = 0.039$).

This is the first study examining the CAT-262C/T polymorphism and its influence on plasma CAT activity in AIS. Bearing in mind that the presence of -262T allele in AIS patients significantly decreased plasma catalase activity compared to CC genotype carriers, further studies should be focused on the testing of the potential protective role of the -262CC genotype in ischemic stroke.

Acta Medica Medianae 2018;57(4):110-116.

Key words: *acute ischemic stroke, catalase, CAT-262C/T polymorphism*

[1]University of Niš, Faculty of Medicine, Department of Biochemistry, Serbia
[2]Clinic of Neurology, Clinical Centre Niš, Niš, Serbia
[3]University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Jelena Bašić
University of Niš, Faculty of Medicine
Bulevar dr Zorana Djindjića 81, 18000 Niš, Serbia
E-mail: jelena.basic@medfak.ni.ac.rs

Introduction

Acute ischemic stroke (AIS) is an episode of neurological dysfunction, caused by focal cerebral, spinal or retinal infarction (1). Stroke is the third most common cause of death, after cardiovascular disease and cancer, and is the leading cause of disability in the developed countries worldwide. Two-thirds of stroke patients develop some form of cognitive impairment, and every fifth patient meets the criteria for dementia (2).

A number of studies are focused on identifying complex molecular mechanisms underlying cerebral ischemia. The main cause of the reduced perfusion and ischemic brain damage is thrombosis and/or embolism. Impaired local cerebral blood flow leads to reduced oxygen and glucose delivery, which results in the disturbance of cell homeostasis and the subsequent oxidative, nitrosative stress and inflammation. Numerous studies clearly demonstrate the association between the oxidative stress and the development of neuronal cell death in brain ischemia (3, 4). It is believed that there are several crucial factors for the generation of reactive oxygen species (ROS), as well as reactive nitrogen species (RNS) in the ischemic area of the brain. Among them, the most prominent ones are the impaired mitochondrial function, the activation of neuronal nitric oxide synthase (nNOS) and the activation of neutrophils (5). The last process leads to the activation of NADPH oxidase. The reaction catalysed by this enzyme primarily generates superoxide anion radical which, through dismutation (a reaction catalyzed by superoxide dismu-

tase - SOD) produces hydrogen peroxide (H_2O_2). The interaction of superoxide anion radical and H_2O_2 leads to the formation of the most potent $OH^{\bullet}$ radical, which causes cell damage and neuronal death (6).

To prevent damage, the cells contain antioxidant enzymes (SOD, catalase and glutathione peroxidase) which neutralize superoxide anions, hydrogen peroxide and lipid peroxide. Catalase (EC 1.11.1.6) (CAT), is an oxidoreductase, a tetramer of four identical subunits, each containing a polypeptide chain of 527 amino acids and a heme molecule. CAT is one of the most active and most widespread enzymes (liver, kidneys), localized in either peroxisomes or the cytosol (red blood cells - RBC). It catalyzes the decomposition reaction of H_2O_2 to water and molecular oxygen (7). Although numerous studies point to the increased production of ROS in AIS, the results of antioxidant enzymes activities in this disease are contradictory (8-10).

The CAT gene is located on chromosome 11p13, it has 13 exons and 410 genetic variations identified so far (11). A large number of these variations results in disturbed CAT gene expression and the change of its activity in RBC and plasma (7). Genetic variation CAT-262C/T (rs1001179) is located in the promoter region of the CAT gene. Although the results of different studies showed the correlation of this polymorphism with the risk of developing diabetes mellitus, breast cancer, hepatocellular cancer, ulcerative colitis (12-16), its functional significance in the ischemic stroke has not yet been established.

Aims

The aim of this study was to investigate the distribution of CAT-262C/T (rs1001179) genetic variation in the catalase gene in AIS patients, compared to healthy subjects, and to examine the impact of this polymorphism on patient's plasma CAT activity.

Material and methods

The study was conducted on 34 patients with ischemic stroke at the acute phase of the disease, diagnosed and treated at the Clinic of Neurology, Clinical Center Niš. Among them, there were 20 women and 14 men, average age of 62.34 ± 2.41. The control group consisted of 32 healthy subjects, whose gender and age corresponded to those of the affected patients. The research was conducted at the Laboratory for Functional Genomics and Proteomics, Medical Faculty, University of Niš. The present study was approved by the Ethical Committee of the Medical Faculty University of Niš, Serbia (No 12-9808-2/1).

Blood samples were taken within 7 days after ischemic attack. From all the blood samples (with EDTA as anticoagulant), we separated 200 μL of blood, which was used for DNA isolation. The blood samples were then centrifuged at 3500 rpm for 10 minutes at +4°C, after which the plasma was separated and frozen at -80°C.

The isolation of DNA was performed using a commercial kit for DNA isolation (Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit, Thermo Fisher Scientific). We examined the polymorphism CAT-262 C/T, using the polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP) (17).

The fragment of 185 base pairs (bp) was amplified using a forward (5'-AGA GCC CGC TCG CCC CGG ACC G-3') and a reverse primer (5'-TAA CTG GAG AGA CAT AAG AGC T-3'). The PCR reaction mixture in a volume of 25 μL contained: 12.5 μL of KAPA 2G Fast HS Ready-Mix PCR kit solution (KAPA Biosystems, Germany), 0.5 μL of primer (10 pmol/μL) (Fermentas GmbH, St. Leon-Rot, Germany) and 20 ng of DNA. The PCR conditions were: the initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 63°C for 15 seconds, elongation at 72°C for 15 seconds, and termination at 72°C for 30 seconds. The amplified PCR products were visualized under UV light after agarose gel (2%) electrophoresis.

PCR products were cut into smaller fragments by SmaI restriction enzyme (Fermentas GmbH, St. Leon-Rot, Germany) at 37°C overnight and analyzed by a vertical polyacrylamide gel (8%) electrophoresis.

Homozygous for the C allele (wild type) was detected as two fragments of 155 and 30bp (genotype CC), while the polymorphic homozygous (TT) was shown as one fragment (185 bp). Heterozygous (CT) was confirmed by the presence of three fragments on the gel (185, 155 and 30 bp).

The plasma CAT activity was determined by Goth's spectrophotometric method (18), based on the ability of CAT to decompose the substrate (H_2O_2), whereby the enzymatic reaction was stopped by the addition of ammonium molybdate, and the resulting yellow complex of H_2O^2 and molybdate was measured at 405 nm against the reagent blank. The enzyme activity was expressed in kU/L.

The frequency of alleles and genotypes in the patients and controls was analyzed and compared using χ^2 test or Fisher exact test, but we also determined the possible deviation from the expected values of Hardy-Weinberg equilibrium tests for the patients and the control group. CAT activity in plasma was expressed as mean ± standard deviation. The statistically significant differences in values between the patients and the control groups, as well as between the different genotypes within patients, were determined by Student's t-test for two independent samples. $P < 0.05$ value was considered statistically significant. The statistical analysis was conducted using the SPSS software package version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

The frequency of genotypes of the CAT-262C/T polymorphism does not deviate from the

normal distribution of Hardy-Weinberg equilibrium in patients and control groups ($p > 0.05$).

The results shown in Table 1 indicate that the CC genotype was present in 16 (47%) patients, while TT genotype was present in 4 (11.8%) patients. Fourteen (41.2%) patients were heterozygous. The distribution of genotypes of the CAT-262C/T polymorphism in patients with AIS did not show a statistically significant difference compared to the control group ($\chi^2 = 2.371$, $p = 0.306$). Although the patients with the diagnosis of AIS had a higher frequency of -262T allele in comparison to the group of the healthy subjects, the difference was not statistically significant ($\chi^2 = 2.453$, $p = 0.117$; Table 2).

The plasma CAT activity in the patients with AIS (12.95 ± 2.86 kU/L) was significantly lower than in the healthy subjects (25.58 ± 13.50 kU/L, $p < 0.001$; Graph 1).

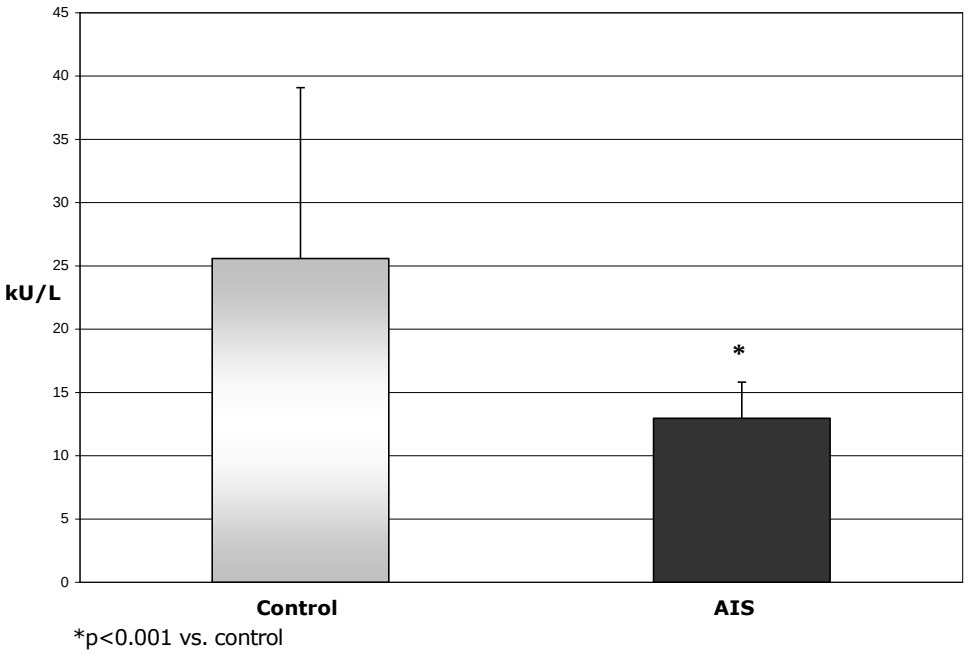
The patients, carriers of the polymorphic -262T allele (CT and TT genotype), had a significantly lower CAT activity in the plasma (11.93 ± 2.82 kU/L) compared to the CC genotype carriers (13.99 ± 2.59 kU/L, $p = 0.039$; Graph 2).

Table 1. Genotype frequencies of the CAT-262C/T polymorphism in AIS patients and controls

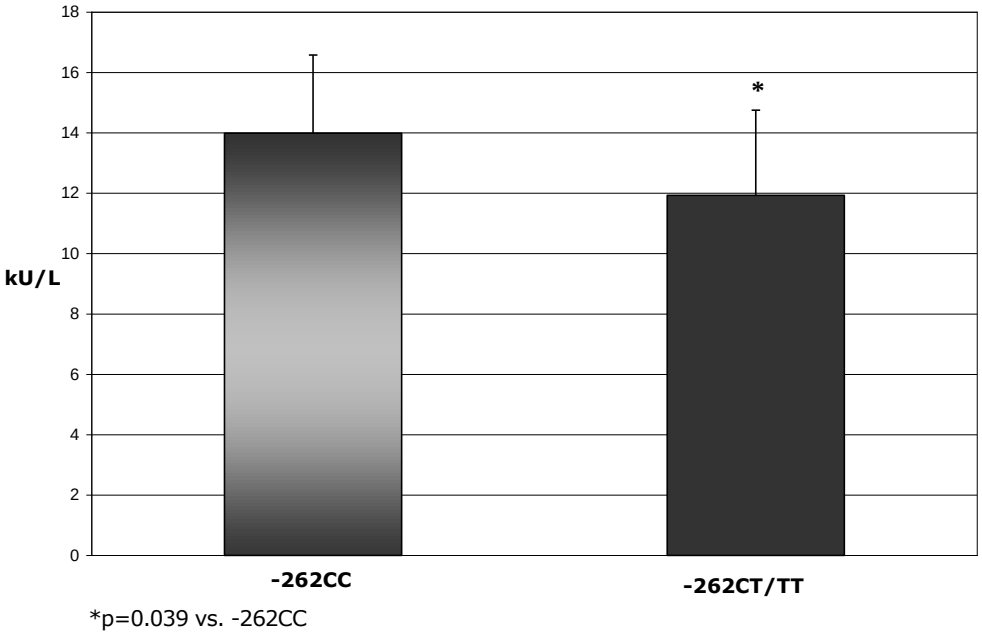
Genotype	AIS n = 34	Controls n = 32	P value (χ^2 test)
CC	16 (47.0%)	21 (65.6%)	
CT	14 (41.2%)	9 (28.1%)	0.306
TT	4 (11.8%)	2 (6.3%)	

Table 2. Allele frequencies of the CAT-262C/T polymorphism in AIS patients and controls

Allele	AIS 2n = 68	Controls 2n = 64	P value (χ^2 test)
C	46 (67.6%)	51 (79.7%)	0.117
T	22 (32.4%)	13 (20.3%)	



Graph 1. Plasma CAT activity in AIS patients and controls



Graph 2. Plasma CAT activity in AIS patients with -262CC and -262CT/TT genotypes of the CAT-262C/T polymorphism

Discussion

Although the CAT-262C/T polymorphism is one of the most analyzed CAT gene polymorphisms, there are no published data in regards to the role of this polymorphism in AIS. The results of this study showed no statistically significant differences in the distribution of genotypes of this polymorphism in AIS patients compared to the control group. Although the frequency of polymorphic T allele was higher in the patients than in healthy subjects, the difference was not statistically significant.

The plasma CAT activity in patients with AIS in this study showed a statistically significant decrease compared to the control group.

Although it is known that oxidative stress is one of the important pathophysiological mechanisms in ischemic stroke and that it is followed by an increased concentration of lipid peroxidation products (malondialdehyde) (9, 10, 19, 20), the results of different studies regarding the antioxidative enzymes activities in this disease are contradictory.

While one group of authors (10) found an increased CAT activity in RBC, 24h, 7 days and 3 months after the ischemic stroke attack, Sheikh et al. (21) did not find a significant difference in serum CAT activity in patients with AIS compared to that of the healthy subjects.

The results of this study are in agreement with the data gathered by the three groups of authors that found a significantly lower CAT activity in the RBC of patients, 24 h, 3, 5 and 7 days after the diagnosis of ischemic stroke, when compared to the control groups (9, 22, 23).

It is assumed that in stroke patients oxidative stress occurs not only because of the increased production of ROS, but also due to the impaired antioxidant defense system. CAT as an antioxidative enzyme is directly involved in the process of H_2O_2 elimination, which might lead to the conclusion that its high activity in plasma and RBC can have a protective effect on the neuronal damage caused by oxidative stress in stroke (24). Bearing in mind the role of oxidative stress in this disease, it is possible that the decline in CAT activity in our study was the result of the excessive accumulation of ROS generated in the brain and oxidative modification of the enzyme. On the other hand, the study results of Min et al. (25) showed that there was a reduced expression of CAT gene during ROS exposure caused by the hypermethylation of CpG region in the enzyme gene promoter, and Lehane et al. (26) indicated the correlation between the ischemic attacks and protein synthesis inhibition, it is possible that the decrease in the CAT activity in this study was the result of the reduced expression of this enzyme's gene in the conditions of oxidative stress.

So far there are no data on the functional significance of CAT-262C/T polymorphism and its possible impact on the CAT activity in AIS. The results of this study showed that patients, carriers of the polymorphic -262T allele (CT and TT genotype), have a significantly lower CAT activity in the plasma in relation to the carriers of the CC genotype.

Polymorphism at position -262 in the promoter region of CAT gene, wherein there is a substitution of cytosine by thymine, is one of the most widely analyzed polymorphism of the CAT gene. It has been shown that the polymorphism of this gene may affect its transcriptional activity by modulating the binding site for transcription factors, resulting in changes in the enzyme activity in RBC and plasma.

While Forsberg et al. (18) found a higher CAT activity in the RBC of 29 healthy donors, carriers of the polymorphic T allele, than in the CC genotype carriers, Ahn et al. (27) and Bastaki et al. (28), while conducting a study on a large number of healthy subjects (266 and 231, respectively), showed a lower CAT activity in the RBC of the polymorphic homo - and heterozygous than in the CC genotype carriers.

The reduced CAT activity in this study's patients with AIS, carriers of the polymorphic -262T allele, can support the study results which indicate a low enzyme activity in the CT and TT genotypes of this polymorphism. It is possible that the decrease in the plasma CAT activity in patients with AIS can be the result of not only the oxidative modification of CAT, but also of the presence of CAT-262C/T polymorphism. A research on a large number of patients is certainly necessary in order to investigate a possible association of this polymorphism with the risk of stroke, its effect on CAT activity, and the mutual influence with the variations in other genes associated with this disease. Furthermore, future research should focus on testing the potential protective effect of CAT-262GG genotype in ischemic stroke, as well as the role of this polymorphism in the possible selection of patients for antioxidant therapy.

Conclusion

This is the first study examining the CAT-262 C/T polymorphism and its influence on plasma CAT activity in ischemic stroke patients. Although patients diagnosed with AIS had a higher frequency of -262T allele compared to a group of healthy subjects, this difference did not reach significance. The plasma CAT activity in AIS patients was significantly lower as compared to the control group. The presence of polymorphic CAT-262T allele in patients with ischemic stroke significantly decreased the plasma CAT activity compared to the CC genotype carriers.

Acknowledgments

The financial support to this work by the Ministry of Education and Science of the Republic of Serbia (Project III41018) is gratefully acknowledged.

References

1. Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJ, Culebras A, et al. AHA/ASA Expert Consensus Document An updated definition of stroke for the 21st century a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2013; 44:2064-89. [CrossRef] [PubMed]
2. Suwanwela N, Koroshetz WJ. Acute ischemic stroke: overview of recent therapeutic developments. Annu Rev Med 2007; 58:89-106. [CrossRef] [PubMed]
3. Tsai NW, Chang YT, Huang CR, Lin YJ, Lin WC, Cheng BC, et al. Association between oxidative stress and outcome in different subtypes of acute ischemic stroke. Biomed Res Int 2014:256879. [CrossRef]
4. Chen SD, Yang DI, Lin TK, Shaw FZ, Liou CW, Chuang YC. Roles of oxidative stress, apoptosis, PGC-1a and mitochondrial biogenesis in cerebral ischemia. Int J Mol Sci 2011; 12:7199-215. [CrossRef] [PubMed]
5. Guzik TJ, Korbut R, Adamek-Guzik T. Nitric oxide and superoxide in inflammation and immune regulation. J Physiol Pharmacol 2003; 54:469-87. [PubMed]
6. Warner DS, Sheng H, Batinic-Haberle I. Oxidants, Antioxidants and the Ischemic Brain. J Exp Biol 2004; 207(18):3221-31. [CrossRef] [PubMed]
7. Kodydková J, Vávrová L, Kocík M, Žák A. Human catalase, its polymorphisms, regulation and changes of its activity in different diseases. Folia Biol 2014; 60 (4):153-67. [PubMed]
8. Paspalj D, Nikic P, Savic M, Djuric D, Simanic I, Zivkovic V, et al. Redox status in acute ischemic stroke: correlation with clinical outcome. Mol Cell Biochem 2015; 406(1-2):75-81. [CrossRef] [PubMed]
9. Milanlioglu A, Aslan M, Ozkol H, Çilingir V, Nuri Aydın M, Karadas S. Serum antioxidant enzymes activities and oxidative stress levels in patients with acute ischemic stroke: influence on neurological status and outcome. Wien Klin Wochenschr 2016; 128(5-6):169-174. [CrossRef] [PubMed]
10. Žitňanová I, Šiarnik P, Kollár B, Chomova M, Pazderova P, Andrezalova L, et al. Oxidative Stress Markers and Their Dynamic Changes in Patients after Acute Ischemic Stroke. Oxid Med Cell Longev 2016; 2016: 9761697.[CrossRef] [PubMed]
11. Available from: URL: http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000121691;r=11:34456149-34456964;t=ENST00000241052
12. Chistiakov A, Zotova EV, Savosťanov KV, Bursa TR, Galeev IV, Strokov IA, et al. The 262T>C promoter polymorphism of the catalase gene is associated with diabetic neuropathy in type 1 diabetic Russian patients. Diabetes Metab 2006; 32:63-8. [CrossRef] [PubMed]

13. Quick SK, Shields PG, Nie J, Platek ME, McCann SE, Hutson AD, et al. Effect modification by catalase genotype suggests a role for oxidative stress in the association of hormone replacement therapy with postmenopausal breast cancer risk. Cancer Epidemiol Biomarkers Prev 2008; 17(5):1082-7. [CrossRef] [PubMed]
14. Ezzikouri S, El Feydi AE, Afifi R, Benazzouz M, Hassar M, Pineau P, et al. Polymorphisms in antioxidant defence genes and susceptibility to hepatocellular carcinoma in a Moroccan population. Free Radic Res 2010; 44(2):208-16. [CrossRef] [PubMed]
15. Khodayari S, Salehi Z, Fakhrieh Asl S, Aminian K, Mirzaei Gisomi N, Torabidalivandan S. Catalase gene C-262T polymorphism: importance in ulcerative colitis. J Gastroenterol Hepatol 2013; 28:819-22. [CrossRef] [PubMed]
16. Hebert-Schuster M, Fabre EE, Nivet-Antoine V. Catalase polymorphisms and metabolic diseases. Curr Opin Clin Nutr Metab Care 2012; 15:397-402. [CrossRef] [PubMed]
17. Forsberg L, Lyrenas L, de Faire U, Morgenstern R. A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. Free Radic Biol Med 2001; 30:500-5. [CrossRef] [PubMed]
18. Goth L. A simple method for determination of serum catalase activity and revision of reference range. Clin Chim Acta 1991; 196(2-3):143-52. [CrossRef] [PubMed]
19. Nanetti L, Raffaelli F, Vignini A, Perozzi C, Silvestrini M, Bartolini M, et al. Oxidative stress in ischaemic stroke. Eur J Clin Invest 2011; 41(12):1318-22. [CrossRef] [PubMed]
20. Ozkul A, Akyol A, Yenisey C, Arpaci E, Kiylioglu N, Tataroglu C. Oxidative stress in acute ischemic stroke. J Clin Neurosci 2007; 14(11):1062-6. [CrossRef] [PubMed]
21. Sheikh N, Tavilani H, Rezaie A, Vaisi-raygani A, Salimi S. Relationship between estradiol and antioxidant enzymes activity of ischemic stroke. J Biomed Biotechnol 2009; 1-5: 841468. [CrossRef] [PubMed]
22. Cojocaru IM, Cojocaru M, Sapira V, Ionescu A. Evaluation of oxidative stress in patients with acute ischemic stroke. Rom J Intern Med 2013; 51(2):97-106. [PubMed]
23. Kocaturk PA, Akbostanci MC, Isikay C, Ocal A, Tuncel D, Kavas GO, et al. Antioxidant status in cerebrovascular accident. Biol Trace Elem Res 2001; 80:11524. [CrossRef] [PubMed]
24. Leinonen JS, Ahonen JP, Lonnrot K, Jehkonen M, Dastidar P, Molnár G, et al. Low plasma antioxidant activity is associated with high lesion volume and neurological impairment in stroke. Stroke 2000; 31:33-9. [CrossRef] [PubMed]
25. Min JY, Lim SO, Jung G. Downregulation of catalase by reactive oxygen species via hypermethylation of CpG island II on the catalase promoter. FEBS Lett 2010; 584(11):2427-32. [CrossRef] [PubMed]
26. Lehane C, Guelzow T, Zenker S, Erxleben A, Schwer CI, Heimrich B, et al. Carbimazole is an inhibitor of protein synthesis and protects from neuronal hypoxic damage in vitro. J Pharmacol Exp Ther 2013; 347 (3): 781-93. [CrossRef] [PubMed]
27. Ahn J, Nowell S, McCann SE, Yu J, Carter L, Lang NP, et al. Associations between catalase phenotype and genotype: modification by epidemiologic factors. Cancer Epidemiol Biomarkers Prev 2006; 15(6):1217-22. [CrossRef] [PubMed]
28. Bastaki M, Huen K, Manzanillo P, Chande N, Chen C, Balmes JR, et al. Genotype-activity relationship for Mn-superoxide dismutase, glutathione peroxidase 1 and catalase in humans. Pharmacogenet Genomics 2006; 16:279-86. [CrossRef] [PubMed]

Originalni rad **UDC: 577:616.831-005.1**
doi:10.5633/amm.2018.0415

UTICAJ CAT-262 C/T POLIMORFIZMA NA AKTIVNOST KATALAZE U PLAZMI BOLESNIKA SA ISHEMIJSKIM MOŽDANIM UDAROM

Jelena Bašić[1], Vuk Milošević[2], Milena Despotović[1], Tatjana Jevtović-Stoimenov[1], Tatjana Cvetković[1], Milica Živanović[1], Miroslava Živković[2,3], Dušica Pavlović[1]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za biohemiju, Niš, Srbija
[2]Klinika za neurologiju, Klinički centar Niš, Srbija
[3]Univerzitet u Nišu, Medicinski fakultet,Niš, Srbija

Kontakt: Jelena Bašić
Medicinski fakultet Univerziteta u Nišu
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: jelena.basic@medfak.ni.ac.rs

Iako je poznato da oksidativni stres ima važnu ulogu u etiopatogenezi akutnog ishemijskog moždanog udara (AIMU), uticaj -262C/T polimorfizma gena za katalazu (CAT) na njenu aktivnost u plazmi ovih bolesnika još uvek nije poznat.

Cilj rada bio je ispitivanje distribucije C gena za katalazu kod bolesnika sa AIMU u odnosu na zdrave ispitanike, kao i ispitivanje uticaja ovog polimorfizma na aktivnost katalaze u plazmi bolesnika.

Istraživanje je obavljeno na 34 bolesnika sa dijagnozom AIMU i 32 zdrava ispitanika. Polimorfizam CAT-262C/T ispitivan je metodom polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). Aktivnost katalaze u plazmi određena je spektrofotometrijskom metodom po Gothu.

Iako bolesnici sa dijagnozom AIMU imaju višu frekvencu polimorfnog -262T alela ispitivanog polimorfizma od zdravih subjekata, razlika nije statistički značajna ($p = 0,117$). Kod bolesnika sa dijagnozom AIMU značajno je niža aktivnost katalaze u plazmi (12,95 ± 2,86 kU/L) u odnosu na kontrolu (25,58 ± 13,50 kU/L, $p < 0,001$), pri čemu bolesnici koji su nosioci polimorfnog -262T alela imaju značajno nižu aktivnost enzima (11,93 ± 2,82 kU/L) u odnosu na nosioce CC genotipa (13,99 ± 2,59 kU/L, $p = 0,039$) CAT-262C/T polimorfizma.

Ovo je prvo istraživanje koje ispituje CAT-262C/T polimorfizam i njegov uticaj na aktivnost katalaze u plazmi bolesnika sa AIMU. Imajući u vidu da prisustvo polimorfnog -262 T alela kod bolesnika značajno smanjuje aktivnost katalaze u plazmi u poređenju sa nosiocima CC genotipa, buduća istraživanja trebalo bi usmeriti na ispitivanje potencijalnog protetektivnog efekta nosilaštva -262CC genotipa u AIMU.

Acta Medica Medianae 2018;57(4):110-116.

Ključne reči: *ishemijski moždani udar, katalaza, CAT-262C/T polimorfizam*

Review article

UDC: 616.853:577.175.6
doi:10.5633/amm.2018.0416

CATAMENIAL EPILEPSY- UPDATE ON PRACTICAL MANAGEMENT

Stevo Lukić

Catamenial epilepsy is a type of epilepsy that is characterized by aggravation and seizures clustering in a perimenstrual or periovulatory periods. Neuroactive properties of reproductive steroids and cyclic variations in their concentrations are important pathophysiological factors. Recent researches have demonstrated and confirmed the presence of at least three forms of catamenial aggravation of the attacks: perimenstrual and periovulatory in ovulation cycles and pattern throughout the whole luteal phase in anovulatory cycles. Rational models have identified that approximately one-third of women with epilepsy may have catamenial aggravation of the seizures. Open studies using cyclic natural progesterone as add-on therapy, medroxyprogesterone and gonadotropin-releasing hormone analogues have shown therapeutic benefits in certain forms of catamenial epilepsy. Therefore, it is important for the physician to consider catamenial epilepsy as a common type of epilepsy in women and recognize a particular pattern of this condition with the potential for good therapeutic response.

Acta Medica Medianae 2018;57(4):117-121.

Key words: *epilepsy, reproductive hormones, seizure clustering, menstruation*

Clinic of neurology, Clinical center Niš, Serbia
University of Niš, Faculty of Medicine, Niš Serbia

Contact: Stevo Lukić
Clinic of Neurology, Clinical Center Niš,
48 Dr. Zorana Djindjića Blvd. 18000 Niš, Serbia
E-mail: slukic@medfak.ni.ac.rs

Introduction

The term catamenial epilepsy refers to the aggravation of attacks during the various stages of the menstrual cycle in women with epilepsy. Catamenial epilepsy occurs in one-third to one half of women with epilepsy (1, 2) and is described in almost one-third of women with pharmacoresistent focal epilepsy (3). The basic principles have been described in the previous publications of the Yugoslav Union of Leagues Against Epilepsy (4, 5), and the aim of this paper is to make a brief overview of the latest achievements in this area.

Endogenic hormons and catamential epilepsy

It has long been known that estradiol has a provocative effect that makes women more susceptible to the seizures. On the contrary, progesterone and some of its metabolites reduce the incidence of epileptic attacks in women with epilepsy, which is associated with its anticonvulsant effects. Therefore, catamenial epilepsy may result from decrease in progesterone levels and/or a relative increase in estradiol/progesterone ratio (6).

In women with normal menstrual cycles, the most significant hormonal variations for catamenial exacerbation of the attack are the onset of a fast estrogen pulse on the 13th day (which induces ovulation) and a rapid drop in progesterone and estrogen from 26 to 28 days, just before the onset of menstrual bleeding.

The most common forms of catamenial epilepsy are:

1) *The perimenstructural pattern (C1)* is the most common form of catamenial epilepsy and is defined as maximum frequency of attacks during the menstrual phase (from the 25th day of the cycle to the third day of the next cycle). This pattern relates to decline in progesterone in perimenstrual periods.

2) *The periovulatory pattern (C2)* is the second most frequently observed pattern and is characterized by the maximum occurrence of attacks during ovulation (10-15 days). This form correlates with a rapid increase of estrogen in mid phase of ovulations.

3) *Luteal pattern (C3)* is characterized by the maximum frequency of attacks during ovulatory, middle luteal and menstrual phase (day 10 to day 3 of the next cycle), compared to the frequency of attacks in the middle follicular phases (3-10 days) in cycles with inadequate luteal phase. This is the third most commonly observed pattern and associates with a low level of progesterone seen in anovulatory cycles.

These three most frequent patterns of catamenial attacks have been confirmed in several studies. El-Khayat et al. described the case of women with catamenial attacks that correlate with a reduction in progesterone levels (7). Quite the opposite, another study pointed to variations in estrogen concentrations as an indicator of catamenial attacks, with no significant changes in progesterone levels (8). Several other studies have indicated the importance of the hormone changes in the onset and cyclical patterns of seizures, including anovulatory cycles in women with epilepsy (9-11). Therefore, both estrogen and progesterone are involved in mechanisms of catamenial epilepsy.

Furthermore, recent clinical studies have shown that women with catamenial epilepsy have better control of attacks during pregnancy, which is probably a consequence to the absence of cyclical hormone variations and the increase in circulating progesterone levels (12).

Specific animal models of catamenial epilepsy have been developed (13). They also approve the significance of neurosteroids in pathophysiological mechanisms.

The effects of neurosteroids that bring out the onset of catamenial epilepsy attacks include:

- premenstrual decline of anticonvulsant effects of neurosteroids via action on GABA-A receptors,
- alteration of the GABA-A receptor subunit and consequent changes in neural inhibition
- sudden peak of estrogen in days before ovulation and
- an increase in the frequency of anovulatory cycles due to the deregulation of the hypothalamic-pituitary-gonadal axis and, subsequently the luteal phase with a low progesterone level.

For example, the experimental model of catamenial epilepsy developed in female rats by hippocampal kindling method, points to the following mechanisms as the key to the onset of catamenial attacks: the premenstrual reduction of progesterone combined with plasticity and changes in GABA-A receptor function during menstrual phase (perimenstrual in humans) leads to exacerbation of seizures (13).

Treatment

Hormonal therapy

The majority of hormonal treatments for catamenial epilepsy involved add-on therapy with natural progesterone, synthetic forms of progesterone or therapy based on menstrual cycle suppression.

The degradation of natural progesterone to allopregnanolone, a neurosteroide with anticonvulsant effects, provides a potential therapeutic option for controlling the attacks. Two small, open studies evaluated natural progesterone as add-on treatment in women with complex partial seizures (14, 15). A study with a long-term follow-up (16) suggests that the use of natural progesterone, in the form of vaginal suppositories or oral lozenges, starting at the time of ovulation (day 14) to the beginning of the next cycle, is an effective treatment for catamenial epilepsy.

A randomized, double-blind, placebo-controlled multicenter study evaluated progesterone treatment in the form of 200 mg oral lozenges twice a day from 14^{th}-28^{th} day of the cycle (11). The positive response, defined as 50% or more reduction of seizures, did not differ between the progesterone and placebo groups when all patients were analyzed, or when comparing patients with or without catamenial epilepsy.

However, the secondary analysis indicated that the perimenstrual (C1) pattern of catamenial epilepsy is a predictor for the response to progesterone. If a woman has three or more times frequent seizures during the C1 phase (days -3 to +3) compared to other days of cycles, then 37.8% have a positive therapeutic response to progesterone while it was case in only 11.1% of patients in the placebo group. For women who have eight or more times frequent seizures during the C1 phase, almost 70% of them will have a good therapeutic response to progesterone. These results suggest that the degree of perimenstrual pattern of the attacks (C1 pattern) is a significant predictor for the response to progesterone therapy. Evidence supporting the efficacy of progesterone in patients with C1 type of attack suggests that benefits increase as the period of perimenstrual occurrence of attacks is shorter and closer to perimenstrual days. These results are in accordance with molecular and *in vivo* models (13, 17, 18). However, this hypothesis also points that catamenial worsening of attacks during the ovulation period cannot be effectively treated with progesterone.

Medroxyprogesterone acetate is a synthetic progesterone, usually used as contraceptive agent. The mechanism of its effect on reducing the seizures is unclear, but it is believed to be connected to interruption of cyclic variations in estrogen and progesterone levels. In patients with catamenial epilepsy, the use of medroxyprogesterone acetate was associated with a reduction in seizure frequency of 39% after one year of follow-up (19, 20).

Medroxyprogesterone acetate is administered in the form of an intramuscular injection and stops the regular menstrual cycle. The standard dose is 150 mg i.m. every 12 weeks. Some clinicians propose a dosing frequency every 10 weeks in order to reduce the risk of interaction with inductor AEDs. Additionally, medroxyprogesterone acetate is associated with higher risk of osteoporosis, as well as with therapy with inductor AEDs (21). Moreover, beyond cessation the medroxyprogesterone acetate therapy, endogenous hormones can vary significantly over the next few months, which can lead to worsening of the seizure control and a prolong period for return to normal fertility.

Some clinicians use a strategy to inhibit normal cyclic release of reproductive hormones by using continuous oral contraceptive pills that suppress ovu-

lation. So far, there is no data on the effectiveness of this strategy from controlled clinical studies.

Non-hormonal therapy

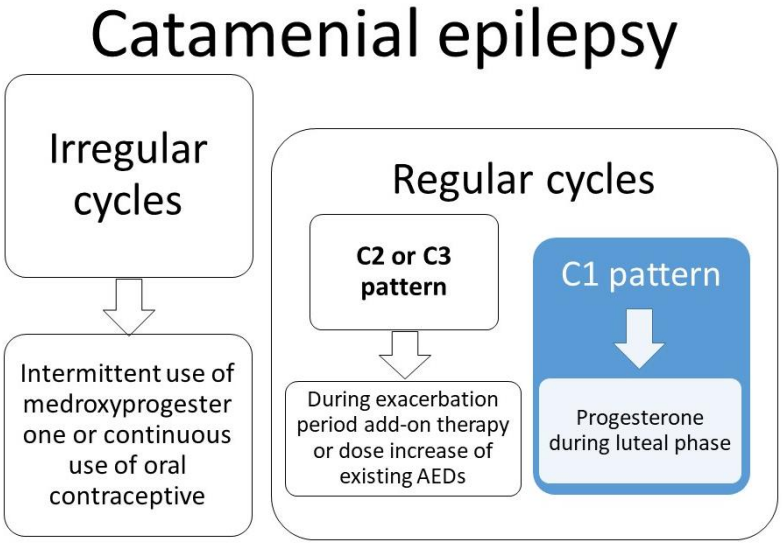
Most non-hormonal based medications were evaluated in the premenstrual (C1) pattern of catamenial epilepsy. Women with regular menstrual cycles are good candidates for these interventions, because medication must be taken a specific number of days after the onset of menstrual bleeding. Generally, treatment starts at some point during the second phase of the cycle (14^{th}-26^{th} day), depending on the individual form of the seizures, bearing in mind that the luteal phase lasts 14 days.

Acetazolamide is used to treat catamenial epilepsy for 60 years (22), although it has never been evaluated in randomized studies. Doses of 250-500 mg per day have been effective when administered 3-7 days before the start of the cycle (22, 23).

Benzodiazepines were used mainly for cessation of seizure clusters but were administered intermittently due to the risk of habituation and/or tolerance during chronic use. Clobazam is the only benzodiazepine which is formally evaluated for the treatment of catamenial epilepsy. In a double-blind, placebo-controlled, cross-sectional study, clobazam was associated with better control of the attack than placebo. Complete control of attacks was observed in most patients during the 10-day treatment (24).

Temporarily increasing the dose of existing AED therapy at the specific time during the menstrual cycle is another rational option. It is an empirical approach, although phenytoin should not be increased due to the risk of toxic effects associated with its non-linear kinetics.

Summary of therapeutic strategies for catamenial epilepsy is presented on Figure 1.



AED- Antiepileptic drugs;
C1- Perimenstrual pattern of catamenial epilepsy;
C2- Periovulatory pattern of catamenial epilepsy;
C3- Luteal pattern of catamenial epilepsy.
(For more details see text)

Figure 1. Summary of therapeutic strategies for catamenial epilepsy

Conclusion

As with most therapeutic approaches for treatment of epilepsy, there are no medication that fits for all patients. Adequate analysis of frequency and time of occurrence of attacks is the key to understanding pathophysiological mechanisms and the implementation of adequate therapy. In women with a clear perimenstrual pattern of seizures, the possibility of using natural forms of progesterone should be considered, with the initiation of treatment in accordance to the anticipated period of aggravation of the attacks. Therapeutic strategies must be made in agreement with the patient in terms of considering potential adverse effects of increasing existing therapy or short-term use of benzodiazepines or acetazolamide. Women with irregular cycles are probably not good candidates for intermittent interventions; they should consider options for therapy based on suppression of menstrual cycles, after discussing long-term consequences and side effects.

References

1. Foldvary-Schaefer N, Falcone T. Catamenial epilepsy: pathophysiology, diagnosis, and management. Neurology 2003;61(6 Suppl 2):2-15. [CrossRef] [PubMed]
2. Morrell MJ. Epilepsy in women: the science of why it is special. Neurology 1999;53(4 Suppl 1):42-8. [PubMed]
3. Herzog AG. Menstrual disorders in women with epilepsy. Neurology 2006;66(6 Suppl 3):23-8. [CrossRef] [PubMed]
4. Spasić M, Lukić S. Katamenijalna epilepsija. 3. Kongres epileptologa sa međunarodnim učešćem, Beograd 23.-26.04.2009. Savez liga za borbu protiv epilepsije Jugoslavije, 2009 (Beograd; Grafički dizajn).- elektronski optički disk (CD- ROM) ISBN 978-86-83665-05-1, COBISS.SR- ID 158066700:152-4. [CrossRef] [PubMed]
5. Spasic M, Lukic S. Uticaj steroidnih hormona na neuronsku ekcitabilnost i epilepsije u žena. U: Jović N. (urednik.) Odabrane teme iz epileptologije 1. XVI Jugoslovenski Simpozijum o epilepsiji sa međunarodnim učešćem. Savez Liga za borbu protiv epilepsije Jugoslavije. Grafomarket, Beograd 2001;176-89. [CrossRef] [PubMed]
6. Najafi M, Sadeghi MM, Mehvari J, Zare M, Akbari M. Progesterone therapy in women with intractable catamenial epilepsy. Adv Biomed Res 2013; 2:8. [CrossRef] [PubMed]
7. El-Khayat HA, Soliman NA, Tomoum HY, Omran MA, El-Wakad AS, Shatla RH. Reproductive hormonal changes and catamenial pattern in adolescent females with epilepsy. Epilepsia 2008; 49:1619-26. [CrossRef] [PubMed]
8. Hussain Z, Qureshi MA, Hasan KZ, Aziz H. Influence of steroid hormones in women with mild catamenial epilepsy. J Ayub Med Coll Abbottabad 2006; 18:17-20.
9. Murialdo G, Magri F, Tamagno G, Ameri P, Camera A, Colnaghi S, et al. Seizure frequency and sex steroids in women with partial epilepsy on antiepileptic therapy. Epilepsia 2009; 50:1920-6. [CrossRef] [PubMed]
10. Quigg M, Fowler K, Herzog A, NIH Progesterone Trial Study Group. Circalunar and ultralunar periodicities in women with partial seizures. Epilepsia 2008; 49:1081-5. [CrossRef] [PubMed]
11. Herzog AG, Fowler KM, Smithson SD, Kalayjian LA, Heck CN, Sperling MR. et al. Progesterone Trial Study Group. Progesterone vs. placebo therapy for women with epilepsy: a randomized clinical trial. Neurology 2012; 78:1959-66. [CrossRef] [PubMed]
12. Cagnetti C, Lattanzi S, Foschi N, Provinciali L, Silvestrini M. Seizure course during pregnancy in catamenial epilepsy. Neurology 2014; 83:339–44. [CrossRef] [PubMed]
13. Reddy DS. Catamenial epilepsy: discovery of an extrasynaptic molecular mechanism for targeted therapy. Front Cell Neurosci 2016; 10:1-15. [CrossRef] [PubMed]
14. Herzog AG. Intermittent progesterone therapy of partial complex seizures in women with menstrual disorders. Neurology 1986; 36:1607-10. [CrossRef] [PubMed]
15. Herzog AG. Progesterone therapy in women with complex partial and secondary generalized seizures. Neurology 1995; 45:1660-2. [CrossRef] [PubMed]
16. Herzog A. Progesterone therapy in women with epilepsy: a 3-year follow-up. Neurology 1999; 52:1917-8. [CrossRef] [PubMed]
17. Smith SS, Shen H, Gong QH, Zhou X. Neurosteroid regulation of GABA A receptors: focus on the α4 and δ subunits. Pharmacol Ther 2007; 116:58-76. [CrossRef] [PubMed]
18. Gulinello M, Gong QH, Li X, Smith SS. Short-term exposure to a neuroactive steroid increases α4 GABA A receptor subunit levels in association with increased anxiety in the female rat. Brain Res 2001; 910:55-66. [CrossRef] [PubMed]
19. Zimmerman AW, Holden KR, Reiter EO, Dekaban AS. Medroxyprogesterone acetate in the treatment of seizures associated with menstruation. J Pediatr 1973; 83:959-63. [CrossRef]
20. Mattson RH, Cramer JA, Caldwell BV, Siconolfi BC. Treatment of seizures with medroxyprogesterone acetate: preliminary report. Neurology 1984; 34:1255-8. [CrossRef] [PubMed]
21. Pitts CJ, Kearns AE. Update on medications with adverse skeletal effects. Mayo Clin Proc 2011; 86:338-43. [CrossRef] [PubMed]
22. Ansell B, Clarke E. Acetazolamide in treatment of epilepsy. Br Med J 1956; 1:650-61. [CrossRef] [PubMed]
23. Poser CM. Modification of therapy for exacerbation of seizures during menstruation. J Pediatr 1974; 84:779-780. [CrossRef]
24. Feely M, Calvert R, Gibson J. Clobazam in catamenial epilepsy: A model for evaluating anticonvulsants. Lancet 1982; 2:71-3. [CrossRef] [PubMed]

Revijalni rad **UDC: 616.853:577.175.6**
doi:10.5633/amm.2018.0416

KATAMENIJALNA EPILEPSIJA- NOVINE U LEČENJU

Stevo Lukić

Klinika za neurologiju, Klinički centar Niš, Srbija
Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

Kontakt: Stevo Lukić
Klinika za neurologiju, Klinički centar Niš
Bul. dr Zorana Đinđića 48, 18000 Niš, Srbija
E-mail: slukic@medfak.ni.ac.rs

Katamenijalna epilepsija predstavlja tip epilepsije koja se karakteriše pogoršanjem i grupisanjem napada u perimenstrualnom ili periovulatornom periodu. Neuroaktivna svojstva reproduktivnih steroida i ciklična varijacija u njihovim koncentracijama važni su patofiziološki faktori. Najnovija istraživanja su pokazala i potvrdila postojanje najmanje tri obrasca katamenijalnog pogoršanja napada: perimenstrualno i periovulatorno kod ovulatornih ciklusa i obrazac tokom cele lutealne faze kod anovulatornih ciklusa. Racionalni modeli su identifikovali da približno jedna trećina žena sa epilepsijom može imati katamenijalno pogoršanje napada. Otvorene studije koje su koristile ciklično dodavanje prirodnih progesteronskih preparata, depo medrokiprogesteron i analoge gonadotropin-oslobađajućeg hormona pokazale su terapijske benefite kod određenih formi katamenijalne epilepsije. Stoga je važno da lekar ima na umu katamenijalnu epilepsiju kao česti oblik epilepsije kod žena i da prepozna specifične kliničke obrasce koji mogu imati dobar terapijski odgovor.

Acta Medica Medianae 2018;57(4):117-121.

Ključne reči: *epilepsija, reproduktivni hormoni, grupisanje napada, menstruacija*

Original article

UDC: 614.2:004.45
doi:10.5633/amm.2018.0417

ANALYSIS OF THE LEVEL OF USE AND ACCEPTANCE OF THE MEDICAL INFORMATION SYSTEM IN PRIMARY HEALTH CARE

Petar Rajković, Dragan Janković, Aleksandar Milenković, Ivana Kocić

This paper presents the analysis of use and acceptance of the medical information system (MIS) within the primary healthcare. Analysis is mostly based on data from the Health Care Center Niš, although the conclusion was made on data from a dozen health care centers which use the medical information system MEDIS.NET developed within the Laboratory for Medical Informatics at the Faculty of Electronic Engineering in Niš. Analysis of the use of MIS is based on calculating the percentage of successfully entered records of visits, provided medical services, recipes, referral letters and physical examinations. In the context of the analysis of the use of MIS, successfully entered medical service is actually the service that was not changed or deleted later. Results of this analysis are significant for further technical development of the medical information system, and support the identification of these functionalities that are hardly accepted by the end-users and should be further developed. The acceptance of MIS is analyzed in the light of the technology acceptance model. Registration of provided services and keeping the record of physical examinations are taken as representative functionalities. Registration of provided services has been observed as a functionality that is accepted by the users due to simplicity of use (perceived ease of use), while the registration of physical examinations is observed as functionality is presumed to be accepted by the users as useful (perceived usefulness). For the functionalities with the expected acceptance based on the simplicity of use, the rate of correct data input is over 90% in each of the category. However, the rate of correct data inputs for visits and provided services is more than 99%. This is very significant having in mind the fact that these functionalities are often used and the high rate of incorrect inputs would slow down the work of doctors. On the other hand, the percentage of use of special functionalities for input of physical examinations varies considerably. Specially designed functionality for the most common physical examinations of children is used in more than two thirds of cases (sometimes more than 97%), while for the registration of adult's physical examinations the percentage is lower than 20%. Since the users could input data on physical examinations using the form for visit input, as well as with special form, they will probably use the other option only when the frequency of use is high enough or when the improved functionality of the specialized form provides improved system performances. Under users of MIS we consider the medical staff which uses MIS functionalities in accordance with their duties and privileges (doctors, nurses, medical technicians, etc.).

Acta Medica Medianae 2018;57(4):122-136.

Key words: *medical information system, technology acceptance model (TAM), assumed functionality usefulness, assumed simplicity of system use*

University of Niš, Faculty of Electronic Engineering,
Laboratory for Medical Informatics, Niš, Serbia

Contact: Petar Rajković
Aleksandra Medvedeva 14, lab 534, 18000 Niš, Serbia
E-mail: petar.rajkovic@elfak.ni.ac.rs

Introduction

Medical Information Systems (MIS) are designed in order to improve the work of healthcare institutions, enable better resources management and be a good basis for generating various types of reports (1). Even though they are developing during the last half century, their acceptance from the potential users did not always go smoothly (2). Globally, the trend of acceptance of medical information systems and their efficient use starts mid nineties (3), while the final expansion was actually during the first decade of the 21st century (4). Massive use of the medical information systems within the primary healthcare of the Republic of Serbia starts from 2010-2011 with the great support of the Ministry of Health. Health Care Center in Niš, as the leading regional health center, started the implementation of the medical information system as the pilot project with the Faculty of Electronic Engineering in Niš (5,

6). Information system, named MEDIS.NET had been in full use since January 2012, and after that was installed in twenty more healthcare centers in South and East Serbia.

After a four-year period, and active use of MEDIS.NET, it is possible, from the point of technology acceptance model, to analyse the effects of use of MIS. Our goal was to assess the level of acceptance of medical information system in general through the analysis of collected data, as well as to assess its individual parts that were given special attention during the development phase. Our basis for analysis was technology acceptance model (8) which considers the system acceptance through two categories of functionality – the ones presumed to be easy to use and the ones that enhance the efficiency of the healthcare institution.

There are a large number of papers dealing with the analysis of the MIS functionality acceptance. Within this research, the particular importance was given to the part (9) on detailed analysis of the use of medical records in primary health care. Beside this, the authors presented the overview of great number of positive and negative aspects of introducing the medical information systems as the collection of implementation strategies that contribute to system acceptance from end users.

In general, the usefulness of the system is commmonly marked as the key element of system acceptance (10). For users, a well designed software system that does not follow their working processes and needs is less significant. During the system development and in communication with potential users it was very important to mark the most useful things (11) and to develop the system in this direction. After the development phase is finished, and starts the system use phase, it is necessary to monitor the users' behaviour and make adaptations of critical parts.

This paper is the result of such monitoring and the results will be the basis for improvement of the most important parts of the MIS. As mentioned in (12) and (13), the acceptance of medical information systems is not a linear process and after initial analysis it is necessary to monitor users' behaviour and react to their changes, suggestions and recommendations.

Potential users could have different opinions on certain parts of the system, and the promptness of functionalities that are not considered as basic could vary a lot (14, 15). Although it is nowadays considered that the medical workers are determined to accept information technologies in their everyday work, a various operative inefficiencies of realized softwares could result with complete rejection of some initially good functionalities. On the other hand, some simple functionality could be identified by users as extremely important and generally could be very quickly accepted.

Material and methods

From the technical point of view, it was of great interest to analyse the system use by medical workers, and in accordance with this to define the guidelines for further development and adaptation of the existing functionalities, as well as forming internal recommendations for more efficient realization of new MIS segments. In order to get objective results, we followed the analysis of collected data for the period from 01/01/2012 to 31/12/2015. Results were processed through the prism of technology acceptance model (TAM). Figure 1 presents block scheme of TAM. Previous experience, domain knowledge and social context could be defined as so called external variable that affects the acceptance of a system. Accordingly, users will accept different system functionalities either because they perceive them as ease of use (Perceived Ease of Use - PEOU), or because they find them useful (Perceived Usefulness – PU). From the point of view of these two categories, the reaction of users considering the functionality will affect the level of functionality acceptance. Another measure for the level of acceptance is the number of mistakes, or corrected records. Ideally, the percentage of corrected records should be the lowest as possible and with the entities that are more frequently created should be lower.

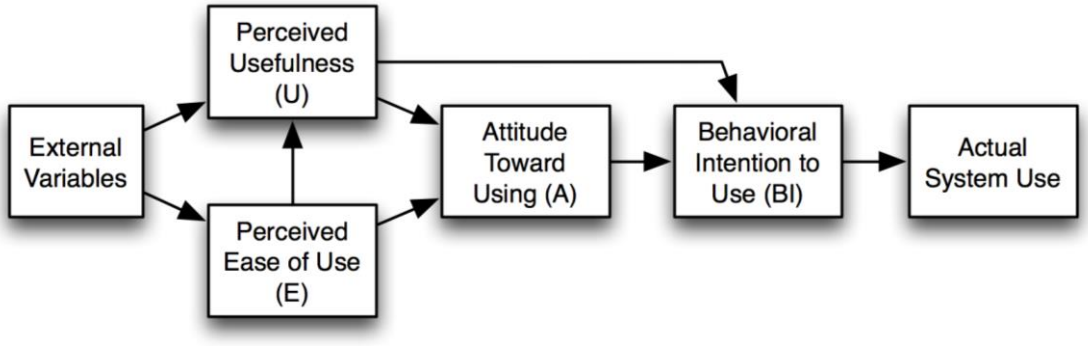


Figure 1. Block scheme of the Technology Acceptance Model (TAM) (8)

In this paper, the primary focus was given to three, from our point of view, the most significant set of entities processed by the medical information system, and these are provided services, referral letters and recipes. The most important entity defined at the level of MIS is provided service that presents the record on provided medical services to patients. They present the basis for all further actions, such as creation of report or invoicing of provided medical services. Also, they present the parent entity for all medical documents such as referral letters and recipes. Provided medical services are modeled in a way that every change upon them is recorded and kept in database as provided service. Once the provided service is changed, the previous version stays recorded as non-valid provided service and could be seen only through the process of revision and generating the report. Secondarily, we have analysed the use of specially defined forms for physical examinations, as specific categories that have specially defined forms for registration and data view. Basic part of the physical examinations is recorded as the entity of provided service, while the specially defined fields that are specific for physical examinations are kept separately.

For both focus points of our paper, the elements of MIS were mainly developed in such a way as to provide full support of established practice and to make it possible for the users to transfer from paper to electronic form of record keeping. Through PEOU approach, our idea was to enable users to initially accept MIS functionalities with simple and easy training, and later with system finishing, to focus on PU in order to increase the work efficiency and recognize the advantages of MIS use.

Results

First important thing that we have analysed is the percentage of successfully entered records for entities that describe provided services. Since MIS users in primary healthcare mainly have large number of patients, it is very important for system to be designed in such a way that users change or delete records as less as possible. Each of these actions is unnecessary waste of time and the general goal is to have less of these actions. For entities such as provided services that percentage should be less than 5%. The level of 95% of valid records means that users had to change or delete on average one out of twenty provided services. Having in mind that data from our MIS could not be deleted, but marked as non-valid in case of deletion or update, absolutely all created records are available from the database special reports or revision.

At the end of period of our research, in the fourth quarter of 2015, the percentage of valid provided services was 99.23 % (Table 1), the best quarterly result since the beginning in 2012. Initially, the percentage of valid provided services reached the level of 95% already in the second quarter of 2012.

Table 1. Percentage of valid provided services: A) quarter, B) year, C) number of registered provided services, D) number of valid provided services, E) percentage of valid services, F) number of active departments, G) number of active users, H) number of users who had non-valid services, I) percentage of users with non-valid services.

A	B	C	D	E	F	G	H	I
1	2012	50475	46870	92.86	16	136	95	69.85
2	2012	708596	678567	95.76	30	342	261	76.32
3	2012	679229	668100	98.36	31	339	232	68.44
4	2012	1234478	1218322	98.69	31	344	262	76.16
1	2013	1269571	1253785	98.76	31	342	253	73.98
2	2013	1212871	1190815	98.18	31	336	249	74.11
3	2013	1091818	1074589	98.42	31	338	238	70.41
4	2013	1389613	1368269	98.46	31	344	239	69.48
1	2014	1387759	1372180	98.88	32	319	229	71.79
2	2014	1263009	1251536	99.09	32	310	220	70.97
3	2014	1150759	1140641	99.12	32	305	203	66.56
4	2014	1464404	1451603	99.13	32	303	209	68.98
1	2015	1379097	1368216	99.21	32	303	213	70.30
2	2015	1321209	1310318	99.18	32	298	188	63.09
3	2015	1157166	1148156	99.22	33	296	195	65.88
4	2015	1486557	1475066	99.23	34	298	202	67.79

Initially, this percentage was 92.86% which was satisfactory. It is important to mention that during the first quarter of 2012, the system was used by 136 users who worked in 16 organizational units. At the same time, the training was provided for new users and many initially noticed disadvantages were corrected.

Already in second quarter of 2012, the number of users went up to 342, while the number of active departments went up to 30. Over time, the percentage of successfully recorded services had the mild uptrend, which could be explained by the fact that the medical staff was getting more and more secure and more easily accepted the provided technology. During the third quarter of 2012, the percentage of successfully recorded services went up to 98%, and in the second quarter of 2014 went over 99%.

With the percentage of correctly entered provided services, important factor is their total number, as well as the moment of increase of these recorded data. At the beginning (January – March 2012), the number of recorded visits was around a couple of tens of thousands a month. In the first quarter of 2012, over 50 thousand provided services were recorded. But, in the second quarter of 2012, a significant increase of registered visits arose and went over 700 thousand. In the third quarter of 2012 this number went down a bit, while in the fourth quarter, there were over a million registered records.

Also, each of these great lumps in number of registered services included a great lump in the number of users, as well as in number of departments that use MIS. For example, in April 2012 not only the number of services went up from 45 to 170 thousand, but the number of departments that use the system went up from 14 to 29, and the number of active users from 225 to 376. The biggest number of users in one month was 377, and it was registered in July 2013. After this we had the trend of decrease of users. In July 2014 the number of users went down to around 300 and stayed there until the end of 2015. The percentage of users who registered non-valid visits was around 70%. Although, a great number of users, from time to time, made mistakes during the recording of provided services, the general trend was positive.

On the other hand, the trend for referrals and recipes was a bit different. Table 2 presents general statistics related to referral letters. Having in mind the total number of created referrals, the trend was going up. The biggest number of registered referrals was recorded in the last quarter of 2015. Also, the number of valid referrals was bigger, but their percentage went down ovrt time. During the system development, the number of supported types of referrals was increased from initial 9 to current 13. Table 3 presents distribution of referral letters by type. Even though the number of created referrals was different from type to type, the highest percentage of non-valid was among the categories that were least used, while within the categories that cover the highest percentage of crated entities the percentage of non-valid was 10%. Similar trend could be recognized for recipes.

Table 2. Statistics related to the referral letters

Month	Year	Referral letters	Valid	Valid %	Type of referral letters	Urgent	Out of institution	Out of institution (%)
1	2012	3700	3481	94.08	9			
2	2012	47551	46838	98.50	10	16	7266	15.51
3	2012	59007	57443	97.35	10	26	27087	47.15
4	2012	70116	67739	96.61	11	79	32548	48.05
1	2013	67906	64941	95.63	11	72	30045	46.27
2	2013	55133	52392	95.03	11	71	23917	45.65
3	2013	59553	56250	94.45	11	76	26092	46.39
4	2013	69085	64425	93.25	11	91	28649	44.47
1	2014	68246	63619	93.22	11	100	28092	44.16
2	2014	61439	56839	92.51	11	133	26790	47.13
3	2014	61399	56336	91.75	12	78	26591	47.20
4	2014	79029	71709	90.74	12	97	33172	46.26
1	2015	81259	73057	89.91	12	70	33344	45.64
2	2015	78473	69892	89.07	12	66	32630	46.69
3	2015	73881	65183	88.23	12	59	30942	47.47
4	2015	92811	81930	88.28	12	86	38348	46.81

Table 3. Distribution of referral letters according to the type

Name	No. of referral letters	No. of non-valid	% of non-valid
Medical specialist report	717	65	9.07
Referral for intervention	5617	245	4.36
Referral for transport	665	35	5.26
General laboratory referral	112836	8750	7.75
Certificate on travel need for healthcare purposes	1107	49	4.43
Registration of disease	82	9	10.98
Registration of infectious disease	1515	58	3.83
Transfer referral - laboratory	82	13	15.85
Referral to specialist	595694	56628	9.51
Referral to medical commission	10368	372	3.59
Laboratory referral	137267	3046	2.22
Radiology referral	33752	3076	9.11
Stationary treatment referral	57637	4974	8.63

Table 4. Recipes: A) month, B) year, C) total number of recipes, D) number of valid recipes, E) percentage of valid recipes, F) number of patients, G) number of users, H) number of different prescribed medications, I) number of different diagnosis, J) number of recipes marked as the recipes of specific importance, K) percentage of recipes of special importance

A	B	C	D	E	F	G	H	I	J	K
1	2012	9092	8742	96.15	2889	80	603	362	634	7.25
2	2012	232432	222887	95.89	36838	148	923	1138	5532	2.48
3	2012	288942	267636	92.63	34441	142	885	1117	4557	1.70
4	2012	384943	352155	91.48	41254	145	893	1164	9476	2.69
1	2013	371961	342602	92.11	42504	147	930	1114	9943	2.90
2	2013	354373	325683	91.90	39755	146	1002	1111	7800	2.39
3	2013	361392	328673	90.95	40096	158	1045	1145	6666	2.03
4	2013	433702	395611	91.22	47068	161	1080	1218	7506	1.90
1	2014	422912	389569	92.12	46144	157	1059	1235	6685	1.72
2	2014	422006	386098	91.49	41994	156	1037	1227	6231	1.61
3	2014	402450	368834	91.65	44341	150	1042	1223	5918	1.60
4	2014	483711	442390	91.46	49933	154	1053	1320	6818	1.54
1	2015	448923	408087	90.90	49661	150	1068	1293	6394	1.57
2	2015	455558	412450	90.54	43728	150	1101	1274	6555	1.59
3	2015	422875	382723	90.50	42552	148	1096	1291	6381	1.67
4	2015	504459	456506	90.49	50705	152	1125	1331	7335	1.61

Table 4 presents general statistics for recipes. As the number of recipes went up, the percentage of non-valid was also increasing, similar like referrals.

Further, forms for physical examinations were addressed in this analysis as an addition to the system developed on the request of users in order to support one specific category of entity. Physical examinations could generally consist of few provided services. Table 5 presents data related to services that are usually registered through the form of phy-

sical examinations. It is important to mention that it is possible for users to register any kind of service, even the physical examination, through the standard form for registration of provided services. The level of acceptance is the best with the physical examinations for school children. The lowest percentage is with adults, where the most various services were registered with the form of physical examinations.

Table 5. Overview of using the forms for physical examinations:
A) number of recorded examinations through specific form, B) total number of recorded examinations,
C) percentage of examinations recorded through specific form

Type of examination	A	B	C
Infants (37 different services)			
Preventive examination of newborns and infants during the first year of life	23028	35967	64.03
Physical examination of newborns and infants until the first year of life	5697	10359	55.00
Physical examination of small children from age one to six years	865	11129	7.77
Preventive examination of children from age one to school age	1234	17746	6.95
Others	863		
Pre-school children (29 different services)			
Preventive examination of children from age one to school age	15299	17746	86.21
Physical examination of small children from age one to six years	3973	11129	35.70
Control examination of children, school children and youth	8406	32853	25.59
Preventive examination of newborns and infants during the first year of life	4711	46326	10.17
Control examination of children, school children and youth (regular, in case of monitoring of disabilities)	2918	32626	8.94
Preventive examination before referring to residential institution for children, school children and youth	1134	49263	2.30
Medical examination before referring to residential institution (kindergarten, summer school)	167	11208	1.49
Others	1787		
School children (24 different services)			
Preventive examination of school children and youth	38875	39727	97.86
Physical examination of school children and youth	8303	10142	81.87
Physical examination of children age from one to six years	1235	11129	11.10
Preventive examination of children age from one year to enrolment in school	715	17746	4.03
Others	520		
Adults (42 different services)			
Preventive examination of adults	7095	41089	17.27
Physcial examination of adults	1367	13088	10.44
Others	161		

Analysis and discussion

In the light of the TAM, registration of visits has been treated as PEOU. Users should find easy to use the forms for entity registration which they already are familiar with – in this case visits and provided services (Figure 2). As already mentioned, the percent-age of successfully created provided services is over 99%.

The difference in percentage of non-valid provided medical services (changed and deleted) is significant if we look at the departments which have

medical records and the ones that do not have (Table 6 and Table 7). In Primary Health Center Niš, a total of 7 departments have registration of services through medical records, while 33 departments do not have separate medical record. Also, within the 7 departments that have medical records, certain number of medical services is registered as direct medical services. Medical recording, from the point of view of TAM, could be identified as a significant external variable. Medical recording requires larger number of administrative operations than simple registration of provided medical services to patients. Users, who have the obligation to lead electronic medical record within MIS, have more contacts with the system and due to large scope of work they accept the MIS functionalities more quickly.

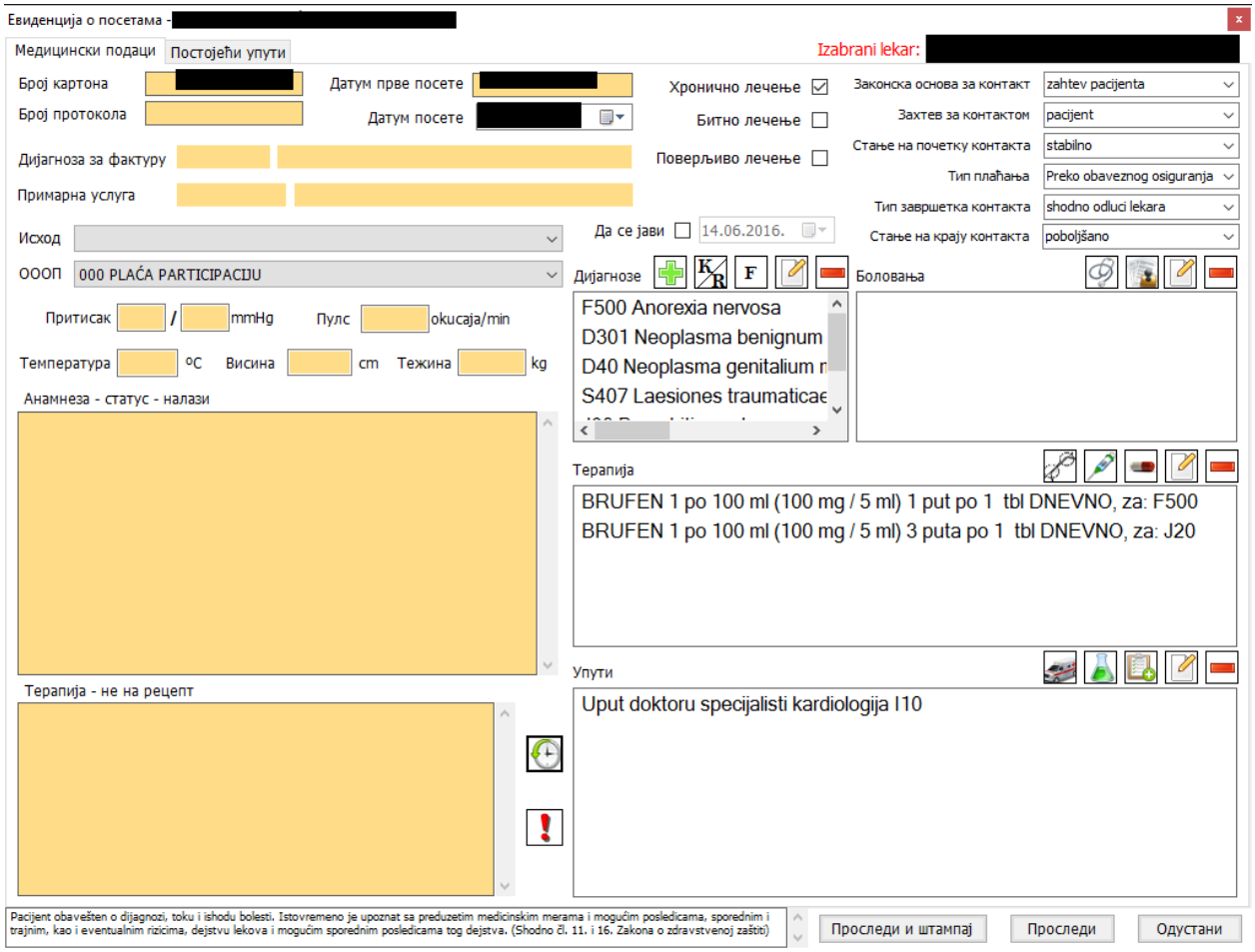


Figure 2. Example of entering the provided medical service using the form for entering the new visit from the electronic health record of the patient

In December 2015, 122 users recorded services that were registered using electronic medical record of the patient, while 298 registered services directly. Users that record services through electronic medical record of patient added on average 3264.71 services during December 2015, with an average of 6.61 non-valid records. This made 0.2% of changed or deleted services. Considering the services recorded out of patients' record, for the same month, average number of recorded services was 1275.84 where 13.02 were non-valid and that was 1.02%. At the beginning of MIS use, in January 2012, this percentage was 15.48% within the departments that had electronic medical records and 48.5% within the departments that did not have this type of recording. After three months this percentage went down below 5% within both categories.

Considering the recipes and referrals, the percentage of unchanged records is lower and it goes around 90%. Furthermore, after the initial percentage of valid records of over 95%, the increase of records, as well as the number of users that create these entities has lead to decrease of successfulness. Here, however, the records that are marked as invalid, and that could be excluded from the analysis (so called false positives) must be taken into account. We can divide them into following categories:

- Recipes and referrals that belong to deleted provided services and visits,
- Recipes and referrals created by coping the existing ones,
- Non-copied recipes and referrals deleted during the forming of provided service or visit.

Table 6. Statistics for departments that have medical records:
A) month, B) year, C) total number of registered visits, D) number of valid visits,
E) number of departments that have medical records, F) number of various registred services,
G) number of patients, H) number of MIS users, I) number of users with non-valid visits,
J) number of registered services by user, K) average number of non-valid services by user,
L) relative percentage of non-valid services.

A	B	C	D	E	F	G	H	I	J	K	L
1	2012	1374	1335	4	13	708	170	20	12.59	1.95	15.48
2	2012	2941	2854	6	34	2062	83	15	40.58	5.80	14.29
3	2012	7970	6781	5	42	5087	128	30	351.30	39.63	11.28
4	2012	29563	25366	5	72	17829	159	93	1077.47	45.13	4.19
5	2012	79105	70001	6	85	47645	170	146	1659.90	62.36	3.76
6	2012	80366	74987	7	75	50776	154	143	1656.46	37.62	2.27
7	2012	66563	64939	6	84	44766	145	130	1514.77	12.49	0.82
8	2012	52737	51607	6	74	36711	142	127	1548.35	8.90	0.57
9	2012	78407	76957	7	82	51312	145	136	1653.26	10.66	0.64
10	2012	101447	99146	7	93	61950	151	138	2990.11	16.67	0.56
11	2012	91998	89719	6	95	57594	148	143	2758.57	15.94	0.58
12	2012	87347	84903	5	94	55373	146	135	2566.46	18.10	0.71
1	2013	86529	84513	6	95	54670	148	140	2499.09	14.40	0.58
2	2013	97700	94616	7	139	60254	151	142	2818.68	21.72	0.77
3	2013	98966	96127	7	100	61102	154	144	3078.48	19.72	0.64
4	2013	95616	93318	5	98	59972	153	143	3071.34	16.07	0.52
5	2013	77343	75556	5	92	52798	150	141	2556.03	12.67	0.50
6	2013	76194	74620	5	84	50101	147	131	2445.93	12.02	0.49
7	2013	79197	77322	5	85	50477	147	130	2507.99	14.42	0.58
8	2013	75809	74360	6	101	50307	160	149	2117.28	9.72	0.46
9	2013	95000	92947	7	203	59880	166	158	2315.54	12.99	0.56
10	2013	115525	112259	6	118	66961	169	165	2902.99	19.79	0.68
11	2013	103787	101356	6	117	63037	167	163	2642.60	14.91	0.56
12	2013	111131	108123	7	117	65767	170	163	2692.31	18.45	0.69
1	2014	97017	95498	7	108	59893	165	155	2495.44	9.80	0.39
2	2014	109740	108530	7	114	66497	167	147	2783.47	8.23	0.30
3	2014	112076	110810	7	113	67589	166	147	3079.35	8.61	0.28
4	2014	101036	100012	7	111	64371	165	144	2635.93	7.11	0.27
5	2014	96364	95385	7	106	62577	164	141	2583.94	6.94	0.27
6	2014	93753	92922	6	106	59391	157	129	2575.25	6.44	0.25
7	2014	86060	85390	7	105	54774	157	129	2398.20	5.19	0.22
8	2014	84468	83741	7	104	53751	155	125	2250.10	5.82	0.26
9	2014	103496	102672	6	108	62846	153	123	2780.89	6.70	0.24
10	2014	124118	123154	7	114	71195	156	135	3333.49	7.14	0.21
11	2014	105864	104990	6	112	63841	161	148	2860.75	5.91	0.21
12	2014	119290	118384	7	119	69084	158	135	3062.01	6.71	0.22
1	2015	94558	93780	7	105	58907	154	134	2615.43	5.81	0.22
2	2015	101716	100943	7	111	63037	154	131	2862.98	5.90	0.21
3	2015	122722	121870	7	113	71275	155	138	3454.34	6.17	0.18

4	2015	105166	104372	7	117	64113	154	136	2914.14	5.84	0.20
5	2015	102323	101492	7	109	63359	152	130	2902.74	6.39	0.22
6	2015	100566	99667	7	107	61409	149	125	2894.06	7.19	0.25
7	2015	89848	89143	7	109	55785	149	129	2516.68	5.47	0.22
8	2015	85574	84895	7	112	54645	148	120	2383.06	5.66	0.24
9	2015	102971	102236	7	116	62752	152	130	2825.58	5.65	0.20
10	2015	120037	119078	7	116	69796	154	141	3270.90	6.80	0.21
11	2015	110859	109646	7	122	65989	155	140	3097.24	8.66	0.28
12	2015	122567	121635	7	122	70053	154	141	3264.71	6.61	0.20

Table 7. Statistics for departments that do not have medical records or have visit records out of the medical records.
A) month, B) year, C) total number of registered visits, D) number of valid visits,
E) number of departments that do not have medical records, F) number of various registered services,
G) number of patients, H) number of MIS users,
I) number of users with non-valid visits, J) number of registered services by user,
K) average number of non-valid services by user, L) relative percentage of non-valid services

A	B	C	D	E	F	G	H	I	J	K	L
1	2012	767	663	14	144	216	93	26	8.25	4.00	48.50
2	2012	427	359	16	131	147	53	24	8.06	2.83	35.17
3	2012	36996	34878	14	189	10267	136	95	272.03	22.29	8.20
4	2012	141755	138475	29	523	35470	344	232	412.08	14.14	3.43
5	2012	203078	199351	30	548	53442	352	268	576.93	13.91	2.41
6	2012	174729	170387	30	543	45453	342	261	510.90	16.64	3.26
7	2012	153078	151157	30	523	38752	331	182	462.47	10.55	2.28
8	2012	167128	164430	29	515	46395	337	228	495.93	11.83	2.39
9	2012	161316	159010	31	575	41738	339	232	475.86	9.94	2.09
10	2012	350059	346599	31	622	55188	351	266	997.32	13.01	1.30
11	2012	316271	313036	31	632	52444	350	274	903.63	11.81	1.31
12	2012	287356	284919	31	619	47973	344	262	835.34	9.30	1.11
1	2013	283336	280672	31	597	45159	339	242	835.80	11.01	1.32
2	2013	327920	325468	31	667	52341	338	245	970.18	10.01	1.03
3	2013	375120	372389	31	623	58251	342	253	1096.84	10.79	0.98
4	2013	374299	369126	31	620	57122	346	265	1081.79	19.52	1.80
5	2013	306061	300343	31	613	50048	340	257	900.18	22.25	2.47
6	2013	283358	277852	31	604	43637	336	249	843.33	22.11	2.62
7	2013	289478	285340	31	589	42519	376	242	769.89	17.10	2.22
8	2013	262955	258938	31	585	39775	375	228	701.21	17.62	2.51
9	2013	289379	285682	31	970	42725	338	238	856.15	15.53	1.81
10	2013	375081	370163	31	624	53166	336	270	1116.31	18.21	1.63
11	2013	337527	333430	31	617	48892	343	270	984.04	15.17	1.54
12	2013	346562	342938	31	614	48261	344	239	1007.45	15.16	1.51
1	2014	314730	311670	32	583	42449	331	227	950.85	13.48	1.42
2	2014	355100	350282	32	583	47309	323	252	1099.38	19.12	1.74
3	2014	399096	395390	32	588	51886	319	229	1251.08	16.18	1.29
4	2014	333893	330863	32	594	45898	331	235	1008.74	12.89	1.28

5	2014	327402	324810	32	587	45142	323	230	1013.63	11.27	1.11
6	2014	310561	307544	32	565	41115	310	220	1001.81	13.71	1.37
7	2014	290458	287773	32	559	38540	305	218	952.32	12.32	1.29
8	2014	264297	261945	32	560	34517	300	180	880.99	13.07	1.48
9	2014	321980	319120	32	576	42079	305	203	1055.67	14.09	1.33
10	2014	395907	392559	32	595	50869	306	226	1293.81	14.81	1.14
11	2014	354717	351297	32	580	43623	307	218	1155.43	15.69	1.36
12	2014	364508	361219	32	592	44911	303	209	1203.00	15.74	1.31
1	2015	308218	305898	32	588	38866	304	193	1013.88	12.02	1.19
2	2015	339183	336484	32	606	43312	303	205	1119.42	13.17	1.18
3	2015	412700	409241	32	601	50712	303	213	1362.05	16.24	1.19
4	2015	343611	340561	32	609	44465	302	212	1137.78	14.39	1.26
5	2015	338894	336191	31	605	43635	299	208	1133.42	13.00	1.15
6	2015	330649	328035	32	615	41488	298	188	1109.56	13.90	1.25
7	2015	285137	282989	33	607	35958	293	195	973.16	11.02	1.13
8	2015	267119	265167	32	604	34501	291	171	917.93	11.42	1.24
9	2015	326517	323726	32	619	40176	296	195	1103.10	14.31	1.30
10	2015	383682	380671	32	617	46632	304	221	1262.11	13.62	1.08
11	2015	369213	366467	32	612	44449	300	209	1230.71	13.14	1.07
12	2015	380199	377569	33	631	45384	298	202	1275.84	13.02	1.02

When provided service or visit is deleted, that action necessarily leads to deletion of all related documents. In this way all related referrals and recipes will be marked as deleted, even though they were not directly deleted. Having in mind the fact that the number of this kind of services and visits is very low, the percentage of recipes deleted in this way is around 3.65% (total number is 18575, and the number of non-valid recipes is over 509 thousand, Table 8).

Copying of existing referrals and recipes into the new visit or provided service is the functionality created to accelerate the work of system users. The main purpose is to enable the prescription of the chronic therapy to the patient (prescription of recipes for continuation of existing therapy) or the creation of another referral for patients who suffer from chronic disease, in cases where it is necessary (Figure 3). The basic mode for use is that the user should choose one of the existing recipes or referrals from the list and copy it within the existing visit. If in this case the user decides to change some of the parameters after the copying, one non-valid entity will be created. This category gives around 10% of the total number of non-valid recipes.

Also, since the data are not physically erased from the database during the work in MIS, prescribed recipes and referrals, either they were erased or updated during the creation of new visit or provided service, will be registered as non-valid. These entities do not get valid protocol numbers until the moment when the complete visit is not saved, so we can ignore them from the total number of non-valid entities. This is the largest group of non-valid recipes and it actually presents one third of the total number of non-valid ones.

When we eject the above mentioned recipes from the total number of recipes, the result is that the percentage of invalid recipes goes within the acceptable 5%. During a couple of quarters, the number of changed and deleted recipes goes over 5% and it is not significant (Table 8). From the point of accepting technology, these are expected results since all the above mentioned categories are entities created within the well known processes and there was no need to explain to users any additional elements. Therefore all the observed functionalities that are classified into PEOU category are accepted in satisfying way.

As the representative of PU category we have chosen the overview of the physical examinations. During the system development and considering the demands of users, they were mainly focused on specific design of input forms and comparative view of values (Figure 4). The whole functionality was developed with the aim to be used instead of standard function for data input on visits for physical examinations recording. Generally, a user can input data on physical examination both through specific and standard functionality. Specific functionality was supposed to be the first choice in most of the cases in order to justify its PU nature.

Table 8. Potential false positives for non-valid recipes: A) quarter, B) year, C) total number of non-valid, D) non-valid from erased services and visits, E) non-valid from copied entities, F) non-valid generated during the creation of provided service or visit, G) number of non-valid after taking out potential false positives, H) percentage of non-valid after deletion of false positives records.

A	B	C	D	E	F	G	H
1	2012	350	44	139	142	25	0.27
2	2012	9545	906	2281	3453	2905	1.25
3	2012	21306	300	3254	7926	9826	3.40
4	2012	32788	1197	3729	10060	17802	4.62
1	2013	29359	1172	3508	11044	13635	3.67
2	2013	28690	1090	3124	9669	14807	4.18
3	2013	32719	1257	2670	10103	18689	5.17
4	2013	38091	1863	3293	10872	22063	5.09
1	2014	33343	1759	3036	10143	18405	4.35
2	2014	35908	1544	3003	13065	18296	4.34
3	2014	33616	1157	3443	10989	18027	4.48
4	2014	41321	1327	3056	12488	24450	5.05
1	2015	40836	1190	3790	13324	22532	5.02
2	2015	43108	1304	3787	15395	22622	4.97
3	2015	40152	1088	4390	14370	20304	4.80
4	2015	47953	1377	4464	18615	23497	4.66

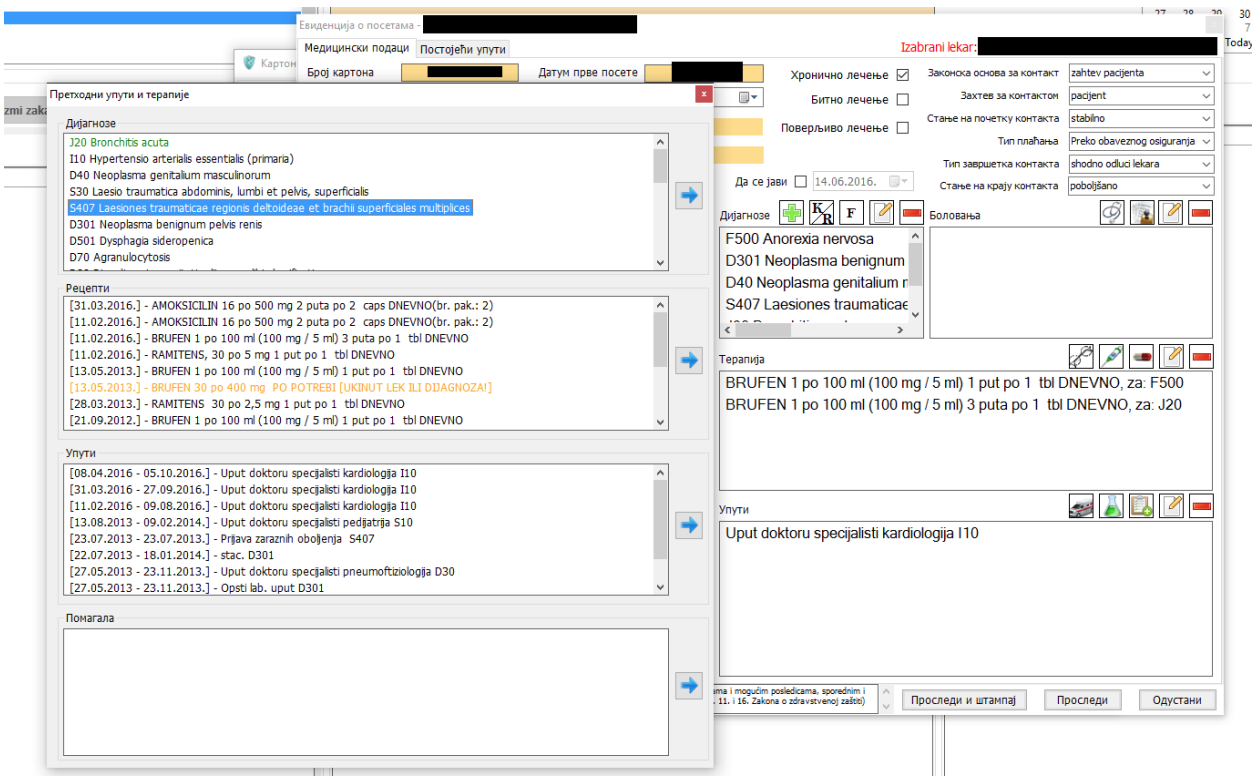


Figure 3. Copying the existing diagnosis, referral letters and recipes into the new visit/provided service

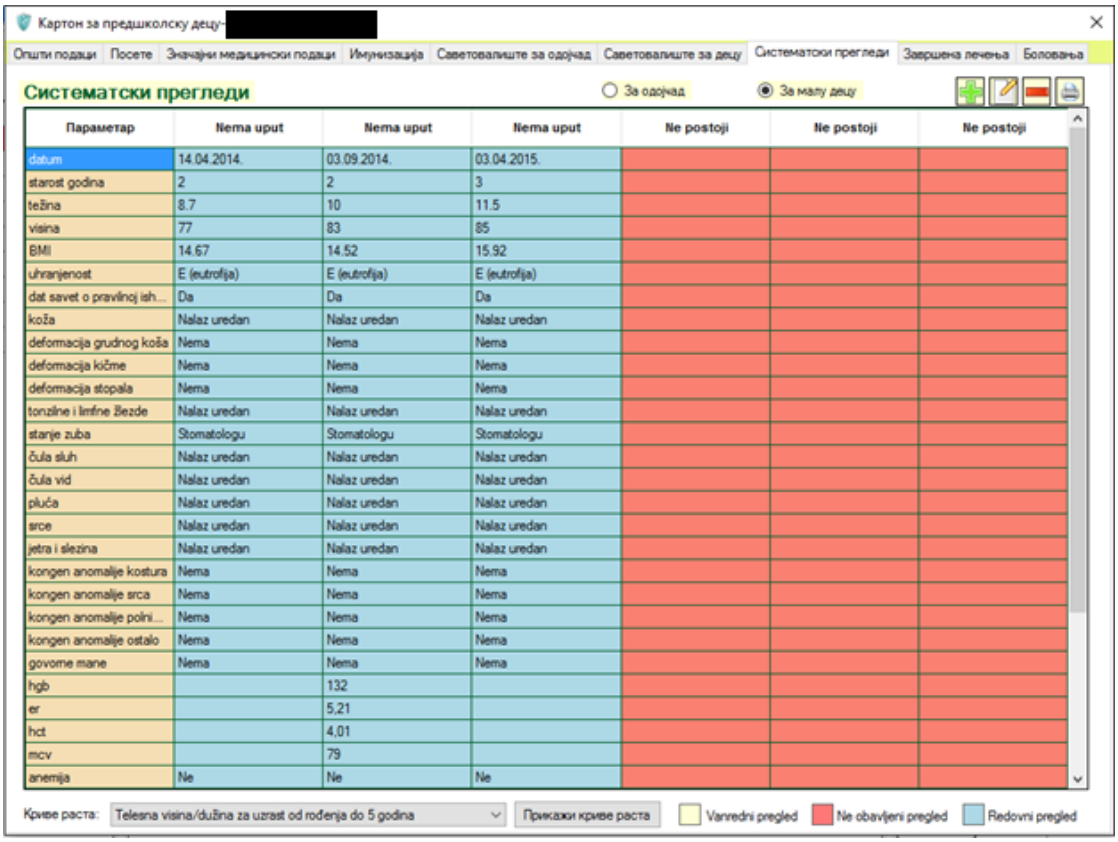


Figure 4. Comparative review of data obtained after the physical examinations.

Usage results may vary from department to department, as well as from the type of physical examination. The best percentage of acceptability is within physical examinations (81.87%) and preventive (97.86%) examinations of school children (Table 5). Within the physical examinations of school children there were totally 24 various services registered, at it is important to mention the examinations related to school enrolment. Their percentage is lower 11.1% and 4.03% while the percentage of use with all other registered services is insignificant. From the remaining 20 services there are 520 records that are made.

Considering the physical examination of pre-school children, the best indicator of acceptance is within the preventive examinations of children – 86.21%. None of the remaining services does have the usage percentage more than 50%, and only 7 of them is on the level of 1% and higher. Considering the examinations of infants, percentage of usage for PU functionalities is 64% for preventive and 55% for physical examinations. Here we can notice great number of various services for which the functionality has been used, but with the small percentage.

For preventive and physical examinations of adults, the percentage of acceptance for PU functionality is the lowest – 17.27%, or 10.44%. For physical examinations of adults there are totally 42 different services registered, but similar like with infants it has low percentage, less than 1%.

It is important to point out that these services are registered through various types of physical examinations; therefore in the overview given by the categories they have lower percentage of use for PU based functionality. For example, these services are *Preventive examination of children from age 1 to school age* and *Physical examination of children from age 1 to 6 years*. These services are registered through physical examinations of infants and pre-school and school children. There are totally 17746 registered preventive examinations, of which 15229 physical examination of pres-school children, 715 examinations of school children and 1234 infant examinations. It is totally 17248 from 17746 examinations. It represents more than 97% of all registered ones. For physical examinations the total percentage of registered ones is 55% (6023 out of 11129).

For registration of physical examinations through specific PU functionalities it can be concluded that the functionality itself has been used for large number of various services through each of offered forms, but only for couple of characteristic ones the percentage of use was significant. Users are usually using the above mentioned functionality for preventive and physical examinations of children, since they are carried out according to a predefined model and there is a detailed record about it. Unfortunately, there is no large number of registered physical examinations of adults, so the percentage of use for PU functionality is on the lower level than expected.

Conclusion

During the exploitation phase of the medical information system, very important activity is constant system improvement in order to achieve higher level of the efficiency and satisfaction of users. In order to improve MIS, it is necessary to conduct the data analysis on how the system is used after the appropriate period of exploitation. This paper shows the results of this analysis and adequate conclusions.

Considering the number of users and number of generated records we could be satisfied with the scope of system acceptance. The percentage of valid records is on the extremely high level considering the provided services (more than 99%), while the referrals and recipes are on satisfying 90%, or 95% when we drop out the records marked as false positives. It is confirmed that users do accept much easier and on a larger scale these functionalities which are considered easy to use, especially if they follow in detail the existing working processes. What we did not cover with this analysis is the quality of collected medical data. The validity of records has been measured only through users' actions aimed at their creation, deletion and updating.

Considering the acceptance of additional functionalities that are expected to provide additional system quality, on the example of physical examinations we have received high level of acceptance for those examinations which are more frequent and in larger number. The level of acceptance for physical and preventive examinations is much higher with physical examinations of children than with adults. Unfortunately, even the number of provided physical examinations of adults is lower due to the fact that there are many various examinations (our users have registered even 42 types of different physical examinations) and does not create the sense of need for use of special functionalities.

As already mentioned in (14), when accepting the new technology, the level of functionality acceptance, if presented through the acceptance model as simple, is very high. This is also significant because these functionalities are the ones that are often used and the high level of bad records would significantly slow down the work of doctors. In cases where users have the choice between basic and improved functionalities that presumably could contribute with their usefulness, the users will choose the other one only when the usage frequency is high enough or the improved functionality offers the obvious improvement of system performances.

References

1. Jha AK, Doolan, D, Grandt D, Scott T, Bates DW. The use of health information technology in seven nations. Int J Med Inform 2008; 77(12):848-54. [CrossRef] [PubMed]
2. Holroyd-Leduc JM, Lorenzetti D, Straus SE, Sykes L, Quan H. The impact of the electronic medical record on structure, process, and outcomes within primary care: a systematic review of the evidence. J Am Med Inform Assoc 2011; 18(6), 732-7. [CrossRef] [PubMed]
3. Li YC, Yen JC, Chiu WT, Jian WS, Syed-Abdul S, Hsu MH. Building a National Electronic Medical Record Exchange System – Experiences in Taiwan. Comput Methods Programs Biomed 2015; 121(1), 14-20. [CrossRef] [PubMed]
4. Boas SJ, Bishop TF, Ryan AM, Shih SC, Casalino LP. Electronic health records and technical assistance to improve quality of primary care: Lessons for regional extension centers. Healthcare 2014; 2(2):103-6. [CrossRef] [PubMed]
5. Rajković P, Janković D, Tošić V. A software solution for ambulatory healthcare facilities in the Republic of Serbia. In e-Health Networking, Applications and Services, 2009. Healthcom 2009. 11th International Conference on (pp. 161-168). IEEE. [CrossRef]
6. Rajković P, Janković D, Stanković T, Tošić V. Software tools for rapid development and customization of medical information systems. In e-Health Networking Applications and Services (Healthcom), 2010 12th IEEE International Conference on (pp. 119-126). IEEE. [CrossRef]
7. Boddy D, King G, Clark JS, Heaney D, Mair F. The influence of context and process when implementing e-health. BMC Medical Informatics and Decision Making 2009; 9(1):9.

https://doi.org/10.1186/1472-6947-9-9
8. Davis FD. (1989). Perceived usefulness, perceived ease of use, and user acceptance of information technology. MIS 1989; 319-40. [CrossRef]
9. Goetz GD, Kuzel AJ, Feng LB, DeShazo JP, Love LE. EHRs in primary care practices: benefits, challenges, and successful strategies. Am J Manag Care 2012; 18(2):48-54. [PubMed]
10. Jones SS, Rudin RS, Perry T, Shekelle PG. Health information technology: an updated systematic review with a focus on meaningful use. Ann Intern Med 2014; 160(1):48-54. [CrossRef] [PubMed]
11. Leonard, Kevin, and Dean Sittig. Improving information technology adoption and implementation through the identification of appropriate benefits: creating IMPROVE-IT. Journal of medical Internet research 9.2 (2007): e9. [CrossRef] [PubMed] https://www.jmir.org/2007/2/e9/, DOI: 10.2196 /jmir.9.2.e9
12. Kim J, Park HA. Development of a health information technology acceptance model using consumers' health behavior intention. J Med Internet Res 2012; 14 (5): 133. [CrossRef] [PubMed]
13. Ketikidis P, Dimitrovski T, Lazuras L, Bath PA. Acceptance of health information technology in health professionals: An application of the revised technology acceptance model. Health Informatics J 2012; 18 (2): 124-34. [CrossRef] [PubMed]
14. Meulendijk M, Spruit M, Drenth-van Maanen C, Numans M, Brinkkemper S, Jansen P. General practitioners' attitudes towards decision-supported prescribing: an analysis of the Dutch primary care sector. Health Informatics J 2013; 19(4):247-63. [CrossRef] [PubMed]
15. Dünnebeil S, Sunyaev A, Blohm I, Leimeister JM, Krcmar H. Determinants of physicians' technology acceptance for e-health in ambulatory care. Int J Med Inform 2012; 81(11):746-60. [CrossRef] [PubMed]

Originalni rad **UDC: 614.2:004.45**
doi:10.5633/amm.2018.0417

OSVRT NA KORIŠĆENJE I PRIHVATANJE MEDICINSKOG INFORMACIONOG SISTEMA U PRIMARNOM ZDRAVSTVU REPUBLIKE SRBIJE

Petar Rajković, Dragan Janković, Aleksandar Milenković, Ivana Kocić

Univerzitet u Nišu, Elektronski fakultet, laboratorija za medicinsku informatiku, Niš, Srbija

Kontakt: Petar Rajković
Aleksandra Medvedeva 14, lab 534, 18000 Niš, Srbija
E-mail: petar.rajkovic@elfak.ni.ac.rs

U ovom radu prikazana je analiza korišćenja i prihvatanja medicinskog informacionog sistema (MIS) u Domu zdravlja Niš. Analizirani su podaci prikupljeni u periodu od 1. januara 2012. do 31. decembra 2015. godine. Analiza uspešnosti korišćenja sistema bazirana je na računanju procenta uspešno unetih zapisa o posetama, pruženim medicinskim uslugama (datim uslugama), receptima, uputima i sistematskim pregledima. U kontekstu analize korišćenja sistema, uspešno uneta medicinska usluga je usluga koja kasnije nije menjana ili brisana. Kako se tokom rada iz MIS-a zapravo ne brišu zapisi, već se samo označavaju kao promenjeni ili obrisani, može se tačno odrediti broj i procenat zapisa koji nisu menjani nakon svog prvog snimanja. Rezultati ove analize su od značaja za dalji tehnički razvoj medicinskog informacionog sistema i pomažu u identifikaciji onih funckionalnosti koje krajnji korisnici teže prihvataju i koje je potrebno dalje usavršavati. Samo prihvatanje MIS-a je analizirano u svetlu modela prihvatanja tehnologije (technology acceptance model). Kao reprezentativne funkcionalnosti uzete su registracija datih usluga i vođenje evidencije o sistematskim pregledima. Registrovanje datih usluga je posmatrano kao funkcionalnost koju korisnici prihvataju zbog jednostavnosti korišćenja (perceived ease of use), dok je registrovanje sistematskih pregleda posmatrano kao funkcionalnost za koju se pretpostavlja da će biti ocenjena od strane korisnika kao korisna (percieved usefulness). Za funkcionalnosti kod kojih se očekuje prihvatanje na osnovu jednostavnosti korišćenja, stopa ispravnih unosa podataka je preko 90% u svakoj od kategorija. Šta više, stopa ispravnih unosa podataka kod poseta i datih usluga je više od 99%. Ovo je značajno zato što su te funkcionalnosti najčešće korišćene i visok stepen loših unosa bi u mnogome usporio rad lekara. Sa druge strane, procenat korišćenja posebne funkcionalnosti za unos sistematskih pregleda znatno varira. Dok se za najčešće sistematske preglede kod dece posebno dizajnirana funkcionalnost koristi u preko dve trećine slučaja (kod pojedinih i više od 97%), kod registrovanja sistematskih pregleda odraslih bolesnika taj procenat je niži od 20%. Kako korisnici mogu da unesu podatke o sistematskom pregledu i pomoću forme za unos posete, kao i pomoću posebne specijalizovane forme, korisnici će se opredeliti za korišćenje druge, tek onda kada je frekvencija korišćenja dovoljno velika ili kada unapređena funkcionalnost specijalizovane forme pruža očito poboljšanje performansi sistema. Pod korisnicima MIS-a smatraćemo medicinsko osoblje koje u skladu sa zaduženjima i privilegijama koje ima koristi funkcionalnosti MIS-a (lekari, medicinski tehničari,...).

Acta Medica Medianae 2018;57(4):122-136.

Ključne reči: *medicinski informacioni sistem, model prihvatanja tehnologije (TAM), pretpostavljena korisnost funkcionalnosti, pretpostavljena jednostavnost korišćenja sistema*

Original article

UDC: 159.953:811.111'367.625
doi:10.5633/amm.2018.0418

PROCESSING ENGLISH PREFIXED VERBS

Nikola Tatar

This paper takes for its main aim to question whether prefixed verbs (and other morphologically complex verbs) are more demanding for processing than "regular" ones through the reading experiment that was conducted among the senior students of the English Department at the Faculty of Philosophy. It was expected that the respondents would need more time for treating verbs with additional elements and mostly they did. However, while examining the results, our attention was drawn by the fact that besides semantic priming one has to take into consideration and account for the so-called structural or morphological priming. What is more, conclusions drawn from this research might be applicable to language processing in general, since it is almost widely agreed that language is processed in the same way regardless of the modality of the input signal, which may, as it is widely known, be visual or auditory. There is only one exception: written language employs the visual cortex as an input pathway, while spoken language makes use of the auditory cortex.

Acta Medica Medianae 2018;57(4):137-143.

Key words: *prefixed verbs, reading, language processing, priming*

Centre for Foreign Languages, Faculty of Philosophy, University of Niš, Serbia

Contact: Nikola Tatar
Jahorinska 1, 18000 Niš, Serbia
E-mail: nikolatatar@gmail.com

Introduction

In order to elucidate the experiment which was conducted and arrive at certain conclusions, it is palpably clear that prefixation and the recognition of written words will have to be dwelled upon. Since prefixation can be dealt with in brief for the purpose of this paper, it will be discussed in the introductory part, along with language processing. However, when it comes to the visual recognition of words, it will be debated in a separate section.

Therefore, if a prefix is defined as an affix that precedes its base, then prefixation can be very easily defined as the process of adding a prefix (dis + appear => disappear). The logical question that arises here is what happens when prefixes are attached to verbs in English. Unlike in Serbian, the only special phenomenon that happens in English is the creation of a new word, because all English prefixes are of derivational nature.

Our assumption in this paper would be that verbs with no additional elements will take the smallest amount of time to be recognized and that prefixed verbs would need more time because of an added element. Then we finally reach verbs which combined with prefixes give nonwords and which should, we believe, require the largest amount of time for visual recognition. By doing all this, certain conclusions concerning language processing in general will be arrived at because language is processed in the same way regardless of the modality of the input signal, which may, as it is widely known, be visual or auditory (1, 2, 3). Accordingly, before visual word recognition is dealt with, we ought to explain what is meant by language processing.

The above-mentioned phenomenon refers to the system how human beings process writing or speech and understand it as language. It should be highlighted that it does not matter what kind of stimulus is offered (visual or auditory), language processing will operate in an utterly similar way, with only one exception: spoken language employs the auditory cortex as an input pathway, while written language makes use of the primary visual cortex. Contemporary theories support the idea that this process is done completely by and inside the brain. The auditive organ receives acoustic stimuli and they are converted to bioelectric signals on the organ of Corti. These electric impulses are then transported through scarpa's ganglion (vestibulocochlear nerve) to the primary auditory cortex, on both hemispheres. What is important here is that each hemisphere treats it in a different way. While the right side is in charge of taking over prosodic characteristics and

melodic information, the left side recognizes distinctive parts such as phonemes. After this, the signal is transported to Wernicke's area on the left hemisphere where the already noted analysis takes part (the information which was being processed on the right hemisphere is able to cross through inter-hemispheric axons). The signal then travels from this area to Broca's area through what is referred to as the arcuate fasciculus. Broca's area is responsible for interpreting the information provided by Wernicke's area (using the pars triangularis) and it also transmits the information to the closely located motor-related areas of the brain for production of speech (relying on the pars opercularis) (4).

Written word recognition

Scientists have always wanted to know what processes the brain engages in when making the way from a visual input of crisscrossed lines and curves to making contact with meaning. This question has brought about a considerable amount of research, and it has generated findings that inform us of not only psycholinguistics but also of domains as diverse as computational modelling, automatic and attentional processes, pattern recognition, and the neural substrates of language processing. (5) Research at the word level is particularly manageable and illuminating as words are well-defined units which can be investigated and processed at different levels (i.e., spelling, sound, grammar, meaning) (6).

Researchers have developed many procedures in order to study the processes involved in word recognition. For instance, with the procedure of perceptual identification, words are visually degraded by masking or brief presentations, and subjects are asked to identify them, and in this particular case identification accuracy is the dependent measure. There are also eye-tracking studies, in which subjects' eye movements (e.g., fixation, location, and duration) are tracked as they read text. With semantic categorization tasks, researchers ask examinees to classify words (e.g., Is dog an animal?), and here response latency and accuracy are the dependent measures. In neuroimaging studies, conclusions about the processes included in word recognition are created from on-line measures of the time course and location of neural activity via event related potentials, positron emission tomography*, or functional magnetic resonance imaging. Individuals with isolated disruptions in reading (specific subtypes of dyslexia) are also studied by researchers so that they could better understand normal reading (5).

Although benefits could be found in each of these approaches, there are also some costs (7). Therefore, two relatively simple tasks (lexical decision and naming) are most heavily relied on when researchers want to investigate isolated word recognition. With the task called speeded lexical decision, examinees are presented with either a real word or a nonword (e.g., flirp), and their task is to make the word/nonword discrimination as quickly and as accurately as possible. There is also speeded naming, in which words (and very often nonwords) are visually presented to interviewees and they are asked to utter the words aloud as quickly and accurately as they can. In both tasks, scientists' primary interest is in how quickly people can name words or make lexical decisions in different experimental conditions. There is always the assumption that naming and lexical decision latencies reflect processes included in accessing lexical representations (9).

It would be useful to point out here that many models have been put forward which would explain how word recognition takes place. The logogen model was one of the earliest models that was proposed, and it postulates that there is a word detector (logogen) for every word in the reader's lexicon. Every logogen has a certain resting level of activation, and when a word is presented, the logogen for that particular word gathers evidence till a certain threshold is reached. It should be underlined that word recognition occurs at this juncture (10).

McClelland and Rumelhart used the logogen framework as a basis to develop a very influential model of word recognition. They named it the interactive activation model (IAM) and it includes three processing levels (visual, letter, word), while a node represents respective units within each level. When there is a visual input, it first stimulates feature-level nodes, which then activate letter level nodes, and finally word-level nodes (which correspond to logogens) are activated. Eventually, every node reflects the activation spreading across the units, and the effect of a variable can be tested by studying the value of a node after the specified amount of time has elapsed. The afore-mentioned model explains many findings in the literature. A good example would be the finding that people can identify frequently encountered words (world) more quickly than rarely encountered ones (such as glitch). This is the effect of word frequency, and it suggests the fact that high-frequency word nodes have lower recognition thresholds than low-frequency ones. So, the former need less evidence to be recognized, while the latter require more evidence (11).

There is one important aspect of the IAM that needs to be mentioned here and that is its parallel and cascadic nature. In particular, in the course of word recognition, we do not simply recognize a word independently of other words stored in our lexicon. Actually, it is many words that receive activation, and the model finally arrives at the appropriate representation across time, through a set of facilitatory and inhibitory pathways. One finding is interesting enough because it is in accordance with the activa-

* Positron Emission Tomography (PET) represents a technique for imaging internal body tissues in nuclear medicine. PET requires a cyclotron as an on-site source of short-lived positron-emitting isotopes. The isotopes are injected into the patient along with a glucose-related compound, and the positrons collide with electrons in body tissues to produce photons. The photons are tracked by a tomographic scintillation counter, and the information is processed by a computer to provide both images and data on blood flow and metabolic processes within the tissues that are observed. What everybody agrees on is that PET scans are particularly convenient and practical for diagnosing brain tumors and the consequences which strokes may have on the brain, along with various mental illnesses. PET scans also found their use in brain research and the mapping of brain functions (8).

tion of multiple words en route to recognition. It is called the orthographic neighbourhood effect. An orthographic neighbour stands for a word that can be produced by another word if only a single letter is changed (12). The following example is suitable to illustrate this point. The word can has the neighbours cap, cat, pan, man, among many others. The orthographic neighbourhood effect implies that words with more orthographic neighbours yield faster response latencies than words with few orthographic neighbours. This effect is larger for low-frequency words. Based on this, it does not come as a surprise that multiple lexical units seem to be activated when one word is presented. Of course, just the opposite pattern might have been expected, because of inhibition of competitors, a prediction from the original IAM. Even though contemporary embellishments of components of the interactive activation model can accommodate orthographic neighbourhood effects (5, 13), this is an important area that is still being actively researched and therefore we cannot include it in our experiment.

The experiment

This portion will begin with the description of the experiment, and the presentation of the results in two ways will follow. The results will be represented by using numbers (Table 3) and then the results will be discussed and certain inferences will be drawn.

When it comes to our experiment, which is of behavioural character, since a stimulus (a word snake) is offered to our participants and their latencies, or reaction times, are recorded, it should also be said that it represents a modification of self-paced reading. The test used for the experiment consists of six word snakes. Each word snake is comprised of five content words with no spaces between them, as it can be illustrated in Table 1. Every snake contains two nouns, two verbs and one adjective or adverb. It is of vital importance for this experiment to mention here which principles guided our choice of words.

Table 1. Problems (word snakes) presented to examinees

1)	yawnopportunitybulletcompeteawkwardly
2)	alternativebouncestifflyluggageinvestigate
3)	deliberatelyoverbuildartificiallypredominatepension
4)	genuinelyrearrangelaughterjewellerydisregard
5)	pronunciationpreimposerhythmquotealphabetically
6)	environmentallyelevatorurgeunbelievecircumstance

Table 2. Answers to word snakes

	Answers:
1)	yawn ı opportunity ı bullet ı compete ı awkwardly
2)	alternative ı bounce ı stiffly ı luggage ı investigate
3)	deliberately ı overbuild ı artificially ı predominate ı pension
4)	genuinely ı rearrange ı laughter ı jewellery ı disregard
5)	pronunciation ı preimpose ı rhythm ı quote ı alphabetically
6)	environmentally ı elevator ı urge ı unbelieve ı circumstance

English examples were chosen from the list of three thousand words which are offered by Oxford. The keywords of the Oxford 3000 have been carefully selected by a group of language experts and experienced teachers as the words which should receive priority in vocabulary study because of their importance and usefulness. All the words that make up the Oxford 3000 can be found at the appropriate website (14).

This group of words was chosen because it was thought that every candidate focused on them during his or her studies. As for examinees, there were ten of them and all of them were senior students of the English Department at the Faculty of Philosophy in Niš. Their task in this small-scale research was to make 5 meaningful units (words) by dividing each word snake with only four vertical lines. The amount of time which candidates needed to com-

plete each word chain was measured. The answers can be found in Table 2.

Before we move on to see the solutions, we should probably point out here that examinees could not see all the word snakes they had to split. The word chains were covered with another piece of paper while the experiment was being carried out. The chains were uncovered one by one as the candidates finished dealing with the previous word chain. In no possible way could they see the word snake which they were about to cope with.

It should also be said that if an interviewee does not complete the task in 15 seconds, he or she will have to stop and proceed to the next word snake. The same rule is applied to mistakes. If an interviewee has made a mistake he or she can rectify it if he or she has got enough time, i.e. if 15 seconds have not elapsed. It can be easily noticed that none of these figures in Table 3 reaches 15.00 seconds, which means that none of the examinees erred while they were "chopping" these strings of words.

Now it seems the right time to mention that these results are going to be contrasted within the language itself. But before we start doing any of this, we should probably first explain what phenomenon each pair of these word snakes questions.

The first two English word snakes simply question how much time it takes to visually recognize and divide verbs without any affixes. As it can be seen, the target verbs are: yawn, compete, bounce and investigate. The average times which are taken from these two examples will serve as some kind of standard value which will be compared to all the other average values. This does not mean that other average values will not be compared to one another, this basically means that the first average value will merely serve as a starting point.

Example 1	yawnopportunitybulletcompeteawkwardly
Example 2	alternativebouncestifflyluggageinvestigate
Example 3	deliberatelyoverbuildartificiallypredominatepension
Example 4	genuinelyrearrangelaughterjewellerydisregard
Example 5	pronunciationpreimposerhythmquotealphabetically
Example 6	environmentallyelevatorurgeunbelievecircumstance

The third and the fourth word snake, or the second pair of examples, is used to show how many seconds our candidates needed to visibly distinguish prefixed verbs. The verbs we are focused on here are: overbuild, predominate, rearrange and disregard. The average values which are calculated based on these two clusters of words will be of vital importance because the figures stand for the main issue we are examining here.

It should be said that the last pair of English examples is very significant for this paper. By offering these two word snakes to our examinees, we hoped to find out how many seconds it took the examinees to visibly diagnose that something is wrong here, i.e. to sight that the prefixes pre- and un- do not create meaningful prefixed verbs with verbs impose and believe (v. Table 3). Despite the facts that the examinees were not told anything about this and that they were expected to reach the maximum of 15 seconds here, our candidates dealt with this pair fascinatingly well. Not a single one of them made a mistake, and we will have to pay a lot of attention to examples 5 and 6 when we start giving explanations.

After a full account of the experiment has been given, and before we commence comparing calculations done based on the results of the experiment, attention should be called to the point that all the conclusions that will be drawn here refer only to the corpus of words that make up the word snakes.

Results

Only after we state a couple of facts about our anticipation of the results, will we start commenting the real results, which are gathered in Table 3. The table shows latency times for all word snakes, which are marked from E1 to E6, and for each candidate. After that, average values for each word snake and average values for pairs of word snakes which questioned the same phenomenon are presented.

It was expected that latency times for responses would grow with the addition of elements (prefixes) to verbs. In other words, we thought that candidates would need more time to graphically parcel out each following word snake or pair of word snakes due to its or their more complex structure. Naturally, it was believed that the largest amount of time would be required for the last two instances. Whether this was really accurate can be checked in the above-mentioned table, which will be used as a reference.

Table 3. Experiment results

CANDIDATES	E1	E2	E3	E4	E5	E6
CANDIDATE 1	11.72	6.44	8.94	13.86	9.28	6.66
CANDIDATE 2	11.34	8.47	9.50	12.87	12.53	8.97
CANDIDATE 3	10.57	7.68	8.83	10.54	10.06	9.28
CANDIDATE 4	12.60	8.19	10.79	9.88	11.50	10.07
CANDIDATE 5	11.54	9.88	11.06	10.48	12. 06	11.38
CANDIDATE 6	13.00	11.95	13.41	12.19	13.06	11.24
CANDIDATE 7	12.59	10.83	11.27	11.03	13.81	12.32
CANDIDATE 8	12.74	11.58	11.99	10.89	13.02	11.76
CANDIDATE 9	9.24	8.71	12.30	11.41	11.21	10.48
CANDIDATE 10	10.74	11.83	12.35	10.68	14.12	13.57
AVERAGE TIME	**11.60**	**9.55**	**11.04**	**11.38**	**12.06**	**10.57**
Average time for two examples	**10.57**		**11.21**		**11.31**	

Discussion

If we cast a quick glance at the average times, we will easily notice that the average times for the first example in each pair are larger than the second one. The only exception is the second pair of examples, in which the average time for the first example is smaller. But, let us get back to the phenomenon that can be ascribed to the remaining two pairs. This phenomenon can be explained by "morphological" priming. By solving the first word snake in one of the two pairs, the examinees must have somehow adapted their apparatuses which are used for visual word recognition for the structure of the target words in the second word snake and found it easier. Therefore, this can be seen as some kind of positive morphological priming. But, as this kind of priming cannot be attributed to the second pair of examples because E4 is larger in value than E3, we have to introduce the concept of negative morphological priming, which characterizes the second English duo.

The average latency times for the starting two examples are 11.60 seconds for the first one and 9.55 seconds for the second one. That makes the average of 10.57 seconds for these two instances. If we compare this result to the arithmetic mean for the second pair of examples which is 11.21, it can effortlessly be spotted that we were right: prefixed verbs necessitated students taking more time to finish the given task. Prima facie, it seems that, on average, the interviewees took additional 0.64 seconds to visually recognize prefixed verbs. However, when we compare E1 to E3 and E2 to E4, we can see that response times for E1 and E3 are almost the same and that E2 and E4 are the ones that create this difference, which can be attributed to the well-known element that is added in front of the verbs, also known as a prefix.

We have already pointed out the fact that there was no positive priming with the second pair of examples. With these two examples we can rather talk about negative priming. The difference between E3 and E4 is not as big as it may seem, it is 0.34 seconds, and some might say that this difference cannot be ascribed to negative priming due to its shortness. On the other hand, we have to take into consideration that E4 is the only second example out of all the pairs of word snakes which increased, i.e. latency times for E2 and E6 decreased in comparison to their respective counterparts, E1 and E5. Thus, it can be concluded that we can talk about negative priming with prefixed verbs. Since in our experiment we dealt with the verbs which with prefixes yield non-words (examples 5 and 6), we should underline that negative priming is only connected to prefixed verbs which have meaning.

When the last pair, or examples 5 and 6, is taken into consideration, it can be seen that E5 and E6 did not bring the discrepancy between our expectations and the statistic for the final pair. It was reckoned that the contestants of the research would most arduously find the solution to the last pair of examples, because the verbs in combination with prefixes yielded non-words. Since we have covered all 6 examples, it can now be said that we have two word snakes with the largest average time, they are E5 and E6. This indicates that these two examples

along with the problem they carry are the most challenging to handle. In comparison with the first pair of examples, it can be perceived that the interviewees were in need of additional 0.34 seconds when they were solving the third pair of examples. It is obvious, but we must mention it again, students found the first two examples easy and the third pair the most difficult due to the facts that the former have "no-additional-element" verbs and the latter are combined with an additional prefix with which they created non-existing words, which must have puzzled the examinees most. The difference between the first and the last pair of is 0.74 seconds on behalf of the last pair, while the difference between E3 and E4 on the one hand and E5 and E6 on the other amounts to 0.10 seconds.

Another aspect of the last pair should be looked at and that is whether positive priming is expressed. From the very first sight, positive priming is noticeable. The first word snake in the last couple was unravelled in 12.06 seconds and the second word snake in 10.57 seconds. Positive priming here adds up to 1.49 seconds, which makes it the second largest in terms of positive priming. The first pair is typified by the largest amount of positive priming and it is 2.05 seconds. As it has already been said, only one pair of examples is characterized by negative priming and that is the second duo: interviewees finished E4 in 0.34 seconds faster than E3.

Conclusion

The main purpose of this paper was not to compare many authors' points of view on the aforementioned topics, but to create a theoretical background for our research. It was reckoned that explaining the word detector model (logogen) and the interactive activation model (IAM) would be sufficient for visual recognition and language processing in general. The results which were obtained only from the examples showed that more complicated verbs were mostly recognized more slowly. However, what is mainly perceived with these instances is the so-called morphological priming. This phrase actually means that spotting the written form of a word which is characterized by a particular morphological structure (in our case, a verb is this word to which structural elements are added, primarily prefixes) would either delay or accelerate visual recognition of a next word which is morphologically the same.

After the experiment, which consisted of ten word chains containing verbs with different extra elements, has been carried out among the senior students of English, we first contrasted the results, and based on this comparison, particular conclusions can be drawn. First, English prefixed verbs are more complicated for recognition and language processing than simple verbs, which can be ascribed to the morphological complexity, i.e. additional elements. Cutler states that bases and affixes (including prefixes) are processed separately, so basically with prefixed verbs students had to deal with two units, not just one (15). Second, nonword prefixed words are even more complicated for processing and recognition, most likely due to their complexity and most of all impossibility to be combined. Finally, morphological priming, be it positive or negative, is a feature that must be taken into consideration with this kind of research.

References

1. Mildner V. The Cognitive Neuroscience of Human Communication. New York: Taylor & Francis Group; 2007.
2. Keith R, Kiefte K Kiefte M. In: Traxler MJ, Gernsbacher AM, editors. Handbook of Psycholinguistics. USA: Academic Press; 2006. p. 153-99.
3. Kutas M, Van Petten CK, Kluender R. In: Traxler MJ, Gernsbacher AM, editors. Handbook of Psycholinguistics. USA: Academic Press; 2006. p. 659-724. [CrossRef]
4. Pinker S. The Language Instinct. New York: W. Morrow and Co; 1994. [CrossRef]
5. Brown K. Encyclopedia of Language and Linguistics. 2nd edition. Elsevier; 2005.
6. Balota DA. Visual word recognition: the journey from features to meaning. In: Gernsbacher M A, editor. Handbook of psycholinguistics. San Diego: Academic Press; 1994. p. 303-48.
7. Balota DA, Cortese MJ, Sergent-Marshall SD, Spieler DH, Yap MJ. Visual word recognition of singlesyllable words. J Exp Psychol Gen 2004; 133(2):283-316. [CrossRef] [PubMed]
8. Microsoft® Student 2009 [DVD]. Redmond, WA: Microsoft Corporation; 2008. [CrossRef] [PubMed]
9. Seidenberg MS. Lexical access: another theoretical soupstone? In: Balota DA., Flores, d'Arcais GB,Rayner K, editors. Comprehension processes in reading. Hillsdale: Lawrence Erlbaum Associates; 1990. p. 33-71.
10. Morton J. A functional model for human memory. In: Norman DA, editor. Models of human memory. New York: Academic Press; 1970. p. 203-60. [CrossRef]
11. McClelland JL, Rumelhart DE. An interactive activation model of context effects in letter perception, part 1: an account of basic findings. Psychological Review 1981; 88: 375–407. [CrossRef]

12. Coltheart M, Davelaar E, Jonasson J, Besner D. Access to the internal lexicon. In: Dornic S, editor. Hillsdale: Lawrence Erlbaum Associates; 1977.
13. Grainger J, Jacobs AM. Orthographic processing in visual word recognition: a multiple read-out model. Psychological Review 1996; 103: 518-65. [CrossRef] [PubMed]
14. Available from: URL: http://www.oup.com/eltnew/catalogue/teachersites/oald7/oxford_3000/oxford_3000_list?cc=global
15. Cutler A, Hawkins JA, Gilligan G. The suffixing preference: A processing explanation. Linguistics 1985; 23: 723-58. [CrossRef]

Originalni rad

UDC: 159.953:811.111'367.625
doi:10.5633/amm.2018.0418

MENTALNA OBRADA ENGLESKIH GLAGOLA SA PREFIKSOM

Nikola Tatar

Univerzitet u Nišu, Filozofski fakultet, Centar za strane jezike, Niš, Srbija

Kontakt: Nikola Tatar
Jahorinska 1, 18000 Niš, Srbija
E-mail: nikolatatar@gmail.com

Glavni cilj ovog rada je da se kroz eksperiment vizuelnog prepoznavanja, koji je sproveden među studentima završne godine Departaman za anglistiku Filozofskog fakulteta, ispita da li su glagoli sa prefiksom (kao i drugi morfološki kompleksni glagoli) teži za mentalnu obradu od onih ,,običnih". Očekivalo se da će ispitanicima biti potrebno više vremena za obradu glagola sa dodatnim elementima i to je uglavnom bilo tačno. Međutim, prilikom obrade rezultata, skrenula nam je pažnju činjenica da pored semantičkog prajminga, treba uzeti u obzir i objasniti takozvani strukturalistički ili morfološki prajming. Štaviše, zaključci doneti na osnovu ovog istraživanja mogli bi se primeniti na razmatranja o obradi jezika u opštem smislu, budući da je opšte poznato da se jezik procesuira uvek na isti način, bez obzira na modalitet inputa, koji, kako je poznato, može biti vizuelni ili auditivni. Postoji samo jedna razlika, pisani jezik angažuje vizuelni korteks kao ulazni put, dok govorni jezik angažuje auditivni korteks.

Acta Medica Medianae 2018;57(4):137-143.

Ključne reči: *glagoli sa prefiksom, čitanje, mentalna obrada jezika, prajming*

Case report **UDC: 618.5-089.888.61:616.853:616.831**
doi:10.5633/amm.2018.0419

SUBDURAL HEMATOMA WITH SYMPTOMS OF EPILEPTIC ATTACKS AFTER SUBARACHNOIDAL ANESTHESIA – A CASE REPORT

Emilija Ivanov[1], Dafina Karadzova[1], Ana Doneva[2], Jordan Nojkov[3], Atanas Sivevski[1]

The study describes a case of subdural hematoma developed after cesarean section in a 34-year-old patient with normal intra-operative course.

During the first twelve hours after the operation, the patient had a headache considered as post-dural puncture headache (PDPH) and was treated in that direction.

After the third operative day the headache was reduced, and on the fifth day the patient was discharged from the hospital in good condition. As soon as the patient was discharged, the headache appeared again with stronger and persisting intensity and at the end it was accompanied by epileptic seizure. MR scan showed subdural hematoma in absorption. After conservative therapy, the condition was improved and the patient was without neurologic consequences. The differences between PDPH and other types of headache, as well as the potential etiopathogenesis of subdural hematoma in obstetric patient, are discussed in this study. We have come to the conclusion that after the long persisting headache, if we take into consideration this complication as a possibility, the early diagnosis and adequate treatment could lead to complete recovery.

Acta Medica Medianae 2018;57(4):144-147.

Key words: *subdural hematoma, cesarean section, post-dural puncture headache, neuraxial block, subarachnoid anesthesia*

[1]University Clinic of Ginecology and Obstetrics, University "Ss.Cyril and Metodius", Skopje, FYR Macedonia
[2]General Hospital 8 September, Skopje, FYR Macedonia
[3]Goce Delčev University of Štip, Štip, FYR Macedonia

Contact: Emilija Ivanov
E-mail: ivanovemilija@gmail.com

Introduction

Spinal anesthesia (SA) is a standard anesthesia for cesarean section both in the world and in the FYR Macedonia. The side effects of this type of anesthesia are headache, sickness, nausea as well as vomiting, bradycardia, hypotension and urinary retention.

The headache after SA is relatively frequent accompanying phenomenon which is usually considered to be the result of dural puncture (PDPH). One should have in mind different diagnoses of headaches of other etiology such as migraine, pains in the temples or frontal pains. However, in everyday work we rarely think of another type of headache as a symptom, except of PDPH due to their infrequent occurrence.

In this study we present a case of post spinal headache, the etiology of which was hemorrhage, i.e., development of subdural hematoma.

Case report

A 34-year-old female, non-smoker patient who did not consume alcohol. There was no medical history of allergy on food and drugs. Familial anamnesis was negative. The performed routine tests including the number of thrombocytes and coagulation status were within normal limits.

The patient was scheduled for elective cesarean section in the 38th week (3rd pregnancy). She underwent elective surgical intervention under spinal anesthesia. Spinal anesthesia was performed at L3-L4 using 26G needle. During puncture there was clear liquid, thus Bupivacain 0.5% 10 mg and Fentanyl 0.002 mg were administered. The block was performed in a seated position. Blood pressure, pulse, number of respirations and oxygen blood saturation (SpO2) were controlled every five minutes the whole time. Throughout the intervention, the vital parameters were stable and in normal limits. The operation was unremarkable and lasted 40 minutes. During the intervention, the patient was administered 2x500 ml 0.9% NaCl and 500 ml Ringer solution. Postoperatively she was administered antibiotic Ceftriaxon 2.0 gr, antiemetic Reglan (Metoclopramide) and Ranitidine 2 x 50 mg, analgesic Ketoprofen 2 x 100 mg,

Tramadol 2 x 100 mg, low molecular weight heparin (enoxaparin) 0.4 ml (40mg) and uterotonic Ergotyl (methylergometrine maleate) 3 x 1. In the period of 24 hours postoperatively, 2000 ml crystalloids were administered intravenously (iv). The patient was recommended to rest 24 hours postoperatively and to take liquids per os after the termination of SA motor block. Anamnestically her past medical history was unremarkable – without previous serious diseases. The patient had two deliveries formerly, both of them performed with cesarean section. Postoperatively, the laboratory results were within normal limits.

Twelve hours after the intervention, there was an onset of a headache, which was considered and treated as post-dural puncture headache (PDPH). Analgesics were administered iv and liquids per os were continued. The headache weakened in a lying position, while it increased while getting up, which led us to the assumption that it was PDPH. The following 48 hours the headache was relieved and as the patient was in good condition she was discharged from the hospital. When she got home the headache was persisting and it did not weaken when the patient was inactive, that is, in a lying position. Since the patient had continuous headache, she went to the Clinic of Neurology where computer tomography was performed. The scan was normal, that is, there were no pathologic substrates. The patient was sent home again, however, she had two epileptic attacks and forty days after the Cesarean section she was hospitalized at the Department of Neurology as an urgent case, due to acute onset seizures (focal motor secondary generalized). During the examination by the physician in attendance, the patient was slightly confused. The neurologic status was as follows: slower reaction of the left pupil of the eye. The remaining cranial nerves were in normal condition. The arms were kept in normal position with discretely pronated right hand, the legs could not be kept all together due to the recent cesarean section. Babinski test showed light reflex asymmetry on the right (positive), the left was negative. MR of the brain was made in standard pulse sequences and planes. Non-contrast TOF angiography of intracranial blood vessels, as well as venography, were additionally performed. Small irregular hypersignal change (residual blood products in chronic resolution), sequela of the previous hematoma, was detected left high parietal cortically.

The following investigation were performed during hospitalization: routine analyses, biochemical analyses, as well as thyroid status, hemostasis factors and urinary status. All the results were within reference values. EEG finding was pathologically changed. EEG with activation methods, unstable, desynchronized basic brain activity, showing degradation and slowing/deceleration during hyperventilation, teta to delta range, as well as separate groups of sharp and slow waves with noticed predilection over the left parietal electrode's. During hospitalization, the patient was treated with anti-edematous therapy – corticosteroids, antiepileptic drugs, antibiotic, gastro protection. During hospital stay, the patient was stable, without seizures. The condition was improved with the therapy. After seven days, control MR scan was made which showed that subdural hematoma was completely withdrawn. There was no indication for surgical treatment. The therapy ordinated by the neurologist continued until the control check-up.

Discussion

Spinal anesthesia is most commonly used for cesarean section both in the world and in the FYR Macedonia. Complications of all types of neuraxial anesthesia are rare, however, they can be very severe. One of the most serious complications of neuraxial anesthesia is the onset of spinal and epidural hematoma. The incidence of these severe complications is really very low, and it is significantly lower in obstetric population than in non-obstetric population. In the study of Rosero and Joshi (1) from 2016, who investigated the complications in more than 3,700,000 epidural anesthesia (out of which more than 2,300,000 were obstetric epidural analgesia), the incidence was 0.6 per 100,000 cases. On the other side, the onset of intracranial subdural hematoma after neuraxial anesthesia,as it is in our case, is even a more rare complication, and the precise incidence is unknown. Several descriptions of cases have been reported, in obstetric population around 60, after neuraxial analgesia, but the real number is probably bigger.

The mechanism of subdural hematoma development is considered to be the same as in the development of post-puncture headache (PDPH)(2). During spinal puncture, an opening in the dura is formed, through which cerebrospinal fluid/liquid is coming out, intraspinal and intracranial pressure is decreased, which causes caudal withdrawing of brain structures and tearing of intracranial and subdural veins consequently by which subdural hematoma is developed. Dural gap/hole or fistula may persist up to 18 weeks after the puncture as a result of spinal puncture (3), while the quantity of liquid that is lost can be even greater than 200 ml per day (4), which exceeds the normal production of liquid.

On the other hand, the cerebral subdural hematoma can develop spontaneously in patients in the course of the delivery where anesthesia and dural puncture are not used. The increased intracranial pressure while coughing, Valsalva maneuver, may cause bleeding to which subdural portions of the connecting veins are particularly sensitive (5). Also, it is known that pregnancy increases the risk of brain stroke, and according to certain studies the incidence of brain bleeding is increased in the first 6 weeks after giving birth (6).

Due to the development mechanism, the symptoms that appear in PDPH are similar to those in subdural hematoma. Most frequently, it is the headache which may be accompanied by pain in the neck, nausea, vomiting, photophobia, diplopia, dizziness, as well as eye and hearing problems (7).

The most characteristic is the type of the headache. The headache develops or it worsens on straightening and getting up posture, while it is lost or improved in a lying position being a result of intra-

cranial hypotension. When subdural hematoma develops, intracranial pressure increases and in one moment the headache can be lost temporarily. It has been described in several cases (8, 9), which also happened to our patient. On the fifth day, her headache was very weak and that is why she was discharged from the hospital. Probably, it was the moment when the subdural hematoma developed.

By increasing of intracranial pressure, the postural headache transforms into non-postural and that is actually the main symptom that should give sign that something serious is happening. In the late review of Cuyperset et al. (10) from 2016 who analyzed 56 cases of intracranial subdural hematoma in obstetric population, this symptom which appeared in 83% of female patients was the most important symptom in differential diagnosis of subdural hematoma. As a result of the increased intracranial pressure, convulsions and other focal neurologic symptoms may appear that point out to serious complications. In our case, there was a transfer from postural to non-postural headache and later on convulsions developed that were a clear indication that something serious was happening. After MR was carried out, the diagnosis was made that it was subdural hematoma.

There are several risk factors that may lead to subdural hematoma development. They are: cerebral atrophy, dehydration, cerebrovascular malformations, multiple punctures, puncture with big needle and use of anticoagulants. Cuyperset et al. (10) presented 56 female patients in their literature review where risk factors were present only in a small number of patients. There are several case reports (11, 12) presenting patients who developed intracranial subdural hematoma after receiving heparin for thromboprophylaxis.

In addition, the description of one case (13) from 2017 shows development of subdural hematoma after long-term consumption of aspirin. One study (14) which investigated the risk for intracranial hemorrhage, demonstrates that long-term consumption of aspirin increases the risk of hemorrhage though the risk is very small. Otherwise, in patients who consume aspirin mortality is higher if hemorrhage develops (15).

In our case, the patient was administered anticoagulant prophylactic therapy the first 7 days postoperatively, the first dose 12 hours after giving birth and afterwards 1 dose in 24 hours. Thus, it is possible that anticoagulant drugs had their role in the development of subdural hematoma.

Conclusion

Intracranial subdural hematoma as a consequence of neuraxial anesthesia in obstetric patients is very rare, however, it is a particularly serious phenomenon. When a patient complains of strong, persistent headache following regional anesthesia, which is not relieved after the use of conservative measures, the possibility of the existence of subdural hematoma should be considered. Awareness and careful examinations in these cases are obligatory if we want to reach an early diagnosis and adequate treatment before there is irreversible neurologic misfortune or even death.

References

1. Rosero EB, Joshi GP. Nationwide incidence of serious complications of epidural analgesia in the United States. Acta Anaesthesiol Scand. 2016; 60: 810-20. [CrossRef][PubMed]
2. Macon ME, Armstrong L, Brown EM. Subdural hematoma following spinal anesthesia. Anesthesiology 1990; 72: 380-1. [CrossRef][PubMed]
3. Gass H, GoldsteinAS, Ruskin R, Leopold NA. Chronic postmyelogram headache. Arch Neurol 1971;25:168-70. [CrossRef][PubMed]
4. Franksson C, Gordth T. Headache after spinal anesthesia and a technique for lessining its frequency. Acta Chir Scand 1946; 94: 443-54. [PubMed]
5. Yamashima T, Friede R L. Why do bridging veins rupture into the virtual subdural space? J Neurol Neurosurg Psychiatry 1984; 47: 121-7. [CrossRef][PubMed]
6. Sharshar T, Lamy C, Mas J L. Incidence and causes of strokes associated with pregnancy and puerperium. Stroke 1995; 26: 930-6. [CrossRef][PubMed]
7. Mokri B. Headaches caused by decreased intracranial pressure: diagnosis and management. Curr Opin Neurol 2003; 16: 319-26. [CrossRef][PubMed]
8. Zeidan A, Farhat O, Maaliki H, Baraka A. Does postdural puncture headache left untreated lead to subdural hematoma? Case report and review of the literature. Int J Obstet Anesth. 2006; 15: 50-8. [CrossRef][PubMed]
9. Yildirim GB, Colakoglu S, Atakan TY, Büyükkirli H. Intracranial subdural hematoma after spinal anesthesia. Int J Obstet Anesth. 2005; 14: 159-62. [CrossRef][PubMed]
10. Cuypers V, Van de Velde M, Devroe S. Intracranial subdural haematoma following neuraxialanaesthesia in the obstetric population: a literature review with analysis of 56 reported cases. Int J Obstet Anesth. 2016; 25: 58-65. [CrossRef][PubMed]
11. Ortiz M, Aliaga L, Baturell C, Preciado MJ, Aguilar J, Vidal F. Intracranial subdural haematoma-a rare complication after spinal anesthesia. Eur J Anaesth. 1991; 8: 245-8. [PubMed]

12. Cantais E, Behnamou D, Petit D, Palmier B. Acute subdural hematoma following spinal anesthesia with a very small needle. Anesthesiology. 2000; 93: 1354-5. [CrossRef][PubMed]
13. 13. Yuri Iwase, Manzo Suzuki, Hiroyasu Bito. A case report of intracranial hemorrhage after spinal anesthesia. JA Clin Rep. 2017; 3: 11. [CrossRef][PubMed]
14. García Rodríguez LA, Martín-Pérez M, Hennekens CH, Rothwell PM, Lanas A. Bleeding risk with long-term low-dose aspirin: a systematic review of observational studies. PLoS One. 2016; 11: e0160046. [CrossRef][PubMed]
15. Saloheimo P, Ahonen M, Juvela S, Pyhtinen J, Savolainen ER, Hillbom M. Regular aspirin-use preceding the onset of primary intracerebral hemorrhage is an independent predictor for death. Stroke. 2006; 37: 129-33. [CrossRef][PubMed]

Prikaz bolesnika

UDC: 618.5-089.888.61:616.853:616.831
doi:10.5633/amm.2018.0419

SUBDURALNI HEMATOM SA SIMPTOMIMA EPILEPTIČNOG NAPADA NAKON SUBARAHNOIDALNE ANESTEZIJE – PRIKAZ SLUČAJA

Emilija Ivanov[1], Dafina Karadžova[1], Ana Doneva[2], Jordan Nojkov[3], Atanas Sivevski[1]

[1]Univerzitetska Klinika za ginekologiju i akušerstvo Univerziteta Sv. Kiril i Metodije, Skoplje, BJR Makedonija
[2]Opšta Bolnica 8 Septembar, Skoplje , BJR Makedonija
[3]Univerzitet Goce Delčev, Štip, BJR Makedonija

Kontakt: Emilija Ivanov
E-mail: ivanovemilija@gmail.com

U radu se opisuje slučaj subduralnog hematoma koji se razvio posle carskog reza kod 34-godišnje bolesnice nakon normalnog intraoperativnog toka. Tokom prvih dvanaest časova nakon operacije, kod bolesnice se javila glavobolja za koju se smatralo da predstavlja postduralnu punkcionu glavobolju (PDPH), pa je u tom smislu i tretirana. Nakon trećeg postoperativnog dana, dolazi do slabljenja glavobolje tako da je petog dana otpuštena iz bonice u dobrom opštem stanju. Neposredno nakon otpusta, glavobolja se ponovo javlja, dugotrajnija i jača po intenzitetu, da bi kasnije progredirala u epileptični status. Snimanjem magnetnom rezonancom utvrđeno je postojanje hematoma u apsorpciji. Nakon konzervativne terapije, dolazi do poboljšanja bez pojave neuroloških posledica. U okviru ovog rada razmatrani su razlika između PDPH i glavobolja drugog porekla, kao i etiopatogeneza subduralnog hematoma u opstetriciji. Došlo se do zaključka da u slučaju dugotrajne, perzistirajuće glavobolje treba imati u vidu i subduralni hematom kao komplikaciju, zato što rana dijagnoza i odgovarajuće lečenje vode ka potpunom oporavku.

Acta Medica Medianae 2018;57(4):144-147.

Ključne reči: *subduralni hematom, carski rez, postduralna punkciona glavobolja, neuraksijalni blok, subarahnoidalna anestezija*

Review article

UDC: 615.015.4:615.2
doi:10.5633/amm.2018.0420

REBOUND PHENOMENON – IMPORTANT AND UBIQUITOUS IN PHARMACOTHERAPY

Maja Koraćević[1], Jelena Lalić[1], Sonja Nedeljković[2], Goran Koraćević[3,4]

The rebound effect represents a common characteristic of the numerous classes of modern drugs and can result in serious and even fatal disorders. For example, prolonged administration of proton pump inhibitor (PPI) leads to moderate hypergastrinemia in 20-25%. This hypergastrinemia will result in rebound gastric acid hypersecretion in 30-40% of patients following the abrupt PPI discontinuation. PPIs are among the most widely used drugs worldwide.

An abrupt cessation of chronic corticosteroid, beta blocker, or opioid treatment may also provoke rebound phenomenon. Even in heart failure patients, beta blocker withdrawal on admission resulted in a significant increase of the probability of in-hospital mortality. The incidence of a rebound phenomenon depends on numerous factors, including the intensity and duration of action of a particular drug and how long it has been applied; the susceptibility of an individual patient (regarding the comorbidities and the severity of the primary disease) and the related circumstances (e.g, co-therapy). The clinical importance of the rebound phenomenon varies from academic to lethal. Even rare rebounds found for some classes of drugs are becoming very important if the drug has been used often globally.

Acta Medica Medianae 2018;57(4):148-152.

Key words: *rebound, proton pump inhibitor, corticosteroid, beta blocker, opioid*

[1]University of Niš, Faculty of Medicine, Department of Pharmacy, Niš, Serbia
[2]Pharmacy "Nevenpharm", Niš, Serbia
[3]University of Niš, Faculty of Medicine, Niš, Serbia
[4]Department for Cardiovascular Diseases, Clinical Center Niš, Serbia

Contact: Maja Koraćević
Adress: 9. brigade 53/50, 18000 Niš, Serbia
E-mail: koracevic.maja@gmail.com

Introduction

Rebound phenomenon is defined as a worsening of symptoms even exceeding baseline levels when the drug is abruptly discontinued or loses effectiveness (1, 2). The rebound effect represents a common characteristic of the numerous classes of modern drugs. It is difficult to find a part of pharmacotherapy without a good example of rebound phenomenon following sudden drug withdrawal. In order to preserve homeostasis, several pathophysiological mechanisms become activated following the introduction of a drug. For example, if a drug blocks a process for enough long period of time, the number of receptors on target cells will increase (up-regulation), aiming to overcome the blockade. Therefore, sudden withdrawal of an efficacious medicament will allow the stimulant to act on increased number of receptors on target cells, resulting in the rebound of symptoms and signs of the disease. This explains (in a simplified way) how can symptoms and signs not only return following the drug cessation, but become even worse compared to pre-treatment period. Depending upon the drug efficacy and eventual important co-therapy on the one hand and severity of the disease (among other factors) on the other hand, the rebound effect can result in serious and even fatal disorders (1).

The aim of the study is to give a short review of the various examples of rebound phenomenon and to illustrate how ubiquitous and important in pharmacotherapy it is.

Literature overview

A typical example of rebound phenomenon occurs following a sudden proton pump inhibitors (PPIs) discontinuation. With commencing PPI, in order to preserve homeostasis and to overcome the gastric acid secretion blockade, numerous pathophysiological mechanisms change, including the stimulation of gastrin secretion, which becomes moderate in 20-25% of chronic PPI users (1, 3, 4). Namely, as a result of prolonged PPI administration, gastric hypoacidity ensues, resulting in the inhibition of the feedback mechanism initiated by antral mucosal acid sensors. Therefore, antral G-cell release of gastrin is

not suppressed, which leads to chronic hypergastrinemia, gastric parietal, and ECL-cell hyperplasia (4, 5). Among other consequences, hypergastrinemia will result in rebound gastric acid hypersecretion in 30-40% of patients following the abrupt PPI termination (1, 5). Due to cell hyperplasia (which is not a short-time abnormality), rebound gastric acid oversecretion may last for weeks. There is no consensus how long this hypersecretion averages, but some estimates suggest 6-8 weeks, but < 26 weeks.

This becomes even more important if we have in mind that PPIs are among the most widely used drugs worldwide, approximating US$13 billion in sales per year. Long-term usage of PPIs is not approved, but they became over-the-counter drugs in the USA, leading to the lack of efficient control in the PPI administration (5). Therefore, sudden PPI withdrawal occurs more often. Moreover, some authors estimate that 25-81% of PPI users lack valid indication. Additionally, among patients with adequate diagnosis requiring PPI (e.g, Helicobacter pylori eradication), many continue PPI utilization despite the reason for it has disappeared meanwhile (6). Pharmacists should help to decrease the inappropriate medication use. One cross-sectional study recommended patients education about the proper drug cessation (e.g. PPI), particularly because the dose tapering is required to diminish the risk of rebound syndrome (6). It has been suggested that PPI dose should be diminished before cessation. After the sudden PPI cease, rebound hyperacidity can occur resulting in the worsening of symptoms; this can be easily misinterpreted as the disease relapse, leading to new whole -length course of PPI treatment, which is clearly unnecessary (6). A proper way to quit PPI therapy seems to be halving the PPI dose for a month or two and then ceasing PPI or to switching to a less effective acid suppressant (H2 blocker). Indeed, an antacid should be prescribed to control dyspepsia (6).

2. As for psoriasis, there is a clear definition of rebound: it is present with either a flare-up > 125% of baseline Psoriasis Area and Severity Index or with a morphological difference (for example, erythrodermic or generalized pustular psoriasis) (7). Sudden withdrawal of a strong, efficient drug without tapering might result in rebound of psoriasis (7, 8). The incidence rebound phenomenon of drug should be reported, such as in a study of etanercept (9).

3. It is well known that an abrupt cessation of chronic corticosteroid treatment may provoke rebound syndrome (10). Rebound syndrome following sudden cessation of corticosteroids is very important, particularly if intravenous route of administrative is used. A recent consensus document on the appropriate use of inhaled corticosteroids in chronic obstructive pulmonary disease has also recommended that the dose of inhaled corticosteroids should be tapered before stopping (10-12).

A step-wise withdrawal of inhaled corticosteroids is recommended based in part on the results of the WISDOM (Withdrawal of Inhaled Steroids During Optimized Bronchodilator Management) trial (2,027 patients finished the trial). The daily dose of an inhaled corticosteroid should be gradually diminished by approximately 50% at randomization and again at 1,5 month; after a three-month treatment with inhaled corticosteroids, the therapy should be completely ceased (10, 12). For the whole period patients should receive tiotropium 18 mg QD and salmeterol 50 mg BID (12). Shortly after withdrawal, patients who have ceased to inhale corticosteroids should be seen again (as outpatients). It is safe to stop inhaled corticosteroids even in severe chronic obstructive pulmonary disease patients. In order to proceed with step-wise dose reduction, it is important to document the absence of decline in FEV1 after decreasing the dose of inhaled corticosteroid from high to medium one (11).

4. Due to high analgesic efficacy, opioid (e.g, morphine) is frequently the drug of choice for the treatment of severe pain. The number of young individuals addicted to opioids has been increasing during the past decade as well as opioid withdrawal syndrome (recognized by respiratory depression, cramps, changes in body temperature, diarrhea and vomiting, tachycardia, arterial hypertension, etc.) (13). Following a prolonged use of opioids, tolerance may occur decreasing their efficacy and patient requires higher doses to maintain pain control (14). This dose escalation parallels a higher incidence of unwanted effects and eventual withdrawal syndrome may become more severe, too. In one study, all 35 patients had opioid rebound syndrome, which indicated physical dependence (14). Endothelin-A receptor antagonists may diminish the dose of opioids and some of their adverse effects such as respiratory depression (13). Rebound syndrome following the abrupt discontinuation of opioid infusion after ≥ 5 days is found in > 30% in pediatric intensive care unit patients (15).

5. Rebound syndromes have been reported after a sudden withdrawal of various drugs used in cardiology, starting from heparin rebound and beta blocker (BB) rebound (14, 17-25).

If there is no contraindication (such as allergy), it seems wise to use a selective and/or vasodilatatory BB. The reason for such recommendation is in the fact that selective BBs have less common unwanted effects and consequently higher compliance, adherence and persistence. Therefore, it is logical to expect fewer withdrawals and possible rebound phenomena (17). Moreover, chances to forget to take a drug are higher if it is prescribed e.g., three times daily (in comparison with once-daily. Thus, long-lasting drugs (including BBs) have lower probability of dose omitting and can be recommended. It is also rational to pay attention to the price of particular BB, because if it is currently or prospectively too high for a given patient, it might favor patient's decision to stop taking it. Indeed, patients will be more complaint to the prescribed BB regimen if they are informed about the risk associated with sudden BB withdrawal. It is particularly important for patients on high BB daily dose as the chances for rebound rise in parallel with the dose (17). Common sense also suggests that proper BB should be administered, which is evidence-based and recommended in contemporary guidelines for this patient's indication. For example, some BBs are recommended for heart failure, while others are not. Prescribing one of adequate BBs for this particular indication

leads rationally to the expectation of an improvement in symptoms (which will enhance the persistence and diminish the dose omission and resultant BB rebound). Moreover, patients should be instructed to take BB as soon as possible in the morning in order to cope with early morning sympathetic activation and subsequent morbidity and mortality risk. Namely, the risk of major adverse cardiac and cerebral events is several-fold higher in the first hour(s) after awaking and it is not rational to raise this risk even more with BB rebound.

BB rebound has been well-known. Patients might even misuse this knowledge to induce factitious hypertension (16). Yet, in heart failure patients with BB withdrawal at admission to hospital, the OR was 1.77 (1.09–3.26) for in-hospital mortality in the BETAWIN-AHF study. It is consonant previous studies including a recent meta-analysis: a risk ratio of 1.59 (1.03–2.45) for death or rehospitalization (18).

In numerous patients with arrhythmic events, despite therapy, non-compliance may be responsible with the resulting BB rebound phenomenon (22). Moreover, in order to avoid BB rebound, BB should not be stopped in the perioperative period. The dose of BB should be adjusted to achieve the heart rate 60-70 beats per min, and systolic blood pressure should be > 100 mmHg (26). Recommendation that the dose of propranolol should be carefully tapered before its discontinuation (together with diminished physical activity) has been adequate for four decades (24).

Conclusions

1. Rebound symptoms and signs following the abrupt discontinuation of the drug use have been registered in numerous (almost all) areas of medicine.

2. The incidence of a rebound phenomenon depends on numerous factors, including the intensity and duration of action of a particular drug and how long it has been applied; the susceptibility of an individual patient (regarding the comorbidities and the severity of the primary disease) and the related circumstances (e.g, co-therapy).

3. The clinical importance of the rebound phenomenon varies from academic to lethal, depending on the drug and clinical scenario.

4. Even rare rebounds found for some classes of drugs are becoming very important if the drug has been often used globally.

References

1. Teixeira MZ. Rebound effects of modern drugs: serious adverse events unknown by health professionals. Rev Assoc Med Bras 2013; 59(6):629-38. [CrossRef] [PubMed]
2. Ooms P, Blankers M, Figee M, Mantione M, van den Munckhof P, Schuurman PR, et al. Rebound of affective symptoms following acute cessation of deep brain stimulation in obsessive-compulsive disorder. Brain Stimul 2014; 7(5):727-31. [CrossRef] [PubMed]
3. Waldum HL, Hauso Ø, Brenna E, Qvigstad G, Fossmark R. Does long-term profound inhibition of gastric acid secretion increase the risk of ECL cell-derived tumors in man? Scand J Gastroentero 2016; 51(7): 767-73. [CrossRef] [PubMed]
4. Nandy N, Hanson JA, Strickland RG, McCarthy DM. Solitary gastric carcinoid tumor associated with long-term use of omeprazole: A case report and review of the literature. Digest Dis Sci 2016; 61(3):708-12. [CrossRef] [PubMed]
5. Sukhovershin RA, Cooke JP. How may proton pump inhibitors impair cardiovascular health? Am J Cardiovasc Drugs 2016; 16(3):153-61. [CrossRef] [PubMed]
6. Pasina L, Urru SA, Mandelli S, Giua C, Minghetti P. Evidence-based and unlicensed indications for proton pump inhibitors and patients' preferences for discontinuation: a pilot study in a sample of Italian community pharmacies. J Clin Pharm Ther 2016; 41(2):220-3. [CrossRef] [PubMed]
7. Daudén E, Puig L, Ferrándiz C, Sánchez-Carazo JL, Hernanz-Hermosa JM. Consensus document on the evaluation and treatment of moderate-to-severe psoriasis: Psoriasis Group of the Spanish Academy of Dermatology and Venereology. J Eur Acad Dermatol 2016; 30(Suppl 2):1-18. [CrossRef] [PubMed]
8. Khemis A, Cavalié M, Montaudié H, Lacour JP, Passeron T. Rebound pustular psoriasis after brodalumab discontinuation. Brit J Dermatol 2016; 175(5):1065-6. [CrossRef] [PubMed]

9. Prinz JC, Puig L, Girolomoni G. Treatment of psoriasis with etanercept: the typical patient profile. J Eur Acad Dermatol 2016; 30(7):1092-9. [CrossRef] [PubMed]
10. Kaplan AG. Applying the wisdom of stepping down inhaled corticosteroids in patients with COPD: a proposed algorithm for clinical practice. Int J Chronic Obstr 2015; 10(1):2535-48. [CrossRef] [PubMed]
11. Alcázar Navarrete B, Casanova C, Miravitlles M, de Lucas P, Riesco JA, Rodríguez González-Moro JM. "Correct use of inhaled corticosteroids in chronic obstructtive pulmonary disease": a consensus document. Arch Bronconeumol 2015; 51(4):193-8. [CrossRef] [PubMed]
12. Magnussen H, Watz H, Kirsten A, Decramer M, Dahl R, Calverley PM, et al. Stepwise withdrawal of inhaled corticosteroids in COPD patients receiving dual bronchodilation: WISDOM study design and rationale. Resp Med 2014; 108(4):593-9. [CrossRef] [PubMed]
13. Bhalla S, Andurkar SV, Gulati A. Neurobiology of opioid withdrawal: Role of the endothelin system. Life Sci 2016; 15:34-42. [CrossRef] [PubMed]
14. Daitch D, Daitch J, Novinson D, Frey M, Mitnick C, Pergolizzi J Jr. Conversion from high-dose full-opioid agonists to sublingual buprenorphine reduces pain scores and improves quality of life for chronic pain patients. Pain Med 2014; 15(12):2087-94. [CrossRef] [PubMed]
15. Weber GM, Smerling AJ, Saroyan JM. Pentobarbital withdrawal and treatment in an infant in the pediatric cardiac intensive care unit. J Clin Anesth 2013; 25(1): 62-5. [CrossRef] [PubMed]
16. Cuadra R, White WB. Severe and refractory hypertension in a young woman. J Am Soc Hypertens 2016; 10(6):506-9. [CrossRef] [PubMed]
17. Koracevic G. Significance of "beta blocker rebound phenomenon" and new suggestions how to avoid it. In: Rivas-Echeverria C, Allegaert K, Wainstein DE, editors. Proceedings of the World Medical Conference; 2011 Sep 26-28; Prague, Czech Republic. p. 79-84.
18. Prins KW, Neill JM, Tyler JO, Eckman PM, Duval S. Effects of beta-blocker withdrawal in acute decompensated heart failure: A systematic review and meta-analysis. JACC-Heart Fail 2015; 3(8):647-53. [CrossRef] [PubMed]
19. Schonberger RB, Feinleib J, Holt N, Dai F, Brandt C, Burg MM. Preoperative depression symptom severity and its impact on adherence to preoperative beta-blocker therapy. J Cardiothor Vasc An 2014; 28(6): 1467-73. [CrossRef] [PubMed]
20. Koracevic G. Acute right ventricular myocardial infarction: a very specific entity. Baylor University Medical Center Proceedings 2007; 20(2):177-8. [CrossRef] [PubMed]
21. Koracevic GP, Dakic SS, Velickovic-Radovanovic R, Apostolović SR, Krstić NH, Tasić IS, et al. Amlodipine as an antiischemic drug is superior to long acting nitrates. Open Med-Warsaw 2015; 10(1):50-6. [CrossRef] [PubMed]
22. Imberti JF, Underwood K, Mazzanti A, Priori SG. Clinical challenges in catecholaminergic polymorphic ventricular tachycardia. Heart Lung Circ 2016; 25(8): 777-83. [CrossRef] [PubMed]
23. Koracevic G. 'Heparin rebound' means the opposite in cardiac surgery (bleeding) and in cardiology (thrombosis). Blood Coagul Fibrin 2010; 21(2):198-9. [CrossRef] [PubMed]
24. Braillon A. Letter: interruption of beta-blockers in patients with cirrhosis - hasten slowly! Aliment Pharm Ther 2016; 43(11):1250-1. [CrossRef] [PubMed]
25. Koraćević G, Andrejević S, Sakač D, Stanojević Z, Stefanović S, Antović J, et al. Heparin rebound phenomenon in acute coronary syndromes: advantage of low molecular weight heparins. Facta Universitatis (Medicine and Biology) 2000; 7(1):62-9.
26. Vats A, Marbaniang MJ, Howell SJ. Perioperative management of the patient with cardiovascular disease undergoing non-cardiac surgery. Surgery (Oxford) 2016; 34(8):392-8. [CrossRef]

Revijalni rad

UDC: 615.015.4:615.2
doi:10.5633/amm.2018.0420

FENOMEN NAGLE OBUSTAVE LEKA – VAŽAN I SVEPRISUTAN U FARMAKOTERAPIJI

Maja Koraćević[1], Jelena Lalić[1], Sonja Nedeljković[2], Goran Koraćević[3,4]

[1]Univerzitet u Nišu, Medicinski fakultet, Odsek za farmaciju, Niš, Srbija
[2]Apoteka „Nevenpharm", Niš, Srbija
[3]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[4]Klinika za kardiovaskularne bolesti, Klinički centar Niš, Srbija

Kontakt: Maja Koraćević
9. brigade 53/50, 18000 Niš, Srbija
E-mail: koracevic.maja@gmail.com

Efekat nagle obustave leka ("rebound efekat") predstavlja čestu karakteristiku brojnih klasa savremenih lekova i može dovesti do ozbiljnih, pa čak i fatalnih poremećaja. Na primer, produžena primena inhibitora protonske pumpe (PPI) dovodi do umerene hipergastrinemije kod 20-25% bolesnika. Ova hipergastrinemia će rezultirati znatnim porastom sekrecije želudačne kiseline kod 30-40% bolesnika nakon naglog prekida uzimanja PPI, koji su među najčešće korišćenim lekovima u svetu.

Nagli prestanak hroničnog tretmana kortikosteroidom, beta blokatorom ili opijatom može takođe izazvati "rebound fenomen". Čak je i kod bolesnika sa srčanom insuficijencijom obustava beta blokatora na prijemu rezultirala značajnim porastom verovatnoće za intrahospitalnu smrtnost.

Učestalost pojave "rebound fenomena" zavisi od brojnih činilaca, uključujući jačinu i trajanje dejstva određenog leka i koliko dugo je primenjivan; podložnost pojedinačnog bolesnika (u vezi komorbiditeta i ozbiljnosti osnovne bolesti) i povezanim okolnostima (npr, koterapija). Klinički značaj "rebound fenomena" varira od akademskog do smrtonosnog. Iako su retki, "rebound fenomeni" nekih klasa lekova postaju veoma važni ukoliko se lek koristi često i globalno.

Acta Medica Medianae 2018;57(4):148-152.

Ključne reči: *rebound, inhibitor protonske pumpe, kortikosteroid, beta blokator, opioid*

JEDINSTVENI KRITERIJUMI ZA OBJAVLJIVANJE NAUČNIH RADOVA U BIOMEDICINSKIM ČASOPISIMA

Ideja o postavljanju jedinstvenih kriterijuma za objavljivanje radova u časopisima za biomedicinske nauke iskristalisana je 1978. godine u Vankuveru. Ovi kriterijumi za rukopise, uključujući pravila za pisanje bibliografije, prvi put su objavljeni 1979. godine. Vankuverska grupa je vremenom prerasla u Međunarodni komitet urednika medicinskih časopisa — International Committee of Medical Journal Editors (ICMJE). Trenutno je na snazi peta revizija kriterijuma za objavljivanje radova u biomedicinskim časopisima, doneta 1997. godine.

Kriterijumi za citiranje i navođenje referenci

Reference se obeležavaju arapskim brojevima u zagradama, pri čemu se reference obele-žavaju brojevima onim redosledom kojim se pojavljuju u tekstu. Reference citirane jedino u tabelama ili legen-di moraju se obeležiti brojem u skladu sa redosledom pojavljivanja u tekstu.

Naslove medicinskih časopisa treba pisati u skraćenom obliku onako kako su navedeni u poglavlju **List of Journals Indexed in Index Medicus**. Lista skraćenih naziva medicinskih časopisa objavljuje se svake godine u januarskom broju ***Index Medicusa***. Ova lista se takođe može naći na adresi **www.nlm.nih.gov**

Izbegavati upotrebu apstrakata kao referenci, već koristiti samo izvorne tekstove (*in extenso* članci). Reference koje se odnose na radove koji su prihvaćeni, ali još nisu odštampani, treba označiti sa "u štampi", pri čemu autor mora imati pismeno odobrenje da citira takve radove i da priloži pismeni dokaz da je citirani rad prihvaćen za štampu. Informacije iz rukopisa koji nisu prihvaćeni za štampanje mogu se citirati u tekstu kao "neobjavljeni rezultati", ali sa pismenom dozvolom autora.

Izbegavati citiranje prethodnih saopštenja (personal communication) ukoliko ona ne obezbeđuju esencijalne rezultate koji još nigde nisu objavljeni. U ovom slučaju, neophodno je u zagradi navesti ime osobe i datum usmenog saopštenja rezultata. Za objavljivanje ovih podataka neophodno je pismeno odobrenje autora.

Kriterijumi za pisanje referenci korišćenih u radu

U ovom pregledu biće obrađena pravila za pi-sanje literaturnih referenci samo za najčešće korišćene tipove publikacija.

Članci u časopisima

1. Standardni članak u časopisu

Navesti prvih šest autora, ukoliko ih je više iza šestog dodati **et al.** ukoliko je referenca na engleskom jeziku ili **i sar.** ukoliko je referenca na srpskom jeziku.

Vega KJ, Pina I, Krevsky B. Heart transplantation is associated with an increased risk for pancreatobiliary disease. Ann Intern Med 1996; 124(11):980-3.

Parkin DM, Clayton D, Black RJ, Masuyer E, Friedl HP, Ivanov E, et al. Childhood–leukaemia in Europe after Chernobyl: 5 year follow-up. Br J Cancer 1996;73:1006-12.

2. Organizacija kao autor

The Cardiac Society of Australia and New Zealand. Clinical exercise stress testing. Safety and performance guidelines. Med J Aust 1996;164:282-4.

3. Članak bez poznatih autora

Cancer in South Africa (editorial). S Afr Med J 1994;84:15.

4. Volumen sa suplementom

Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102 Suppl 1:275-82.

5. Broj sa suplementom

Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1 Suppl 2):89-97.

6. Volumen sa više delova

Ozben T, Nacitarhan S, Tuncer N. Plasma and urine sialic acid in non-insulin dependent diabetes mellitus. Ann Clin Biochem 1995;32(Pt 3):303-6.

7. Broj sa više delova

Poole GH, Mills SM. One hundred consecutive cases of flap lacerations of the leg in ageing patients. N Z Med J 1994;107(986 Pt 1):377-8.

8. Časopisi sa brojem bez volumena

Turan I, Wredmark T, Fellander-Tsai L. Arthros-copic ankle arthrodesis in rheumatoid arthritis. Clin Orthop 1995;(320):110-4.

9. Časopisi bez volumena i broja

Browell DA, Lennard TW. Immunologic status of the cancer patient and the effects of blood transfusion on antitumor responses. Curr Opin Gen Surg 1993:325-33.

10. Reference u obliku apstrakta ili prethodnih saopštenja

Enzensberger W, Fischer PA. Metronome in Parkinson's disease (letter) Lancet 1996;347:1337.

Clement J, De Bock R. Hematological complica–tions of hantavirus nephropathy (HVN) (abstract). Kidney Int 1992; 42:1285.

Udžbenici i monografije

11. Monografija

Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.

12. Autori kao urednici

Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

13. Organizacija kao autor i izdavač

Institute of Medicine (US). Looking at the future of the Medicaid program. Washington: The Institute; 1992.

14. Poglavlje u knjizi

Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

15. Conference proceedings

Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

16. Conference paper

Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors.

MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. p. 1561-5.

17. Istraživački ili tehnički izveštaji

Službeni izveštaji (Issued by funding / sponsoring agency):

Smith P, Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX): Dept. of Health and Human Services (US), Office of Evaluation and Inspections; 1994 Oct. Report No.: HHSIGOEI69200860.

Sponzorisani izveštaji (Issued by performing agency)

Field MJ, Tranquada RE, Feasley JC, editors. Health services research: work force and educational issues. Washington: National Academy Press; 1995. Contract No.: AHCPR282942008. Sponsored by the Agency for Health Care Policy and Research.

18. Magistarske i doktorske disertacije

Kaplan SJ. Post-hospital home health care: the elderly's access and utilization [dissertation]. St. Louis (MO): Washington Univ.; 1995.

Druge vrste publikovanog materijala

Neobjavljeni materijal

19. U štampi (In press)

Leshner AI. Molecular mechanisms of cocaine addiction. N Engl J Med. In press 1996.

Elektronski zapisi

20. Internet članak u elektronskom formatu

Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar "cited 1996 Jun 5"; 1(1)(24 screens). Available from: URL: http://www.cdc.gov/ncidod/EID/eid.htm

21. Monografija u elektronskom formatu

CDI, clinical dermatology illustrated (monograph on CD-ROM). Reeves JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego: CMEA; 1995.

22. Kompjuterski podaci

Hemodynamics III: the ups and downs of hemodynamics (computer program). Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

PROPOZICIJE ZA PISANJE RADOVA U ACTA MEDICA MEDIANAE

Acta Medica Medianae (AMM) je tematski časopis iz oblasti medicinskih nauka. Časopis objavljuje originalne radove koji nisu prethodno publikovani.

U AMM se objavljuju: uvodnici, naučni i stručni članci, prethodna ili kratka saopštenja, revijski radovi tipa opšteg pregleda, aktuelne teme, meta-analize, prikazi slučajeva, prikazi knjiga i drugi prilozi. Radovi se štampaju na srpskom ili engleskom jeziku sa apstraktom na srpskom i engleskom jeziku. Radovi na engleskom jeziku se prezentuju u elektronskom formatu na sajtu Medicinskog fakulteta u Nišu, kao i na međunarodnim sajtovima iz oblasti medicinskih nauka. Acta Medica Medianae izlazi četiri puta godišnje, od 1962 godine.

Svi radovi koji se objavljuju u AMM podležu anonimnoj recenziji, a Uređivački odbor određuje redosled njihovog štampanja. Primedbe i sugestije urednika i recenzenata dostavljaju se autoru radi konačnog oblikovanja. Radovi se predaju u pisanom ili elektronskom obliku na srpskom i engleskom jeziku. Rukopisi radova prihvaćenih za štampu ne vraćaju se autoru.

Rukopis treba predati sa jednostrukim proredom, formata A4, sa levom marginom od 3 cm.

Prva strana rada treba da sadrži: a) naslov rada b) puna imena i prezimena autora c) puni nazivi ustanova i organizacijskih jedinica u kojima je rad realizovan i mesta u kojima se ustanove nalaze, d) znacima *, **, ***, #, ##, ###,…označavaju se redom autori i njihove institucije e) puna adresa, broj telefona i e-mail osobe zadužene za korespondenciju u vezi predatog rukopisa.

Druga strana treba da sadrži samo naslov rada, rezime i ključne reči, bez imena autora i institucija. Veličina rezimea za naučne i stručne članke, revijske radove tipa opšteg pregleda i meta–analize može da bude do 250 reči. Ispod rezimea sa podnaslovom "Klučne reči" navesti 3-5 ključnih reči ili izraza. Poželjno je da autori za ključne reči koriste odgovarajuće deskriptore, tj. definisane termine iz *Medical Subject Heading* (MeSH) liste *Index Medicus-a*. Prva i druga strana se predaju na srpskom i engleskom jeziku i ne obeležavaju se brojevima.

Tekst članka: Naučni i stručni članci, kao i opšti pregledi i meta-analize ne smeju prelaziti 11 stranica sa prilozima; aktuelne teme– 6 stranica; kazuistika 6–stranica; prethodna saopštenja– 5 stranica, a izveštaji sa skupova i prikazi knjiga 2 stranice. Naučni i stručni članci obavezno treba da sadrže poglavlja: uvod, cilj, materijal i metode, rezultati, diskusija i zaključak. Zahvalnost ili komentar povodom sponzorstva rada dati na kraju teksta članka iza poglavlja "zaključak".

U tekstu naznačiti mesta priloga i obeležiti ih onako kako su obeleženi u prilogu.

Literatura se daje u posebnom poglavlju, pri čemu se navodi onim redosledom kojim se citati pojavljuju u tekstu. Broj literaturne reference se u tekstu označava arapskim brojem u zagradi. Za navođenje literature koristiti pravila Vankuverske konvencije. Strane se numerišu arapskim brojevima u donjem desnom uglu

Priloge u vidu teksta, tabela i ilustracija (grafikoni, crteži i dr.) ne unositi u tekst članka, već na kraju teksta, na posebnim stranicama obeleženim u gor-njem levom uglu sa "Tabela, Grafikon, Ilustracija" i arapskim brojem redosledom pojavljivanja u tekstu (npr. Tabela 1, Grafikon 1 i dr.) i svakoj se daje kratak naslov. Kratka objašnjenja i skraćenice daju se u fusnoti. Za fusnotu koristiti sledeće simbole: *, **, ***, #, ##, ###, …itd. Tabele, grafikone i ilustracije treba praviti korišćenjem nekog od programa iz Microsoft Office paketa. Izbegavati upotrebu boja kod izrade grafika.

Za izradu grafičkih priloga može se koristiti bilo koji grafički program, pri čemu slike moraju biti snimljene u jpg formatu rezolucije 300 dpi (u originalnoj veličini). Grafički prilozi se ne unose u Word dokument već se predaju kao posebni JPG fajlovi.

Ukoliko je tabela ili ilustracija već negde objavljena, citirati izvor i priložiti pismeno odo-brenje, ukoliko se radi o zaštićenom materijalu. Ukoliko je na fotografiji prikazan bolesnik tako da se može prepoznati, potrebno je njegovo pismeno odobrenje, u suprotnom, delovi fotografije se moraju izbrisati da bolesnik ne može biti identifikovan.

Uz rad, na posebnom listu, treba dostaviti: a) izjavu da rad do sada nije objavljivan, b) potpise svih autora, c) ime, prezime, tačnu adresu i broj telefona prvog autora.

Rad je preporučljivo predati u elektronskom obliku na e-mail adresu uredništva: acta@medfak.ni.ac.rs ili poslati poštom na CD ili DVD disku sa materijalom u celini na srpskom i engleskom jeziku.

Rad treba otkucati u programu ms office Word verzija 2003. ili novija. Za verziju na engleskom jeziku koristiti font Verdana, veličine 9 pt, kodna stranica (English). Za verziju na srpskom jeziku koristiti font Verdana, veličine 9 pt, kodna stranica (Serbian lat ili Croatian).

U radu je obavezno korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina.

AMM zadržava pravo dalje distribucije i štampanja radova.

SECRETARIAT

GUIDELINES FOR PAPER SUBMISSION TO ACTA MEDICA MEDIANAE

Acta Medica Medianae (AMM) is a thematic journal for medical sciences. The Journal publishes original reasearch articles that have not been published before.

AMM also publishes editorials, observational and experimental articles, procedings or short communications, review articles, meta-analyses, case reports, current topics, articles from the history of medicine as well as other contributions related to medical sciences. All articles are printed in Serbian or English with short abstracts in both Serbian and English. Articles in English are published in electronic form on the University of Nis Medical Faculty website as well as international sites related to medical sciences. Acta Medica Medianae is published four times a year. The first issue appeared as early as 1962.

General Guidelines

Paper Submission

All research articles published in this journal undergo rigorous peer review, based on initial editor screening and anonymized refereeing by at least two anonymous referees. Remarks and suggestions made by the editors and reviewers are sent to the author for final revision. The papers in English are to be submitted by e-mail: acta@medfak.ni.ac.rs. Manuscripts accepted for publication are not returned to authors.

The first page of a research article must contain: a) article title b) full name of author(s) c) full name(s) of institutions and/or address(es) of department(s) where either reasearch was conducted or research article written d) following signs *, **, ***, #, ##, ### signifying author(s) and institutions e) full address, phone number and e-mail of a corresponding author.

The second page should contain only research article title, abstract and key words without names of author(s) and institution(s). Abstract for research and professional articles, review articles and meta-analyses should have up to 350 words while abstract for all other types of publications should consist of 250 words. Key Words section should have up to 5 key words or phrases related to a submitted article. It is desirable that authors use corresponding descriptors from Medical Subject Heading (MeSH) that can be found on Index Medicus list for key words. The first and the second page should not be numbered.

Body of a Research Article – Research and professional articles, as well as general surveys and meta-analyses should not exceed 11 pages altogether; current topics - 6 pages, casuistics - 6 pages and proceding statements - 5 pages, history of medicine articles - 3 pages while conference reports and book reviews - 2 pages. Research and professional articles should comprise the following mandatory chapters: introduction, aim(s), material and method(s), result(s), discussion and conclusion(s). Result(s) and discussion can be comprized into one chapter. Acknowledgments of any kind in a submitted article should be written at the end of the paper after "Conclusion(s)". It is necessary to clearly mark a place for additions in the text.

References should be written in a separate chapter in the same order of appearance as in a research article. Reference numbers that appear in the text should be written in Arabic numerals and put in brackets. All authors should be listed. If there are more than six authors this should be indicated with *et al*. Use the rules of the Vancouver convention when quoting literature. Pages should be enumerated in Arabic numerals in the bottom right corner.

Additions in form of texts, tables and illustrations (photos, drawings, diagrams, etc) should not be inserted in the reasearch article body but at the end of the text on separate pages which should be marked in the upper left corner as "Table(s), Graphic(s), Illustration(s) etc) with Arabic numeral in the same order of appearance as in the text (for instance, Table 1, Graph 1, etc) with a short title. Short explanations and abbreviations should be stated in footnotes where the following symbols should be used: *, **, ***, #, ##, ###, etc. Table(s), graph(s) and ilustration(s) should be drawn in a Microsoft Office Program. Color should be avoided.

Any graphic program can be used for making graphic addition(s) while picture(s) should be saved in .jpg format with 300 dpi resolution (original size). Graphic addition(s) should be sent as separate jpg file(s), and not inserted in the body of a research or any other article submitted to AMM.

If some additions, included in a submitted reasearch article, have already been published, source of publication should be clearly stated, alongside written approval in case the material is copyright protected. Patients on photographs have privacy rights that should not be infringed without their consent. Namely, if a photograph shows a patient who can be recognized, his/her written approval should also be submitted; otherwise, visible and recognisable facial or bodily parts should be blackened so that the patient cannot be identified by readership.

On a separate sheet the author should also enclose: a) his/her statement that a submitted article has not been published before, b) signatures of all authors, c) full name, address, e-mail and phone number of the first author.

Submitted article should be typed in Word Version 2003 for Windows (or more recent ones), font Verdana 9 pt size; code page (English) should be used.

The authors are required to use international measurement standards (SI) and internationally accepted standard terms.

Acta Medica Medianae reserves the right for further distribution and printing of published reasearch articles.