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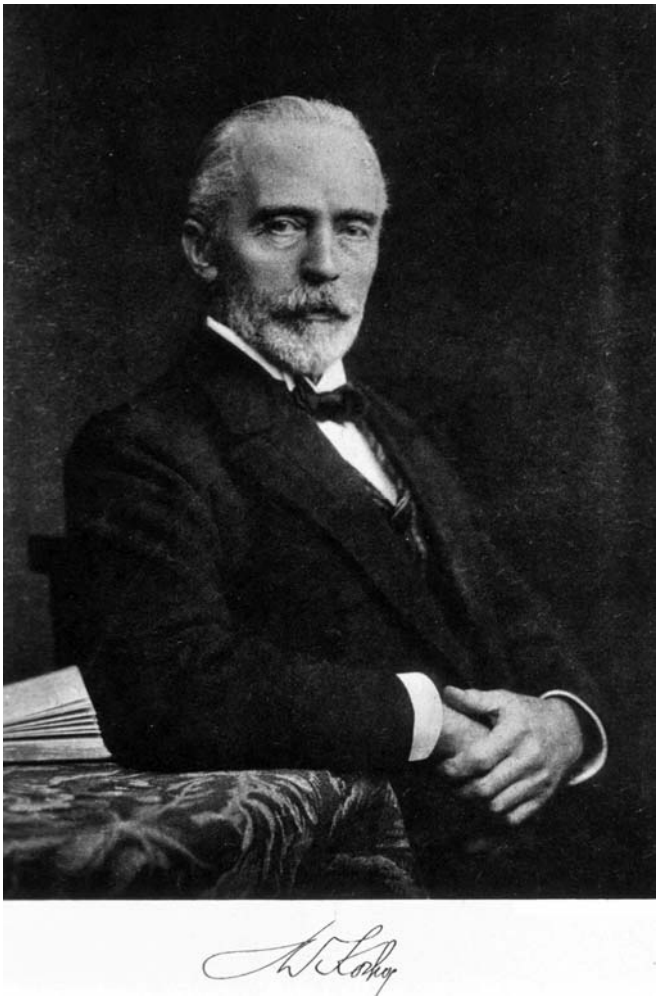

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CONTENTS / SADRŽAJ

ORIGINAL ARTICLES / ORIGINALNI RADOVI

Emil Theodor Kocher (25 August 1841 – 27 July 1917) was a Swiss physician who received the 1909 Nobel Prize in Physiology or Medicine for his work in the physiology, pathology and surgery of the thyroid. He was considered a pioneer and leader in the field of surgery in his time, and was the first Swiss citizen and the first surgeon to ever receive a Nobel prize. Among his many accomplishments, particularly significant are the introduction and promotion of aseptic surgery and scientific methods in surgery, specifically reducing the mortality of thyroidectomies below 1% .
By the end of July this year, 100 years have elapsed since his death.

Emil Teodor Koher (25. avgust 1841 – 27. jul 1917), švajcarski lekar, dobitnik je Nobelove nagrade za medicinu 1909. godine, za rad u oblasti fiziologije, patologije i hirurgije štitaste žlezde. Bio je prvi Švajcarac i prvi hirurg, uopšte, koji je dobio Nobelovu nagradu. Između njegovih brojnih dostignuća, kao izuzetno važno ističe se uvođenje i promocija asepse i naučnih metoda u hirurgiju i, posebno, smanjenje mortaliteta kod tireoidektomije ispod 1%.
Krajem jula ove godine navršilo se 100 godina od njegove smrti.

ORIGINAL ARTICLES

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Carbapenemase production in hospital isolates of multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli* in Serbia

Produkcija karbapenemaza kod bolničkih multirezistentnih sojeva *Klebsiella pneumoniae* i *Escherichia coli* u Srbiji

Anika Trudić*†, Zora Jelesić†‡, Mira Mihajlović-Ukropina†‡, Deana Medić†‡, Branka Zivlak†‡, Vera Gusman†‡, Milan Djilas‡

***Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia; University of Novi Sad, †Faculty of Medicine, Novi Sad, Serbia; ‡Institute for Public Health of Vojvodina, Novi Sad, Serbia**

Abstract

Background/Aim. Carbapenem resistance has escalated in medically important enterobacteria such as *Klebsiella pneumoniae* and *Escherichia coli* worldwide. Multidrug-resistant strains represent an important source of concern as effective therapeutic options of infections they cause are limited or none. There were no comprehensive studies considering the presence of carbapenemase production in enterobacteria in Serbia so far. The aim of the study was to determine carbapenemase production in hospital isolates of multidrug-resistant *K. pneumoniae* and *E. coli* in Serbia. **Methods.** Strains of *K. pneumoniae* and *E. coli* resistant to at least one carbapenem (imipenem, meropenem, ertapenem) were collected from November 2013 to May 2014. Isolates were obtained from clinical samples of patients treated in 14 hospitals in Serbia. Carbapenem resistance was confirmed using phenotypic tests and polymerase chain reaction (PCR) in National Reference Laboratory for Registration and Surveillance of Antimicrobial Resistance of Bacterial Strains in Novi Sad. **Results.** Of 129 collected strains, 121 (93.8%) were *K. pneumoniae* and 8 (6.2%) were *E. coli*. Seventy (54.3%) strains were obtained from urine, 26 (20.2%) from blood, 19 (14.7%) from wound secretions and 14 (10.9%) from lower respiratory tract secretions. Carbapenemase genes were detected in 58 (45%) isolates. The gene bla New Delhi-metallo-beta-lactamases (bla_{NDM}) was found in 33 (27.3%) *K. pneumoniae*, bla oxacillinases-48 (bla_{OXA-48}) in 10 (8.3%), bla *K. pneumonia* carbapenemase (bla_{KPC}) in 1 (0.8%), and 7 (5.4%) strains harbored both bla_{OXA-48} and bla_{NDM}. Seven *E. coli* harbored bla_{NDM} gene. **Conclusion.** In Serbia, the most common type of carbapenemase in both multidrug-resistant *K. pneumoniae* and *E. coli* is NDM. Co-production of OXA-48 and NDM was found in *K. pneumoniae*. To our knowledge, KPC production was detected for the first time in Serbia.

Keywords:
enterobacteriaceae; drug resistance, bacterial; carbapenems; cross infection; genome, bacterial; serbia; beta lactamases.

Apstrakt

Uvod/Cilj. Rezistencija na karbapeneme među medicinski značajnim enterobakterijama kao što su *Klebsiella pneumoniae* i *Escherichia coli* u porastu je širom sveta. Zabrinjavajuća je činjenica da su terapijske mogućnosti kod infekcija uzrokovanih multirezistentnim sojevima ograničene ili ih nema. Do sada nije rađena sveobuhvatnija studija o produkciji karbapenemaza kod enterobakterija u Srbiji. Cilj istraživanja bio je utvrđivanje produkcije karbapenemaza kod bolničkih multirezistentnih sojeva *K. pneumoniae* i *E. coli* u Srbiji. **Metode.** Izolati *K. pneumoniae* i *E. coli* rezistentni na najmanje jedan karbapenem (imipenem, meropenem, ertapenem) prikupljani su od novembra 2013. do maja 2014. Bakterijski sojevi izolovani su iz klinički značajnih uzoraka od bolesnika lečenih u 14 bolnica u Srbiji. Rezistencija na karbapeneme potvrđena je fenotipskim testom i reakcijom lančane polimerizacije u Nacionalnoj referentnoj laboratoriji za registrovanje i praćenje rezistencije bakterijskih sojeva na antimikrobna sredstva u Novom Sadu. **Rezultati.** Od ukupno 129 prikupljenih sojeva, bio je 121 (93,8%) izolat *K. pneumoniae* i 8 (6,2%) izolata *E. coli*. Iz urina je izolovano 70 (54,3%) sojeva, iz krvi 26 (20,2%), iz sekreta rana 19 (14,7%) i 14 (10,9%) iz sekreta donjeg respiratornog trakta. Geni koji kodiraju karbapenemaze su nađeni kod 58 (45%) izolata. Gen *bla New Delhi metallo-beta-lactamases* (bla_{NDM}), dokazan je kod 33 (27,3%) izolata *K. pneumoniae, bla oxacillinases-48* (*bla*OXA-48) kod 10 (8,3%), *bla K. pneumonia carbapenemase* (*bla*KPC) kod 1 (0,8%), a kod 7 (5,4%) izolata su istovremeno nađeni geni *bla*OXA-48 i *bla*NDM. Kod 7 izolata *E. coli* su detektovani *bla*NDM geni. **Zaključak.** Najčešći tip karbapenemaza u Srbiji kod multirezistentnih izolata *K. pneumoniae* i *E. coli* je NDM. Istovremena produkcija OXA-48 i NDM detektovana je kod izolata *K. pneumoniae*. Prema našem saznanju, prvi put je nađena produkcija KPC u Srbiji.

Ključne reči:
enterobacteriaceae; lekovi, rezistencija mikroorganizama; karbapenemi; infekcija, intrahospitalna; genom, bakterijski; srbija; beta-laktamaze.

Correspondence to: Anika Trudić, Institute for Pulmonary Diseases of Vojvodina, Center for Microbiology, Immunology and Virology; Put doktora Goldmana 4, 21 204 Sremska Kamenica, Serbia. Phone: +381(21)4805348. E-mail: anika.trudic@mf.uns.ac.rs

Introduction

Due to increased global transport, there is an increased exposure of people all around the world to diverse Gram-negative bacteria from gut flora, especially *Escherichia coli* and *Klebsiella spp*. Fecal carriage is recognized as the most important for spreading multidrug-resistant strains in the hospital environment [1]. Multidrug-resistant bacteria represent an important source of concern as effective therapeutic options of infections they cause are limited or none [2]. Carbapenems are often used for the treatment of nosocomial infections as the last line therapy [3]. In the last decade, carbapenem resistance has escalated in medically important bacteria [4, 5]. In Europe *Klebsiella pneumoniae* is the most frequently reported carbapenem-resistant enterobacteria [6]. Isolation of carbapenem-resistant *E. coli* is of concern, as it spreads more easily in the community. Also, the treatment of such community-acquired infections might become a challenge [7]. Two main mechanisms are responsible for carbapenem resistance, the first refers to carbapenem-hydrolyzing enzymes (carbapenemases) and the second is usually a combination of deficiency of porin expression and overexpression of beta-lactamases with weak affinity for carbapenems [7]. The most frequently isolated carbapenemases are *K. pneumoniae* carbapenemases (KPC), Verona integron-encoded metallo-beta-lactamases (VIM), imipenemases (IMP), New Delhi metallo-beta-lactamases (NDM) and oxacillinases-48 (OXA-48) [5]. Carbapenemase-encoding genes are usually located on self-conjugative plasmids often accompanied with other non-beta-lactam resistant determinants [8]. Acquisition of genetic material through horizontal transfer explains the urge for proper detection of carbapenemase-producing strains [7, 8]. Unfortunately, the detection of carbapenemase producer cannot rely only on the resistance profile routinely done in microbiology laboratory as their minimal inhibitory concentration (MIC) values may sometimes lay within the susceptibility range. Also, some strains may produce other enzymes and mechanisms responsible for lower resistance to carbapenems [8, 9]. Therefore, multidrug-resistant isolates with lower susceptibility to carbapenems should be tested for the presence of carbapenemase in order to prevent hospital outbreaks. To our knowledge, there were no comprehensive studies considering the presence and the occurrence of carbapenemase production in enterobacteria in Serbia so far.

The aim of the study was to determine carbapenemase production in hospital isolates of multidrug-resistant *K. pneumoniae* and *E. coli* in Serbia.

Methods

The study included 129 nonrepetitive multidrug-resistant strains of *K. pneumoniae* and *E. coli* isolated from a clinical specimen (urine, blood, wound secretion/swab and lower respiratory tract secretions: tracheal aspirate, broncho-aspirate, broncho-alveolar lavage) from November 2013 to May 2014. The strains were collected from microbiology laboratories in Clinical Center Serbia (Belgrade), Clinical Center "Zvezdara" (Belgrade), Clinical Center "Dragiša Mišović" (Belgrade), Institute for Public Health Čačak (Čačak), Institute for Public Health Kikinda (Kikinda), Clinical Center Kragujevac (Kragujevac), Institute for Public Health Kraljevo (Kraljevo), Institute for Pulmonary Diseases of Vojvodina (Novi Sad), Institute for Public Health of Vojvodina (Novi Sad), Institute for Public Health Niš (Niš). Estimated population coverage of the 14 hospitals involved was around 5 million. Collected strains were reported intermediate or resistant to at least one carbapenem (imipenem, meropenem, ertapenem) according to the Clinical and Laboratory Standards Institute (CLSI) [10].

The study was conducted at National Reference Laboratory for Registration and Surveillance of Antimicrobial Resistance of Bacterial Strains in the Institute for Public Health of Vojvodina, Novi Sad, Serbia. Identification of isolated strains was done using VITEK 2 Compact GN cards (BioMérieux, Marcy I Etoile, France). Antimicrobial susceptibility was determined using the disk diffusion method and/or using VITEK 2 AST-GN71 and AST-N240 cards according to CLSI. Susceptibility to fosfomycin was tested by E test strip (AB, Biodisk, Solna, Sweden). For the interpretation of tigecycline MICs, Food and Drug Administration (FDA) breakpoints for *Enterobacteriaceae* were used (susceptible $\leq$ 2 mg/L, intermediate 4 mg/L; resistant $\geq$ 8 mg/L). Phenotypic testing of extended-spectrum beta-lactamases (ESBL) production was done using combined disk tests (CDT) using cefotaxime disk and cefotaxime/clavulanic acid disk and ceftazidime and ceftazidime/clavulanic acid disk (Bio-Rad, France). Enhancement of the zone of inhibition more than 5 mm of the inhibitor-containing disc was considered to be a positive result. Phenotypic testing of carbapenemase production was done by double-disk synergy test (DDST) using tablets containing meropenem (10 μg), cloxacillin, dipicolinic acid, boronic acid (Rosco Diagnostica Neo-Sensitabs, Taastrup, Denmark) according to manufactures' instructions. Enhancement of the zone of inhibition in the area between meropenem disc and the inhibitor-containing disc was considered to be a positive result. Confirmation of carbapenemase production was done using polymerase chain reaction (PCR). PCR reaction of five genes was performed as two separate multiplex reactions and one simplex reaction with Mastercycler personal (Eppendorf, Hamburg, Germany). The first reaction included primers for bla_{NDM} [11] and bla_{KPC} [12] genes, the second included primers for bla_{OXA48} [13] and bla_{VIM} [14] genes. PCR cycling conditions for the first reaction were 1 cycle at 95 ºC for 5 min, 30 cycles of 95 ºC for 30 s, 60 ºC for 30 s, and 72 ºC for 60 s, followed by 1 cycle at 72 ºC for 3 min and holding stage at 4ºC. PCR cycling conditions for the second reaction were 1 cycle at 95 ºC for 5 min, 30 cycles of 95 ºC for 30 s, 58 ºC for 30 s, and 72 ºC for 60 s, followed by 1 cycle at 72 ºC for 3 min and holding stage at 4 ºC. Gene bla_{IMP} [14] was tested separately under following conditions: one cycle at 95ºC for 5 min, 30 cycles of 95 ºC for 30 s, 58 ºC for 30 s, and 72 ºC for 60 s, followed by 1 cycle at 72 ºC for 3 min and holding stage at 4ºC. All primers are shown in Table 1. The PCR-amplified products were analyzed by 2% agarose (MBG, Fischer Scientific, USA) gel electrophoresis and stained with

Table 1

Primers for carbapenemase genes

Primer name	Sequence 5'–3'
*bla*KPC Fw	ATGTCACTGTATCGCCGTCT
*bla*KPC Rw	TTTTCAGAGCCTTACTGCCC
*bla*VIM Fw	GATGGTGTTTGGTCGCATA
*bla*VIM Rw	CGAATGCGCAGCACCAG
*bla*NDM Fw	GGGCAGTCGCTTCCAACGGT
*bla*NDM Rw	GTAGTGCTCAGTGTCGGCAT
*bla*IMP Fw	GGAATAGAGTGGCTTAATTCTC
*bla*IMP Rw	CCAAACCACTACGTTATCT
*bla*OXA-48 Fw	TTGGTGGCATCGATTATCGG
*bla*OXA-48 Rw	GAGCACTTCTTTTGTGATGGC

KPC – *Klebsiella pneumoniae* carbapenemases; VIM – Verona-inkgron encoded metallo-beta-lactamases; NDM – New Delhi metallo-beta-lactamases; IMP – imipenemases; OXA-48 – oxacillinases-48.

ethidium bromide. Images were documented by a BioDocAnalyze system (Biometra, Germany).

The statistical analysis of the results was performed using the Statistical Package for the Social Sciences (SPSS), version 20. Results were expressed through the descriptive statistics, as simple frequencies and percentages. The χ^2 test was used for determination of statistically significant differences. The tested significance level was $\alpha = 0.05$.

Results

The study included 129 isolates of multidrug-resistant *K. pneumoniae* and *E. coli* isolated from a different clinical specimen of hospitalized patients. There were 121 isolates of *K. pneumoniae* and 8 isolates of *E. coli*. The patients from whom the isolates were obtained included 69 (53.5%) males and 59 (45.7%) females. The patients' mean age was 53 (SD ± 24) years. According to the location in hospital in the moment when the sample was taken 71 (55%) were collected from non-intensive care units and 58 (45%) were taken from intensive care units. The distribution of clinical specimen is shown in Table 2.

Table 2

Clinical specimen used for obtaining carbapenemase-producing isolates

Clinical specimen	n	%
Urine	70	54.2
Blood	26	20.2
Wound secretion/swab	19	14.7
Lower respiratory tract secretions	14	10.9
Total	129	100.0

The antimicrobial resistance patterns of collected isolates are presented in Table 3.

Susceptibility to carbapenems of *K. pneumoniae* and *E. coli* is shown separately in Table 4.

Using PCR carbapenemase genes were detected in 58 (45%) isolates. Gene bla_{NDM} was detected in 40 (31%) isolates, bla_{OXA-48} in 10 (7.8%), bla_{KPC} in 1 (0.8%) isolate. Seven (5.4%) tested strains were positive for both bla_{OXA-48} and bla_{NDM}. Genes bla_{VIM} and bla_{IMP} were not detected in tested isolates. Types of carbapenemase genes found in both *K. pneumoniae* and *E. coli* are shown in Table 5.

Table 3

Antimicrobial susceptibility of collected *Klebsiella pneumoniae* and *Escherichia coli* isolates

Tested antibiotic	S%	I%	R%
Ampicillin	0	0	100
Amoxicillin	0	0	100
Amoxicillin with clavulanate	0	0	100
Piperacillin	0	0	100
Cefazoline	0	0	100
Cefuroxime	0	0	100
Ceftriaxone	0	0	100
Ceftazidime	0	0	100
Ciprofloxacin	6.2	0	93.8
Levofloxacin	6.2	0	93.8
Cefepime	0	7	93
Cotrimoxazole	9.3	0	90.7
Piperacillin-tazobactam	0.8	8.5	90.7
Gentamicin	10.9	0	89.1
Amikacin	40.3	26.4	33.3
Tigecycline	82.9	5.4	10.9
Fosfomycin	90.7	0	9.3
Colistin	96.1	0	3.1

Table 4

Susceptibility of tested isolates to carbapenems

Antibiotics	*Klebsiella pneumonia* n (%)	*Escherichia coli* n (%)
Imipenem		
S	67 (55.4)	1 (12.5)
I	5 (4.1)	1 (12.5)
R	49 (40.5)	6 (75)
Meropenem		
S	47 (38.8)	21 (12.5)
I	16 (13.2)	0 (0)
R	58 (47.9)	7 (87.5)
Ertapenem		
S	0	0
I	13 (10.7)	1 (12.5)
R	108 (89.3)	7 (87.5)

S – sensitive; I – intermediate; R – resistant.

Phenotypic tests for ESBL producers and carbapenemase producers were performed in order to enlighten the beta-lactam resistance mechanism of carbapenem-resistant strains. The results are presented in Table 6.

All except one isolate with positive DDST suggested the presence of metallo-beta lactamases. Carbapenemase ge-

Table 5

Carbapenemase genes detected in *K. pneumoniae* and *E.coli*

Carbapenemase genes	*K. pneumoniae*	*E. coli*
	n (%)	n (%)
bla_{KPC}	1 (0.8)	0
bla_{NDM}	33 (27.3)	7 (87.5)
bla_{OXA-48}	10 (8.3)	0
bla_{OXA-48} and bla_{NDM}	7 (5.8)	0
No genes detected	70 (57.8)	1 (12.5)

KPC – *K. pneumoniae* carbopenemases; OXA-48 – oxacillinases; NDM – New Delhi metallo-beta-lactamases.

Table 6

Relation between phenotypic tests and carbapenemase genes detection

Phenotypic testing	Carbapenemase genes detected	No genes detected	Total
CDT for ESBL-P	0	39	39
DDST for CP	41 (89.1%)	5 (10.9%)	46
Negative	17 (38.6%)	27 (61.4%)	44
Total	58	71	129

***CDT for ESBL-P – combined disk test for extended beta-lactamase production; DDST for CP – double-disk synergy test for carbapenemase production.**

nes were detected in 41 (89.1%) DDST-positive isolates. Among strains negative for phenotypic testing 17 (38.6%) carried carbapenemase genes, bla_{OXA-48} was found in 10 and both bla_{OXA-48} and bla_{NDM} in 7 strains.

Among 58 isolates with carbapenemase-encoding genes, 52 (89.7%) were resistant to imipenem. All 58 isolates were resistant to meropenem and ertapenem (Figure 1).

Among 71 isolates without carbapenemase-encoding genes, 3 (4.2%) were resistant to imipenem and 7 (9.9%) were resistant to meropenem.

According to the hospital location in the moment of sampling 37 (63.8%) carbapenemase-producing isolates were collected from patients treated in intensive care units and 21 (29.6%) from other wards ($\chi^2 = 15.848$; $p = 0.007$).

Discussion

K. pneumoniae and *E. coli* are frequently responsible for numerous community and hospital acquired infections [15]. Although originally being human commensals susceptible to almost all antimicrobial agents, in last 15 years we witness a rapid dissemination of multidrug-resistant strains [16]. Resistance to carbapenems is usually a consequence of the acquisition of carbapenemase genes. Being mostly plasmid-encoded, carbapenemase-producing enterobacteria are often accompanied by other resistance genes [15]. The horizontal genetic transfer may cause rapid and extensive dissemination of multidrug-resistant carbapenemase-producing strains not only in healthcare facilities but also within the region or even across borders [17].

On the other hand, carbapenemase-producing enterobacteria are not necessarily clinically resistant to carbapenems thus representing a diagnostic challenge to routine laboratories. Usually, recommendations are either based on epidemiological cutoff values of minimal inhibitory concentrations or clinical breakpoints for carbapenems. It is advised that screening criteria may and should be adjusted depending on the epidemiological situation in a given ecological setting [16]. So far, there have been no comprehensive studies considering the presence and the occurrence of carbapenemase production in enterobacteria in Serbia. Their presence may be missed if different criteria are followed, especially by laboratories lacking the experience in interpreting and performing phenotypic tests.

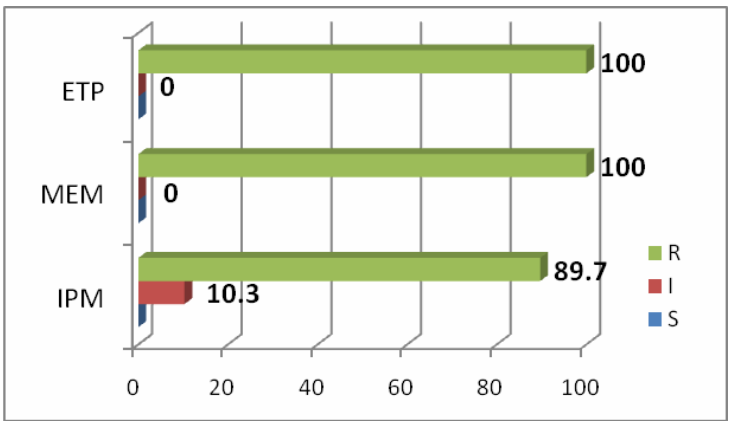

Fig. 1 – Susceptibility to carbapenems of the isolates with carbapenemase encoding genes.
ETP – ertapenem; MEM – meropenem; IPM – imipenem; R – resistant; I – intermediate; S – sensitive.

A total of 129 multidrug-resistant strains with lower susceptibility to routinely used carbapenems was collected in 6 months period from various hospitals in Serbia. *K. pneumoniae* and *E. coli* were isolated mostly from urine and less frequently from blood, wound secretion or swab and lower respiratory tract secretions. Resistance rates for 13 tested antimicrobial agents were very high. Good activity maintained for amikacin, fosfomycin, tigecycline, and colistin.

Collected strains were all intermediately susceptible or resistant to ertapenem. Ertapenem is considered the most sensitive indicator for carbapenemase production, though often with low specificity due to either production of ESBL or overproduction of AmpC beta-lactamases [18]. In our study, none of solely ertapenem intermediate or resistant isolates harbored carbapenemase-encoding genes nor were positive in phenotypic testing for carbapenemase production. All isolates with carbapenemase genes were resistant to meropenem, suggesting that meropenem susceptibility might be an indicator for carbapenemase production among *K. pneumoniae* and *E. coli* in Serbia.

After performing phenotypic testing, 46 strains were suspected for carbapenemase production and 41 carried tested carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}). Positive phenotypic test in 5 isolates that tested negative for carbapenemase genes indicated the presence of metallo-beta lactamase. Additional testing is needed to detect the type of metallo-beta lactamase.

Phenotypic tests for carbapenemase production failed to detect 17 isolates with carbapenemase genes. There are no specific inhibitors for OXA-48 carbapenemase commercially available, therefore 7 strains would be missed without molecular testing. Also, phenotypic tests for carbapenemase production are unreliable when two different mechanisms of carbapenemase production occur [19].

More isolates with carbapenemase production were found among samples collected in intensive care units compared to other wards. Together with various risk factors such as age of patients, underlying illness and co-morbid conditions of the host, the intensive care unit stay and carbapenem resistance are the most important predictors of increased mortality and treatment failure [16].

Carbapenemase genes were detected in 58 isolates. The most prevalent carbapenemase was NDM found in both *K. pneumoniae* and *E. coli*. NDM producing strains are found in 9 hospitals from 7 different cities in Serbia. NDM was first identified in 2008 from *K. pneumonia* in Sweden from a patient treated in India [20] and has been reported worldwide [4]. India is considered to be endemic although after the first comprehensive analyses a smaller percentage of cases were connected to Balkan countries [4]. However, the most Balkan countries were lacking data or were uncertain considering the occurrence of carbapenemases among enterobacteria [6]. In Serbia, NDM was detected for the first time in *Pseudomonas aeruginosa* in 2010 [21]. In 2011 NDM was detected for the first time in *K. pneumoniae* in Belgrade isolated from urine of a 7-month-old baby boy, though without any clinical signs of infection [22]. As for Bulgaria, an outbreak caused by NDM producing *E. coli* was reported [23], but also VIM and KPC producing *K. pneumoniae* were documented showing the diversity of circulating carbapenemases [24]. In Croatia, VIM producing enterobacteria were the most prevalent but NDM producing strains were isolated [25]. In Greece, KPC and VIM reached epidemic proportions [6, 26]. No available data were found considering Albania, Bosnia and Herzegovina and the Republic of Macedonia. NDM producing enterobacteria originated from Montenegro and Kosovo, Serbia was reported in Belgium [27]. Also, NDM and OXA-48 carbapenemase were found in carbapenem-resistant enterobacteria in the neighboring Romania [28]. In general, NDM did not reach such a wide distribution in Europe as KPC according to data from 2013 [6]. United Kingdom has been reporting more NDM isolates comparing to other European countries [17]. Although an outbreak caused by metallo-lactamase producing *Proteus mirabilis* (VIM, IMP) in surgical intensive care unit of Clinical Center Serbia, Belgrade was described [29], none of tested isolates carried bla_{VIM} nor bla_{IMP} genes.

OXA-48 was first detected in *K. pneumoniae* in Turkey followed with hospital outbreaks across the country [30]. Among European countries, OXA-48 was the most frequently detected in Belgium, France and Malta [6]. OXA-48 producing *K. pneumoniae* was reported in Slovenia [31], but no OXA-48 carbapenemase was found in a multicentre study in Croatia [25]. Recently, in Hungary, two isolates of *K. pneumoniae* harboring OXA-48-like carbapenemases were characterized [32]. In our study OXA-48 carbapenemase was found in 10 *K. pneumoniae* isolates obtained from 4 different healthcare facilities from 3 different cities (Belgrade, Niš and Kikinda).

Co-production of OXA-48 and NDM in *K. pneumoniae* is rarely reported. According to published data, both OXA-48 and NDM were found in *K. pneumoniae* isolates in Tunisia [33], Morocco [34] and Turkey [35]. An extensively drug-resistant isolate of *K. pneumoniae* with both OXA-48 and NDM obtained from a rectal swab of a patient transferred from the intensive care unit of a hospital located in Belgrade (Serbia) to Bern University Hospital in Switzerland was described [36]. Among collected isolates, both OXA-48 and NDM were found in 7 *K. pneumoniae* isolates. Isolates were obtained from patients hospitalized in 2 healthcare facilities in Belgrade from different clinical specimens (urine, wound secretion and blood).

The first KPC producing *K. pneumoniae* was identified in 1996 in the eastern part of the United States and has been spreading since [7]. KPC is the most frequently detected carbapenemase among *Enterobacteriaceae* in Europe particularly in Italy and Greece [6]. KPC harboring *K. pneumoniae* has been isolated in the neighboring Hungary [37] and Croatia [25]. In our study, KPC carbapenemase was detected in only one isolate of *K. pneumoniae* from the patient treated in the intensive care unit in the Institute for Pulmonary Diseases of Vojvodina near Novi Sad without previous hospitalization. As far as we know, KPC production was detected for the first time in Serbia.

Conclusion

To our knowledge, this is the most comprehensive national report on carbapenemase-producing enterobacteria in Serbia. NDM carbapenemase is the most prevalent among isolates of *K.*

pneumoniae and *E. coli.* Also, rarely described co-production of OXA-48 and NDM is found in *K. pneumoniae* isolates. KPC production is documented for the first time. Further characterization of detected genes as well as further detailed epidemiological studies are needed. It is of great importance to make a unique and precise guideline for routine microbiology laboratories in order to detect carbapenemase-producing strains adequately and to monitor the epidemiological situation on the national and international level.

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Neurophysiological evaluation of short-term outcome of pharmacological treatment of diabetic neuropathy

Neurofiziološka procena kratkoročnog ishoda farmakološkog lečenja dijabetesne neuropatije

Milan Cvijanović*†, Svetlana Simić*†, Aleksandar Kopitović*†, Ranko Raičević‡§

Clinical Center of Vojvodina, *Neurology Clinic, Novi Sad, Serbia; University of Novi Sad, †Faculty of Medicine, Novi Sad, Serbia; Military Medical Academy, ‡Neurology Clinic, Belgrade, Serbia; University of Defence, §Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. Diabetic polyneuropathy (DPN) is a very frequent and progressive disease that severely impairs the overall quality of life, accompanied by a high rate of disability. For these reasons, the testing of therapeutic agents for this disease is increasing. **Methods.** We tested the most frequently used drugs for diabetic neuropathy in our area, along with electrophysiological monitoring in order to avoid subjectivity and the "placebo effect". A total of 120 patients were divided into four groups: three groups who received alpha-lipoic acid, benfotiamine or gabapentin respectively, and the control group who did not receive any treatment. In all the patients we analyzed motor conduction velocity, distal latency, sensory conduction velocity, F wave and F wave chronodispersion before and after treatment with each drug. **Results.** It is evident that some drugs had a favorable impact on the condition of the peripheral nerves. Alpha-lipoic acid and benfotiamine had an impact on the recovery of the nerve, i.e. pathophysiological processes, whereas gabapentin had no impact on the recovery, similarly to the control group without any treatment. Electrophysiological indicators had different sensitivity to detect conditions of the peripheral neurons. The best effect, in terms of increased sensory conduction velocity, had the patients treated with alpha-lipoic acid. **Conclusion.** The effect of alpha-lipoic acid and benfotiamine on the condition of peripheral nerve was evident. The failure of recovery, i.e. deterioration of electrophysiological parameters in patients who did not receive neuroprotective therapy suggests the need of permanent medication and periodic electrophysiological monitoring of patients with diabetic polyneuropathy.

Keywords:
diabetic neuropathies; electromyography; drug therapy; thioctic acid; thiamine monophosphate; gabapentin; treatment outcome.

Apstrakt

Uvod/Cilj. Dijabetesna polineuropatija (DPN) je veoma česta, progredijentna bolest koja dovodi do grubog urušavanja kvaliteta života praćenog visokom stopom invalidnosti. Iz tih razloga ispitivanje terapijskih sredstava za ovu bolest je u zamahu. **Metode.** Ispitivani su najčešće korišćeni lekovi za dijabetesnu neuropatiju u našem podneblju, uz elektrofiziološko praćenje da bi se izbegla subjektivnost i „placebo efekat“. Kod ukupno 120 bolesnika podeljenih u četiri grupe ispitivana je alfa lipoinska kiselina, benfotiamin i gabapentin, s tim što je elektrofiziološki praćena i grupa bolesnika koji u posmatranom periodu nisu dobijali terapiju. Analizirali smo motornu brzinu provođenja, distalnu latencu, senzitivnu brzinu provođenja, F talas i hronodisperziju F talasa, pre i posle terapije za svako pojedinačno terapijsko sredstvo. **Rezultati.** Evidentno je da su neki lekovi imali povoljan uticaj na stanje perifernog nerva. Alfa lipoinska kiselina i benfotiamini su imali uticaja na oporavak nerva odnosno patofiziološke procese, gabapentin je bio bez uticaja na oporavak, a slično je bilo i kod kontrolne grupe koja je bila bez bilo kakve terapije. Elektrofiziološki pokazatelji su imali različitu osetljivost na detekciju stanja perifernog neurona. Najbolji efekat koji se ogleda u povećanju senzitivne brzine provođenja imali su bolesnici koji su tretirani alfalipoičnom kiselinom. **Zaključak.** Uticaj alfa lipoinske kiselina i benfotiamina na stanje perifernog nerva je evidentno. Odsustvo oporavka, odnosno pogoršanje elektrofizioloških pokazatelja kod ispitanika koji nisu dobijali neuroprotektivnu terapiju, ukazuje na potrebu permanentne medikacije i periodičnog elektrofiziološkog praćenja bolesnika sa dijabetesnom polineuropatijom.

Ključne reči:
dijabetesne neuropatije; elektromiografija; lečenje lekovima; tioktinska kiselina; tiamin monofosfat; gabapentin; lečenje, ishod.

Correspondence to: Milan Cvijanović, Clinical Center of Vojvodina, Neurology Clinic, 21 000 Novi Sad, Serbia.
E-mail: emgnscvij@gmail.com

Introduction

Diabetic neuropathy is a complex group of clinical syndromes that damage the different regions of the nervous system, either individually or in combination [1]. It is the most common and the most unpleasant complication of diabetes, leading to high morbidity and mortality, which results in large economic costs [2].

Assessment of the frequency of this damage in patients with diabetes is not simple and a wide range of various assessments are available depending on the applied criteria and methods for defining neuropathy. However, it can be said that diabetic neuropathy is the most common form of neuropathy, which is responsible for more hospitalizations than all other diabetic complications together. It should not be underestimated, because this "late" complication of diabetes can lead to foot ulcers and gangrene, lower leg and foot amputations, or even sudden death of a patient, presumably due to cardiovascular autonomic neuropathy [3].

The most common type of diabetic neuropathy, accounting for around 80% of all diabetic neuropathies, is a distal symmetric neuropathy, or otherwise called diabetic sensorimotor polyneuropathy [4], with initial symptoms in the feet, spreading upwards. Hyperglycemia is the most important modifiable risk factor for its emergence, as well as for other complications of diabetes, so a maximum control of glucose levels is the primary goal in diabetic patients [5].

The question then arises as to why additional remedies for neuropathy are continuously searched for, considering that a reliable control of blood glucose levels is possible. First, because despite considerable effort, the desired level of metabolic control is often not achieved [6], and second, despite the ideal glucoregulation, a significant number of patients with diabetes still experience neuropathic damage. A recent meta-analysis suggests that a constant glucose control prevents the development of clinical neuropathy only in type 1 diabetes, whereas in type 2 no reduction in incidence is found [7]. The quality of life of these patients is often very poor. This indicates a problem of early diagnosis and a successful treatment action, which should be individualized.

In the clinical practice, a large number of drugs and therapeutic procedures exist, which do not always lead to improvements. Drugs are divided into two groups: symptomatic treatment, or coanalgesics, and a therapy that presumably acts on the pathogenesis of diabetic polyneuropathy. Our observational study dealt with the monitoring of the neurophysiological state in diabetic polyneuropathy. Originally, we planned that the group of patients who received gabapentin for painful diabetic neuropathy serves as controls. However, due to insufficient understanding of the mode of action of this drug in neuropathic pain, we could not conclude with certainty about its possible impact on the status of the peripheral neurons or its potential impact on the pathogenesis. Therefore, the electrophysiological examination was carried out on patients without any therapy that could have had an impact on the state of the peripheral neurons. Gabapentin (GBP) belongs to the group of alfa 2-delta ligands, along with pregabalin, and they have been examined in several studies [8] and are approved for use by the Food and Drug Administration (FDA) and the European Medical Agency. There is data that pregabalin has a more predictable and consistent pharmacokinetic profile and could be titrated faster and easier to use in comparison to gabapentin [9].

Benfotiamine (BENTO) the thiamine monophosphate synthetic analogue, has improved bioavailability compared with thiamine [10]. It also has a preventive effect on microvascular complications in rats, without affecting the glycemic control [11]. Some short-term studies, lasting 8–12 weeks, have suggested its favorable impact on polyneuropathy [12], and along with its widespread use, we chose it for our research. However, probably the longest used and most thoroughly studied drug for diabetic neuropathy (Study 1 Sydney, Sydney 2, etc.), as confirmed by recent research, is alpha-lipoic acid (ALA) [13, 14], which has been shown to have a significant impact on the improvement of diabetic polyneuropathy.

The aim of this study was to study the effects of alpha-lipoic acid, benfotiamine and gabapentin on electrophysiological parameters of the state of peripheral nerves in diabetic polyneuropathy, with a control group of patients without any treatment. In addition, we aimed to determine the differences among the applied treatments and to estimate changes in individual electrophysiological parameters caused by the applied treatment.

Methods

The study was designed as an observational analytical study, with the purpose to determine whether the drugs used in the study have an effect in the treatment of peripheral nerve disorders in diabetic patients, which of them are more effective in the treatment of peripheral nerve injury, and to assess the needs for treatment in the controls in relation to the results of repeated electrophysiological findings.

Our study monitored the neurophysiological state in patients with diabetic polyneuropathy, who were referred to electromyoneurographic examination to the Electromyography Unit of the Neurology Clinic of the Clinical Center of Vojvodina by primary care physicians. Since the first control, the patients were subsequently recommended symptomatic treatment (gabapentin) or causally-related therapy (benfotiamine or alpha-lipoic acid), depending on the clinical picture, and electrophysiological examination at our unit was performed approximately for 3 months.

The choice of the drug was the exclusive responsibility of the neurologist performing an electrophysiological examination, and it is of note that for the last several years we have utilized the above-stated drugs. This was a standard procedure at our unit, however, patient's personal preferences were also taken into account and it could be determined only on the control examination whether the patient received the appropriate treatment. Originally, we had intended to recruit patients who received coanalgetic (gabapentin) as controls, but the control electrophysiological examination showed that a significant number of these patients did not take the recommended treatment, so these patients were taken

as controls. On the other hand, we had the opportunity to observe whether gabapentin still has some influence on the condition of the peripheral neurons.

The research was done as a prospective study. In the period over one year, we examined and analyzed a total of 120 patients with diabetic polyneuropathy, who were selected from patients examined at our Electromyography Unit. Each patient underwent electromyoneurographic examination, and data was collected according to a protocol in two acts: for the first time during the examination of patients before the introduction of the study drug, and the second time on the follow-up examination after three months. The inclusion criteria for the study were: patients with type 2 diabetes mellitus regularly referred by primary care physicians to electromyoneurographic examination aged 40–60 years. The exclusion criteria were: a history of other diseases and conditions that could lead to polyneuropathy and patients who during the study period stopped taking the study drug for some reason.

The criterion for dividing subjects into four groups was the drug they received, including a group who during the three-month period did not receive any medication: patients who received 600 mg of alpha-lipoic acid per day, patients receiving 600 mg of benfotiamine *per* day, patients receiving 900 mg of gabapentin *per* day, and patients who did not receive any medication Each group comprised 30 patients; Neurophysiological examination was performed at the Electromyography Unit in the following way: Electrophysiological indicators were studied by the two-channel MEDELEC system Synergy. We used methods of conventional electromyoneurography without the employment of insertion and denervation activity and innervation sample because it was not possible to exactly compare the quantitative data. Therefore we used stimulation methods of neurography with registering the EP on standard places while keeping the room temperature at around 25° C, and the patient's extremity skin temperature at around 32° C. The following parameters were recorded: motor conduction velocity (MCV) – ref. 48.3 ± 3.9 > 40 m/s, distal latency (DL) – ref. 5.1 ± 2.3 or ≤ 5.0 ms, sensory conduction velocity (SCV) – ref. 58.8 ± 5.8 (47m / s), F wave minimal latency (F min) – ref. for *nervus peroneus* 48.4 ± 4.0 (55 ms, modified for our laboratory), and the time span of F waves or F wave chronodispersion (ChrD) – for our laboratory the reference value of 2.4 ± 1.2 (5 ms). ChrD means the time difference between the minimum and maximum latency (10 consecutive stimulations).

In this study, we used the following statistical methods: for the measurement of central tendency we used descriptive analysis, which included the mean, while measures of variability included the standard deviation; for testing of the statistical hypothesis we used *t*-test; the multivariate analysis of variance (MANOVA) was used for comparisons of sample means of dependent variables.

Results

Patients in all study groups had moderately abnormal electrophysiological parameters, with signs of impairment of long fibers. Patients treated with alpha-lipoic acid or benfotiamine had mild pain, rated on Numeric Pain Rating Scale (NPRS) < 4. Patients treated with gabapentin had NPRS > 4, whereas controls had NPRS < 3. There were 55% of male and 45% female patients, aged 40–60 years, in average 46.5.

Table 1 shows that the initial motor conduction velocity before the applied therapy (MCV-i) and final motor conduction velocity after the three months of treatment (MCV-f) differed in all tested groups. Taking into account the reference values, we can observe that the application of the neuroprotective agents, alpha-lipoic acid and benfotiamine, increased, but not significantly, motor conduction velocity. In addition, the symptomatic treatment by coanalgesic gabapentin reduced motor conduction velocity. In the control group, where no treatment was applied, there was also a reduction in motor conduction velocity.

Table 2 shows that the application of neuroprotective therapy, alpha-lipoic and benfotiamine, caused shortening of distal latency, which is an indicator of improvement, but dif-

Table 1

The effect of the treatment on motor nerve conduction velocity in patients with diabetic polyneuropathy

Treatment	MCV-i (m/s)	MCV-f (m/s)	*t*	*p*
ALA	41.220	42.910	1.534	0.130
Benfo	40.610	41.113	0.670	0.505
GBP	40.980	40.493	0.712	0.479
Controls	40.080	39.690	0.545	0.588

MCV-i – initial motor conduction velocity; MCV-f – final motor conduction valocity; ALA – allpha-lipoic acid; Benfo – benfotiamine; GBP – gabapentin; controls – no treatment.

Table 2

The effect of the treatment on initial distal latency (DL-i) and final distal latency (DL-f) in patients with diabetic polyneuropathy

Treatment	DL-i (ms)	DL-f (ms)	*t*	*p*
ALA	5.350	5.133	0.769	0.445
Benfo	5.458	5.323	0.734	0.466
GBP	5.420	5.557	1.057	0.295
Controls	5.717	5.980	1.668	0.101

ALA – alpha-lipoic acid; Benfo – benfotiamine; GBP – gabapentin; controls – no treatment.

ferences were not statistically significant. On the other hand, the application of gabapentin and no treatment in controls led to prolongation of distal latency, i.e. deterioration of the condition, but also with no statistical significance.

Table 3 increased shows that the sensory conduction velocity after application of alpha-lipoic acid was significantly increased ($p < 0.05$). The application of gabapentin and no treatment in the control group showed a reduction in SCV. In the control group, this reduction was significant.

Table 4 shows that the application of the neuroprotective therapy with alpha-lipoic acid and benfotiamine caused shortening of F waves, whereas in the gabapentin group, as well as in the controls, there was a prolongation of F-waves, i.e. progression of the disease, but none of the differences reached statistical significance.

Table 5 shows that F wave chronodispersion was shorter after the application of the neuroprotective therapy; conversely, it was longer after the application of gabapentin or no treatment. However, these differences were not statistically significant.

lem are fewer. Great psychological and physical problems, as well as the economic costs of the disease, require a targeted, causal therapy. By development of electrophysiological diagnostics, diabetic polyneuropathy is much earlier diagnosed and quantified, although this method is still not widely available, so the clinical criteria are still prevalent. However, over the past few decades, this method has been used in research studies for assessment of severity and type of damage. On the other hand, the lack of an effective drug for the prevention and treatment has encouraged extensive research worldwide aimed at finding an efficient drug that would affect the pathogenesis of peripheral nerve injury [16].

In our observational-analytical study of 120 diabetic patients (type II diabetes) with distal symmetric polyneuropathy, there was a certain preponderance of males (55%), compared to females (45%). Although it is considered that at the age of 30–55 years androgens significantly favor atherogenic [17, 18], and thereby probably neuropathogenic effects in men, there are no significant differences in the clinical picture or therapeutic effects on the existing changes in

Table 3

The effect of treatment on initial sensory conduction velocity (SCV-i) and final sensory conduction velocity (SCV-f)

Treatment	SCV-i (m/s)	SCV-f (m/s)	*t*	*p*
ALA	31.353	35.350	2.292	0.026
Benfo	33.427	35.093	0.787	0.435
GBP	36.867	34.773	1.245	0.218
Controls	36.597	33.173	2.488	0.016

ALA – alpha-lipoic acid; Benfo – benfotiamine; GBP – gabapentin; controls – no treatment.

Table 4

The effect of initial treatment on F waves (Ftl-i) and final F waves (Ftl-f) in patients with diabetic polyneuropathy

Treatment	Ftl-i	Ftl-f	*t*	*p*
ALA	58.610	56.893	0.836	0.407
Benfo	59.697	59.530	0.117	0.907
GBP	59.450	61.197	1.393	0.169
Controls	59.967	62.160	1.682	0.098

ALA – alpha-lipoic acid; Benfo – benfotiamine; GBP – gabapentin; controls – no treatment.

Table 5

The effect of treatment on initial F wave chronodispersion (ChrD-i) and final F wave chronodispersion (ChrD-f)

Treatment	ChrD-i	ChrD-f	*t*	*p*
ALA	10.680	9.290	1.197	0.236
Benfo	12.300	10.757	1.504	0.138
GBP	9.733	13.433	1.533	0.135
Controls	9.353	10.920	1.889	0.064

ALA – alpha-lipoic acid; Benfo – benfotiamine; GBP – gabapentin; controles – no treatment.

Discussion

Diabetic neuropathy as a complication of diabetes leads to microvascular damage, including small blood vessels that supply the nerves of *vasa nervorum*. Therefore, diabetic neuropathy is a degenerative disease that leads to progressive disability, affecting peripheral nerves, namely pain fibers, motor neurons and autonomic fibers [15], and can affect all organs that are innervated. Most treatments are symptomatic, while therapeutic options that eliminate the root of the problem are fewer. Great psychological and physical problems, as well as the economic costs of the disease, require a targeted, causal therapy. By development of electrophysiological diagnostics, diabetic polyneuropathy is much earlier diagnosed and quantified, although this method is still not widely available, so the clinical criteria are still prevalent. However, over the past few decades, this method has been used in research studies for assessment of severity and type of damage. On the other hand, the lack of an effective drug for the prevention and treatment has encouraged extensive research worldwide aimed at finding an efficient drug that would affect the pathogenesis of peripheral nerve injury [16].

In our observational-analytical study of 120 diabetic patients (type II diabetes) with distal symmetric polyneuropathy, there was a certain preponderance of males (55%), compared to females (45%). Although it is considered that at the age of 30–55 years androgens significantly favor atherogenic [17, 18], and thereby probably neuropathogenic effects in men, there are no significant differences in the clinical picture or therapeutic effects on the existing changes in peripheral neurons in diabetic polyneuropathy. The average age of our patients was 46.5 years. The age differences were expected, because of the small age range we used in order to avoid the potential effect of age on the therapeutic response. The duration of DM was on average 8.2 years, ranging between 5 and 15 years. Our results convincingly show improvement of the condition of the peripheral nerves in subjects who received alpha-lipoic acid, which is in agreement with the results of other authors examining the influence of alpha-lipoic acid on larger samples and over longer time [19–21].

Although the statistical significance is not highly significant, it should be noted that this is a short-term study, and the very study design was such that it included subjects with advanced clinical diabetic polyneuropathy (referred by primary care physicians to specialists). Although alpha-lipoic acid showed a definite beneficial effect on all electrophysiological parameters, sensory conduction velocity was found the most sensitive, which may indicate that the effect is the strongest on the sensory nerve fibers. On the other hand, it can be concluded that this electrophysiological indicator is the most appropriate for the assessment of diabetic polyneuropathy. Efficacy of benfotiamine was also demonstrated in all electrophysiological parameters, but with less significance compared to alpha-lipoic acid. The favorable effects of benfotiamine, explained by an activating effect on transketolase and an inhibitory effect on alternative metabolic pathways in diabetic neuropathy, were confirmed in a recent study by the Hungarian author Várkonyi et al. [22], and even more convincing results were obtained by Norwegian researchers in their 24-month study [23], supporting our results. On the other hand, gabapentin had no impact on polyneuropathy and it is definite that this coanalgesic has no effect on the pathogenesis of diabetic neuropathy. In patients who received gabapentin, as well as in patients who did not receive any therapy, we found deteriorated electrophysiological parameters, or progression of polyneuropathy, although not with statistical significance, which is also explained by the relatively short duration of the study. Overall, sensory conduction velocity was proved to be the most sensitive neurophysiological indicator for detecting changes in the peripheral neurons, distal latency also showed a high sensitivity, while the sensitivity of other indicators was less significant. Therefore, it is recommended that the monitoring of diabetic polyneuropathy should involve primarily these two electrophysiological indicators.

Conclusion

Alpha-lipoic acid and benfotiamine had some impact on improving the neurophysiological parameters of peripheral neurons, in patients with diabetic polyneuropathy. Comparing the efficacy of these two drugs, we can conclude that alpha-lipoic acid was more effective. At the same time, we did not find electrophysiological signs of the benefits of the coanalgesic gabapentin on the condition of peripheral nerves or the course of polyneuropathy. Specifically, electrophysiological indicators were slightly worse in patients who received gabapentin, as well as in patients who did not receive any therapy, which indicates the progression of the damage of the peripheral neurons. In a relatively short period of three months there, we saw the deterioration of diabetic polyneuropathy in patients who did not receive therapy or received only symptomatic treatment and right improvement of polyneuropathy in groups of patients who received treatment for diabetic neuropathy. Therefore, an effective, 'neuroprotective' therapy should be applied continuously in this disease. The electrophysiological examination proved to be very precise and sensitive even in the case of small differences in the condition of the peripheral nerves, which indicates the need for regular electrophysiological monitoring of patients with diabetic polyneuropathy.

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Prognostic importance of metabolic tumor parameters on initial FDG-PET/CT in patients with isolated infradiaphragmatic Hodgkin's lymphoma

Prognostički značaj metaboličkih tumorskih parametara na inicijalnom FDG-PET/CT kod bolesnika sa izolovanim infradijafragmalnim Hočkinovim limfomom

Engin Alagoz*, Kursat Okuyucu*, Semra Ince*, Gokhan Ozgur[†], Ozlem Ozmen[‡], Alper Ozgur Karacalioglu*, Mustafa Ozturk[§], Nuri Arslan*

Gulhane Military Medical Academy and School of Medicine, *Department of Nuclear Medicine, [†]Department of Haematology, [§]Department of Medical Oncology, Etlik-Ankara, Turkey; Training and Research Hospital, Ataturk Chest Diseases and Thoracic Surgery, [‡]Department of Nuclear Medicine, Kecioren, Ankara, Turkey

Abstract

Background/Aim. Isolated infradiaphragmatic lymph node involvement is not common and makes up 5–13% of stage I-II Hodgkin's lymphoma. Important subjects about prognostic factors and optimal treatment of isolated infradiaphragmatic Hodgkin's lymphoma (II HL) have not been clearly defined. We aimed to evaluate the prognostic value of metabolic tumor indices on initial 18-fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) through quantitative PET/CT parameters together with the classical predefined risk factors for patients with II HL. **Methods**. This retrospective cohort study conducted between 2004 and 2015 included 21 patients for whom FDG-PET/CT were requested for primary staging. Quantitative PET/CT parameters (maximum standardized uptake value – SUV max) average standardized uptake value – SUV mean, metabolic tumor volume (MTV), and total lesion glycolysis (TLG) were used to estimate disease-free survival and overall survival. **Results**. Univariate Cox regression analysis was performed for all potential risk factors impacting metastasis/recurrence of the disease. Factors which had values of $p < 0.2$ after univariate analysis (sex, age, stage, bulky disease, SUV max, SUV mean, MTV, TLG) were processed with the multivariate model. Sex, TLG and bulky disease were found to be statistically significant risk factors for prognosis of outcome in patients with IIHL after multivariate analysis. **Conclusion**. The existence of bulky disease at the diagnosis and high TLG values on primary staging by FDG-PET/CT are potential risk factors for both disease-free survival and overall survival in Hodgkin's lymphoma with isolated infradiaphragmatic lymph node involvement.

Keywords:
hodgkin disease; lymph nodes; diaphragm; positron-emission tomography; fluordeoxyglucose f18; tomography, x-ray computed; prognosis.

Apstrakt

Uvod/Cilj. Prisustvo izolovanih infradijafragmalnih limfnih čvorova kod Hočkinovog limfoma (HL) nije često i javlja se kod 5–15% svih HL I-II stadijuma. Važni podaci o prognostičkim faktorima i optimalnom lečenju izolovanog infradijafragmalnog HL (IIHL) nisu jasno definisani. Cilj ovog ispitivanja bila je procena prognostičke vrednosti pokazatelja metabolizma tumora na inicijalnom pregledu pomoću 18-fluorodeoksiglukoza-pozitron emisione tomografije /kompjuterizovane tomografije (FDG-PET/CT) preko kvanititativnih PET/CT parametara zajedno sa klasičnim predefinisanim faktorima rizika za bolesnike sa II HL. **Metode**. U ovu retrospektivnu kohortnu studiju, sprovedenu između 2004. i 2015. godine, bio je uključen 21 bolesnik, kod kojih je za određenjivanje primarnog stadijuma bolesti primenjena FDG-PET/CT. Kvantitativni PET/CT parametri [vrednost maksimalnog standardizovanog preuzimanja (*maximum standardized uptake value* – SUVmax), prosečna vrednost standardizovanog preuzimanja (*mean standardized uptake value* – SUVmean), metabolički volumen tumora (metabolic tumor valume – MTV), ukupna glikoliza u lezijama (*total lesion glycolysis* – TLG)] korišćeni su za procenu preživljavanja bez prisustva bolesti (*disease-free survival* – DFS) i sveukupnog preživljavanja (*overall survival* – OS). **Rezultati**. Izvršena je univarijantna Cox-ova regresiona analiza svih potencijalnih faktora rizika koji imaju uticaj na metastaze/recidiv bolesti. Faktori koji su imali vrednost $p < 0,2$ posle izvršene univari-

Correspondence to: Engin Alagoz, Gulhane Military Medical Academy and School of Medicine, Department of Nuclear Medicine, 06018 Etlik, Ankara, Turkey. E-mail: ealagoz@gata.edu.tr

jante analize (pol, dob, stadijum bolesti, raširena bolest (*bulky disease*), SUVmax, SUVmean, MTV, TLG), potom su obrađeni u multivarijantnom regresionom modelu. Posle multivarijantne regresione analize pol, TLG i raširena bolest prepoznati su kao statistički značajni faktori rizika za prognozu bolesti kod bolesnika sa IIHL. **Zaključak**. Prisustvo raširene bolesti u momentu postavljanja dijagnoze i visoka vrednost TLG kod određivanja primarnog stadijuma bolesti pomoću FDG-PET/CT su potencijalni faktori rizika i za DFS i OS kod bolesnika sa IIHL.

Ključne reči:
hodžkinova bolest; limfne žlezde; dijafragma; tomografija, positron-emisiona; fluorodeoksiglukoza f18; tomografija, komjuterizovana, rendgenska; prognoza.

Introduction

Hodgkin's lymphoma (HL) comprises roundly 15% of all lymphoma patients and has two main classifications: classic (90–95%) and nodular lymphocyte-predominant Hodgkin's disease (5–10%). Classic HL has 4 subtypes: nodular sclerosing 65–80%), mixed cellular (15-30%), lymphocyte-rich and lymphocyte-depleted Hodgkin's disease [1, 2]. Isolated lymph node (LN) involvement below the diaphragm is a rare form of HL and called infradiaphragmatic Hodgkin lymphoma.

HL with isolated infradiaphragmatic lymph node involvement (IDHL) is not common and makes up 5-13% (usually less than 10% in series) of all stage I-II HL [3–5]. Approximately 90% of patients have painless, mostly inguinal lymphadenopathy (LAP). Fever, night sweats and weight loss (B symptoms) are present in 25–40% of the cases. The diagnosis is established by biopsy of inguinal lymph nodes in a great majority of the cases by the presence of Reed-Sternberg cells which are only specific for HL pathology.

Computed tomography (CT), 18-fluorodeoxyglucose positron emission tomography (FDG-PET) and 18-fluorodeoxy glucose positron emission tomography/computed tomography (FDG-PET/CT) are used to stage HL [6, 7]. FDG-PET/CT is a superior imaging technique with proved utility especially in the oncologic field and widely used in lymphoma patients. FDG is avidly taken up by Reed-Sternberg cells, inflammatory tissue and cells surrounding them. FDG-PET is able to show functional alterations that precede the anatomical changes. Integration of CT to FDG-PET combines anatomical detail with functional information and yields excellent morphological and functional information increasing accuracy and detection capability. All these advantages of FDG-PET/CT potentially make it a superior imaging modality for primary staging, evaluation of treatment response and restaging in IDHL just like in other types of HL and many of non-Hodgkin's lymphomas. Standard therapy regimen for HL is combined modality treatment (CMT) which includes chemotherapy (CT) + irradiation of the involved fields (RT).

Although important subjects about prognostic factors and optimal treatment of isolated infradiaphragmatic HL have not been clearly determined, it is know that IDHL is characterized by higher male/female ratio, older patients' age at diagnosis and higher prevalence of lymphocyte-predominant histologic subtype in relation to supradiaphragmatic HL [8–13]. Whether pure infradiaphragmatic localization has a worse prognosis than stage I/II supradiaphragmatic disease has still remained controversial [9, 14–17]. Most studies pertaining to IDHL contain limited numbers of patients with different treatment approaches and varying outcomes roughly along a mean of 20-year follow-up [18, 19].

We aimed to evaluate the prognostic value of metabolic tumor indices on initial FDG-PET/CT over quantitative PET/CT parameters together with the classical predefined risk factors for patients with IDHL.

Methods

There were 184 patients with HL for whom FDG-PET were performed. From them our retrospective study included 21 patients with IDHL at stage I,II disease for whom FDG-PET/CT was requested for primary staging in the Nuclear Medicine Department between 2004 and 2015. These patients were treated and followed-up at the Medical Oncology Department of our hospital. Ann-Arbor staging system and definitions were used in this study. Information and data were obtained from clinic follow-up files, radiation therapy records, physician records of other departments at our hospital or personal contact with the patients *via* telephone. The majority of the patients referred with palpable inguinal masses and complaints of fever, night sweats, weight loss, itching in some cases. The diagnosis was established by biopsy from these inguinal masses (lymph nodes or conglomerated lymph nodes) or with excisional biopsy by diagnostic laparoscopy from intraabdominal lymph nodes in a few cases. Clinical staging was performed by physical examination, chest X-ray, thoracic and abdominal CT, FDG-PET (between 2004 and 2010) and FDG-PET/CT from June 2010. When the detected lesions were confined below the diaphragm and no supradiaphragmatic pathologic finding was observed neither on diagnostic images nor with physical examination, these cases were accepted as IDHL. Bulky disease was defined as single lymph node or conglomerated nodal mass of size > 5 cm in axial slice [9]. Patients were treated with adriamycin, bleomycin, vinblastine, dacarbazine (ABVD) protocol and irradiation of involved field 30 Gray (Gy). Patients who didn't complete the whole scheduled treatment owing to comorbidities or toxicity and had inadequate follow-up were excluded from the study.

FDG-PET/CT imaging protocol

Patients fasted for 6 hours and their blood glucose level had to be under 150 mg/dL before the injection of an activity of 370-555 MBq of 18F-FDG according to patient's weight. Image acquisitions were performed 1 hour later with an integrated PET/CT scanner (Discovery 690-GE Healthcare). Unenhanced low dose CT and PET emission data were acquired from mid-thigh to the vertex of the skull in the su-

pine position with the arms raised overhead. CT data were obtained by automated dose modulation of 120 kVp (maximal 100 mA), collimation of 64×0.625 mm, the measured field of view (FOV) of 50 cm, noise index of 20% and reconstructed to images of 0.625 mm transverse pixel size and 3.75 mm slice thickness. PET data was acquired in 3D mode with a scan duration of 2 min *per* bed position and an axial FOV of 153 mm. The emission data was corrected in a standardized way (random, scatter and attenuation) and iteratively reconstructed (matrix size 256 × 256, Fourier rebinning, VUE Point FX [3D] with 3 iterations, 18 subsets).

Visual and quantitative interpretation

Quantitative PET/CT parameters used in the study were maximum standardized uptake value (SUV max), average standardized uptake value (SUV mean), metabolic tumor volume (MTV) and total lesion glycolysis (TLG). They were calculated according to a standard protocol on a dedicated workstation (Volumetrix for PET-CT and AW volume share 4.5, GE Healthcare, Waukesha, WI, USA). SUV max and SUV mean corrected for body weight were computed by standard methods from the activity at the most intense voxel in three-dimensional tumor region from the transaxial whole body images on attenuation-corrected PET/CT images. MTV (cm3) was measured with semiautomatic PET analysis software using an automatic isocontour threshold method based on a theory of being greater than 42% of the SUV max value within the tumor. TLG values were calculated by multiplying MTV and SUV means.

We retrospectively examined demography, clinic, histology, clinical stage, response to treatment and outcome of the patients. Overall survival (OS) was defined as the time from diagnosis to death of any cause including ones other than the disease itself or last follow-up. Disease-free survival (DFS) was defined as the time from diagnosis to detection of relapse or last follow-up. PET/CT of response to treatment was requested later to detect the relapses. This study was approved by our institutional review Board Committee.

Statistical analysis

The whole data were analyzed using the Statistical Package for the Social Science V.21.0 (IBM Inc.) software. Number, percentage, mean, median, standard deviation (SD), minimum (min) and maximum (max) values were used for the description of the continuous data analysis. Univariate and multivariate Cox regression models were performed to determine related factors with disease free survival time. The variables having a value of $p < 0.20$ were included in multivariate analysis. Backward LR elimination method was used to refine regression model. Receiver operating characteristic (ROC) curve was drawn to evaluate the diagnostic value of TLG. TLG was dichotomized by splitting two groups according to ROC curve. Kaplan-Meier method with log-rank test was used to compare disease free survival times of TLG groups.

Results

A total of 21 patients were enrolled in this study. Mean age of the patients at diagnosis was 33 ± 15 years (6–64). Twenty four percent of the patients were female (n = 5), and 76% (n = 16) male (male/female ratio: 3.2). There were 13/21 (62%) of the patients with nodular sclerosing, 3/21 (14%) with mixed cellular, 1/21 (5%) with lymphocyte-rich, 2/21 (9.5%) with lymphocyte-depleted and 2/21 (9.5%) with nodular lymphocyte-predominant Hodgkin's disease. In relation to the disease stage 14% (3/21) of the patients were at stage IA, 5% (1/21) at stage IB, 38% (8/21) at stage IIA, 43% (9/21) at stage IIB; 47.5% (n = 10) of the cases had B symptoms and 33.3% (n = 7) bulky disease. SUV max was 11.74 ± 5.53 (4–21.3), SUV mean 7.27 ± 2.66 (3.5–12.1), MTV 33.56 ± 17.65 cm^3 (9.8–62.3), and mean TLG was 278.4 ± 214.1 (37.2–754, median = 214.5). The diagnosis was established from the inguinal lymph nodes in 17/21 (81%), and intraabdominal lymph nodes in 4/21 (19%) of the cases. Mean follow-up time was 73.7 ± 37.3 (15–133) months. Three (14%) patients died, 15 (71.5%) developed recurrence and/or metastasis during the follow-up (Figure 1). Patient characteristics and demography, clinicopathologic features and follow-up data were detailed in Table 1.

The involved LN groups in the subdiaphragmatic region according to the order of frequency were inguinal, external iliac, internal iliac, paraaortic, common iliac, aortocaval, femoral, obdurate, paracaval, liver hilus, precaval, paravertebral, sacral, juxtaintestinal, interaortocaval and paraaortocaval lymph nodes. One patient died of cardiac event owing to obesity and diabetes mellitus. The other two patients died of the disease itself (widespread metastasis) and its complications. Most patients 14/15 (93%), with the recurrent/metastatic disease were at stage II; 2/15 (13%) of them had splenic involvement, and 3/15 (20%) had supradiaphragmatic (cervical, axillary, supraclavicular, mediastinal) LN involvement. Overall survival at 5 years was 100%, 90.5% at 10 years (Figure 2). DFS at 5 years was 28.5%. Secondary malignancies were detected in 3 cases (colon adenocarcinoma, squamous cell carcinoma of the left lung, follicular thyroid cancer).

Univariate cox regression was performed for all potential risk factors impacting metastasis/recurrence. Factors which had values of $p < 0.2$ after univariate analysis (sex, age, stage, bulky disease, SUV max, SUV mean, MTV, TLG), were processed with the multivariate model. Sex, TLG and bulky disease were found statistically significant after multivariate analysis. Female sex increases recurrence rate 5.8 times in relation to male sex. Recurrence rate increases 16.6 times in bulky disease. One unit increment of TLG amplifies recurrence in 0.6%. The results of univariate and multivariate Cox regression analyses were shown in Tables 2 and 3, respectively. ROC curve was drawn to evaluate the diagnostic value of TLG (Figure 3). Sensitivity and specificity were calculated 100% and 83.3%, respectively when the cut-off value of TLG was taken as 100. TLG was dichotomized by splitting two groups according to ROC curve. Kaplan-Meier method with log-rank test was used to compare disease free survival times of TLG groups. Kaplan-Meier curve was drawn for TLG with a value of 100 (Figure 4).

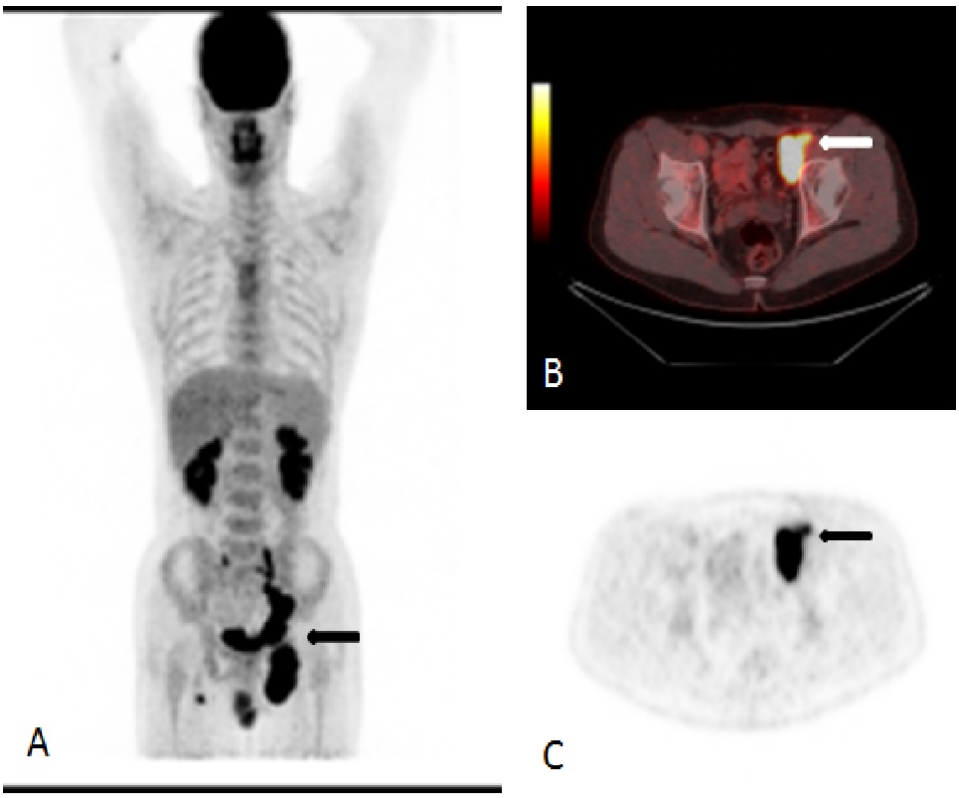

Fig. 1 – A) Maximum intensity projection (MIP); B) 18-fluorodeoxyglucose positron emission tomography/computed tomography (PET/CT) fusion; C) transaxial slice of 18-fluorodeoxyglucose positron emission tomography (FDG-PET) images of a 25-year old male patient with nodular sclerosing Hodgkin's lymphoma (HL) at stage II B disease. Arrows indicate left femoral, inguinal, external iliac, internal iliac, common iliac and paraaortic conglomerated lymph nodes with maximum standardized uptake value (SUV max) 19.5, mean standardised uptake value (SUV mean) 10.6 and metabolic tumor volume (MTV) 53.8 cm^3. The patient had a high total lesion glycolisis (TLG) value of 572.8 and the disease recurred after 15 months.

Table 1

Patient characteristics, demography, clinical, histological features and follow-up data

Patient no	Age	Gender	Histology	Stage	Presentation site	Bulky disease	B Symptoms	Recurrence	SUV max	SUV mean	MTV	TLG	DFS	OS
1	48	M	NS	IIB	L inguinal	+	+	+	13.5	8.2	36.6	298	11	36
2	21	M	NS	IIB	L inguinal	+	+	+	17.4	9.6	52.5	505	18	38
3	46	F	MC	IA	L inguinal	_	_	+	8	5.3	24.7	130.9	30	72
4	53	M	NS	IIA	L inguinal	_	_	+	5.8	4.6	31.2	143.5	21	74
5	20	M	NS	IA	L inguinal	_	_	_	4.7	3.5	10.8	37.8	75	75
6	35	F	NS	IIA	Paracaval	_	_	+	8.8	5.4	27.8	150	20	82
7	21	M	LR	IA	R inguinal	_	_	_	4	3.8	9.8	37.2	84	84
8	20	M	NS	IB	L inguinal	_	+	_	4	3.8	10	38	78	78
9	20	F	LD	IIB	Paraaortic	+	+	+	15	8.9	56.5	502.8	18	133
10	6	M	NS	IIA	Paraaortic	_	_	+	7.8	5	25.2	126	28	84
11	25	M	NS	IIB	L inguinal	_	+	+	19.5	10.6	53.8	572.8	15	48
12	27	M	MC	IIB	R inguinal	_	+	+	9	6.1	45.5	277.5	36	132
13	27	F	NS	IIA	R inguinal	_	_	+	13.5	8.9	38.3	340	24	120
14	23	M	NLP	IIB	L inguinal	_	+	_	14.7	9.2	15.3	140.7	130	130
15	42	M	NS	IIA	R inguinal	_	_	_	7.2	5	8.4	42	15	15
16	20	M	NS	IIA	R inguinal	+	_	+	17.7	9.8	48.4	475	10	17
17	20	M	NS	IIA	L inguinal	_	_	_	11.4	6.9	15	103.5	35	35
18	59	F	MC	IIB	R inguinal	+	+	+	18.1	10.5	37	389.8	7	32
19	64	M	NS	IIB	Paraaortic	_	+	+	7.3	5.5	39	214.5	36	120
20	42	M	NLP	IIA	R inguinal	+	_	+	17.8	10	56.8	568	10	72
21	50	M	LD	IIB	L inguinal	+	+	+	21.3	12.1	62.3	754	8	70

M – male; F – female; NS – nodular sclerosing; MC – mixed cellular; LR – lymphocyte-rich; LD – lymphocyte-depleted; NLP – nodular lymphocyte-predominant; R – right; L – left; SUV max – maximum standard uptake value; SUV mean – mean standardised uptake value; MTV – metabolic tumor volume; TLG – total lesion glycolysis; DFS – disease free survival; OS – overall survival.

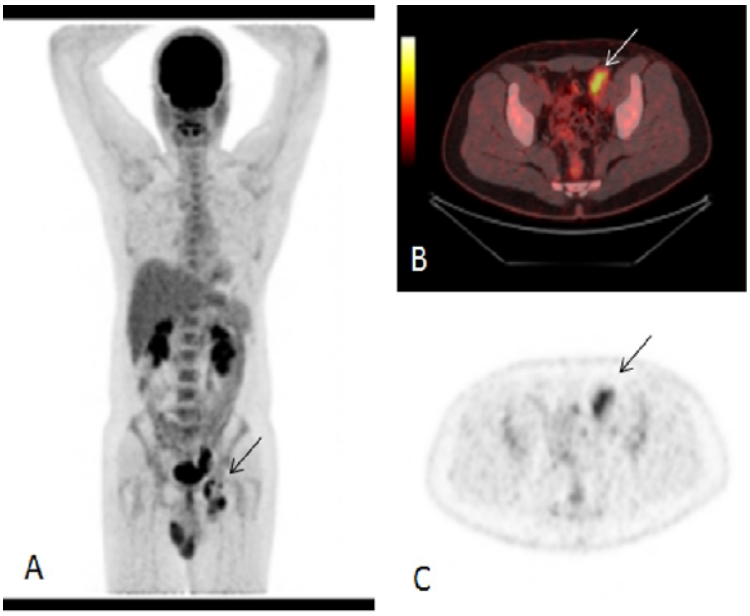

Fig. 2 – A) Maximum intensity projection (MIP); B) 18-fluorodeoxyglucose positron emission tomography/computed tomography (PET/CT) fusion; C) transaxial slice of 18-fluorodeoxyglucose positron emission tomography (FDG-PET) images of a 42-year old male patient with nodular sclerosing Hodgkin's disease HL at stage II A disease. Arrows indicate left inguinal, external iliac, internal iliac, common iliac and obdurate lymph nodes with maximum standardized uptake value (SUV max) 7.2, mean standardised uptake value (SUV mean) 5 and metabolic tumor volume (MTV) 8.4 cm^3. The patient had a low total lesion glycolisis (TLG) value of 42 and a total remission was observed during a 24-month follow-up.

Table 2

Univariate Cox regression analysis

Factors	*p*	Hazard Ratio	95% CI for Hazard Ratio	
			Lower	Upper
Sex *	0.142	2.332	0.753	7.226
Age	0.107	1.024	0.995	1.054
NS	0.507		Reference	
MC	0.722	1.271	0.339	4.765
LR	0.989	0.000	0.000	
LD	0.086	4.183	0.815	21.471
NLP	0.685	0.651	0.081	5.203
Stage I A	0.460		Reference	
Stage I B	0.990	0.000	0.000	
Stage II A	0.128	5.372	0.616	46.842
Stage II B	0.115	5.343	0.663	43.037
SUV max	0.001	1.253	1.099	1.429
SUV mean	0.001	1.513	1.172	1.953
MTV	0.001	1.080	1.034	1.129
TLG	0.000	1.008	1.004	1.012
Bulky disease**	0.000	20.681	3.898	109.709
Site of diagnosis	0.604	1.356	0.429	4.291
B symptoms	0.567	1.352	0.482	3.787

NS – nodular sclerosing; MC – mixed cellular; LR – lymphocyte-rich; LD – lymphocyte-depleted; NLP – nodular lymphocyte-predominant; SUV max – maximum standard uptake value; SUV mean – mean standardised uptake value; MTV – metabolic tumor volume; TLG – total lesion glycolysis;CI – confidence interval.
***Reference group was males **Reference group was non-bulky disease.**

Table 3

Multivariate Cox regression analysis

Factors	*p*	Hazard Ratio	95% CI for Hazard Ratio	
			Lower	Upper
Sex	0.030	5.866	1.190	28.924
TLG	0.015	1.006	1.001	1.011
Bulky disease	0.012	16.648	1.842	150.452

TLG – total lesion glycolysis; CI – confidence interval.

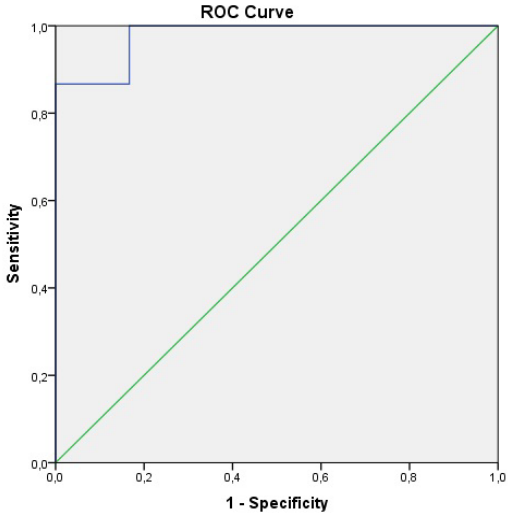

Fig. 3 – Receiver operating characteristic (ROC) curve for total lesion glycolysis (TLG).

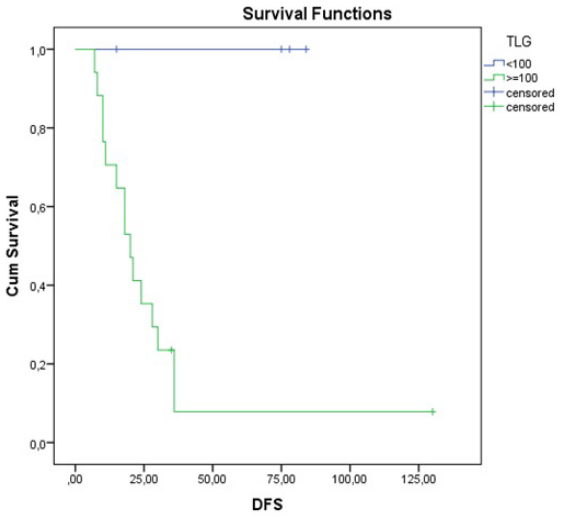

Fig. 4 – Kaplan-Meier curve for total lesion glycolysis (TLG) with a cut-off value of 100.

Discussion

In our patient population with HL 21/184 (11%) had IDHL and this incidence of IDHL is in accordance with literature [3]. IDHL has a higher prevalence of lymphocyte-predominant histologic subtype according to supradiaphragmatic HL. Among 9.5% of our patients with IDHL had nodular lymphocyte-predominant Hodgkin's disease and this is also consistent with the literature (5–10%) [1]. But 5% of supradiaphragmatic HL in our study had nodular lymphocyte-predominant Hodgkin's disease. There is a meaningful difference between them and this is an expected finding according to previous studies. It has been claimed in some studies that the incidence of nodular sclerosing subtype is lower in IDHL in relation to supradiaphragmatic HL [3]. Our incidence of the nodular sclerosing subtype (62%) is a little lower and similar to those ones.

According to literature, IDHL has higher male/female ratio and older age of patients at diagnosis in relation to supradiaphragmatic HL [2, 5]. Mean age of our patients with IDHL at diagnosis was 33 years and male/female ratio was 3.2. Mean age of our supradiaphragmatic HL group was 33 years and male/female ratio 4. There is not a difference between them regarding the age. On the contrary, male/female ratio of our supradiaphragmatic HL patients was higher than that of IDHL patients and this is a disparate finding in relation to literature. Mean age in IDHL patients was declared around 40 years in several studies [14]. Mean age of our IDHL patients (and also of supradiaphragmatic HL ones) were prominently lower than that reported in the literature, because our hospital is serving for recruits aged 18–23 years. Approximately, 30% of our patients were recruits.

The diagnostic site is inguinal lymph nodes in a great majority of the cases and most patients are at stage II [3]. The presentation site was inguinal lymph nodes and patients were at stage II in 81% of our cases and 47.5% of our cases had B symptoms. This is slightly higher incidence than in former studies which is generally between 25–40% [20]. If there is paraaortic LN involvement, careful evaluation of spleen with FDG-PET/CT is very useful [21]. We had two patients with splenic and paraaortic LN involvement. FDG-PET/CT contributed significantly for the delineation of splenic involvement in these patients. Hodgkin's survivors are at increased risk for secondary malignancies [22]. Many secondary malignancies were documented in patients with IDHL during the follow-up in lots of the published series. Hull et al. [18] found 5 secondary malignities in 21 patients during a 32-year follow-up. There were 3 cases of secondary malignancy in 21 patients during the 11-year follow-up in our study.

We observed complete remission in 6 patients. Mean follow-up time of this group was 70 (15–130) months. In 10 out of 15 patients with the metastatic/recurrent disease it occured purely in infradiaphragmatic sites which were an involved component before or a new focus. The affected region was supradiaphragmatic lymph nodes plus previously involved infradiaphragmatic sites in 3 cases; 2 patients had splenic involvement plus an involved area before or a new focus. Overall survival at 10 years gathers around 80% in reported series 4, 13. Vassilakopoulos et al. [3] found it 75% in their big cumulative nationwide historical cohort of 131 cases. Our overall survival at 5 years was 100%, and 90.5% at 10 years. These results are excellent according to other studies reported in the literature. But DFS was 28.5%.

There are controversial results about the prognosis of IDHL in comparison to stage I/II supradiaphragmatic disease in the literature. All the studies compared them with their classical prognostic factors with limited numbers of patients and different treatment approaches [10, 12, 15]. After evaluation of all potential risk factors affecting metastasis/recurrence with univariate cox regression analysis and multivariate model sex, TLG and bulky disease were found to be statistically significant risk factors for disease free survival time in our study.

Bulky disease incidence is higher in supradiaphragmatic HL than in IDHL. Although there are different accepted values for bulky disease, ranging from 5–10 cm in studies,

consensus about it is that it is taken as an advanced form of the disease [3, 23]. We chose 5 cm in axial slice as the size for it and one third of our patients had bulky disease. Bulky disease is related to tumor volume and reflects tumor burden. Ergo, its existence means much more tumor cells which could spare themselves getting rid of treating agents and thus have the potential of recurring or metastasizing later [17, 23, 24]. We found bulky disease a meaningful parameter as a predictor of IDHL ($p = 0.12$).

FDG-PET/CT is being widely used in many cancers and lymphoma patients. Some quantitative metabolic parameters derived from initial staging by PET/CT (SUV max, SUV mean) have also been used in prognosis estimation of many cancers and lymphomas. SUV max is the first one used [24, 25]. More lately increasing recognition of volume-based metabolic parameters (MTV and TLG) emerged for this purpose [24]. Gallicchio et al. [25] in their study of 52 patients found these quantitative parameters helpful in the management of diffuse large B-cell lymphoma [25]. Especially TLG proved its utility in this area and came out as a striking predictor in many cancers and lymphomas. As it combines the assessment of tumor volume and metabolism, it can stratify patients or predict the effectiveness of therapy regimens. Ceriani et al. [26] in their cohort study of 103 patients with diffuse large B-cell lymphoma showed TLG is the most powerful predictor on baseline PET/CT. But there are not studies researching the use of these parameters in a specific group of patients with IDHL and almost all evaluations using FDG PET/CT in HL were qualitative. Song et al. [27] evaluated metabolic tumor parameters in early stage HL to determine the appropriate therapeutic modality [27]. To the best of our knowledge, our study is the first one in literature in which the prognosis of IDHL was predicted over these metabolic indicators. Among the examined prognostic metabolic parameters, TLG remained as the only statistically significant pointer ($p = 0.15$) after multivariate model for DFS in this study. There is a similarity between bulky disease and TLG. Both of them are related to tumor volume. But TLG is superior to bulky disease in that it reflects the metabolically active tumor burden. When we evaluated the diagnostic value of TLG over ROC curve, we observed pretty high sensitivity and specificity (100% and 83.3%, respectively) with a cut-off value of 100. No patient whose TLG value was under 126 had recurrence. On the other hand, two patients suspected to have died from the disease had very high TLG values (502 and 754).

The main limitations of our study were the limited patient number and its retrospective design. First impressions show that metabolic tumor parameters, especially TLG may be used in the management of IDHL. However, our results should be supported with studies of large numbered samples in the future. Though our results showed that female sex increases recurrence rate 5.8 times in relation to male sex, this depends on the fact of quite a few sampling number and is no clinically important. Moreover, there are not studies stating that female sex is a risk factor for IDHL or HL yet. On the contrary, male sex was reported as a risk factor in some studies [9, 11].

Conclusion

The existence of bulky disease at the diagnosis and high TLG values (over 126) on primary staging by FDG-PET/CT are potential risk factors for both disease-free survival and overall survival in IDHL patients. Patients with high TLG have an increased risk of recurrence/metastasis and must be followed-up carefully for a possible change of treatment.

Disclosure

The authors declare that they have no conflict of interest.

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Determination of reference values of acetyl and butyryl cholinesterase activities in Serbian healthy population

Određivanje referentnih vrednosti aktivnosti acetil i butiril holinesteraze kod zdrave populacije u Srbiji

Milica Zlatković*†, Nadežda Krstić*, Vesna Subota‡, Bogdan Bošković*, Slavica Vučinić*†

Military Medical Academy, *National Poison Control Center, ‡Department of Medical Biochemistry, Belgrade, Serbia; University of Defence, †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are important biomarkers of exposure to organophosphorus and carbamate insecticides. Since the estimation of the level of cholinesterase inhibition depends on the normal values which may vary in different populations, it is important to determine them in our population, which so far has not been done. Therefore, the aim of this study was to determine the reference values for AChE and BuChE in a healthy population of adults in the Republic of Serbia. **Methods**. The AChE activity was measured by spectrophotometry (λ = 412 nm), using a modified Ellman's method. BuChE activity was determined by the integrated chemical system (Dimension RxLMax) with ready-made reagent cartridge for analysis. The examinees were healthy voluntary blood donors from the Institute of Transfusiology and Hemobiology, Military Medical Academy in Belgrade, Serbia. Statistical Package for Social Sciences (SPSS) software program was used for data processing. **Results**. In the group of 851 persons, there were 728 males and 123 females. The mean age was 39.1 ± 11.6 years. For all of them, erythrocyte AChE activity was done while BuChE was determined in 205 persons (169 males and 36 females). Their mean value of acetylcholinesterase activity was 8,090.6 ± 1,976.7 IU/L, and of butyrylcholinesterase activity was 14,556.6 ± 4,078.1 U/L. Due to lack of normal data distribution in male group (both enzymes), reference ranges were estimated as 2.5 and 97.5 percentiles. **Conclusion**. The results of this pilot study on cholinesterase in healthy population in the Republic of Serbia which has now been done for the first time, indicate the need for considering their wider ranges of when estimating the severity of poisoning. However, further study for BuChE with the inclusion of a larger number of females and data for body weight of the examinees, in order to get more precise reference limits, is suggested.

Key words:
cholinesterases; butyrylcholinesterases; cholinesterase inhibitors; insecticides; poisoning; reference values; serbia.

Apstrakt

Uvod/Cilj. Acetilholinesteraza (AChE) i butirilholinesteraza (BuChe) su važni biomarkeri ekspozicije organofosfornim i karbamatnim insekticidima. S obzirom na to da procena nivoa inhibicije holinesteraze, koja odgovara težini trovanja ovim agensima, zavisi od normalnih vrednosti, koje mogu varirati u različitim populacijama, bilo je važno odrediti ih u našoj populaciji, što do sada nije urađeno. Stoga je cilj ovog rada bio da se odrede referentne vrednosti za aktivnost AChE i BuChE u zdravoj populaciji odraslih osoba na području Republike Srbije. **Metode**. Aktivnost AChE u eritrocitima merena je spekrofotometrijski (λ = 412 nm). Korišćena je modifikovana Ellman-ova metoda. Aktivnost BuChE u plazmi određivana je na integrisanom hemijskom sistemu (Dimenzion RxLMax) sa gotovim reagens uloškom za analizu. Ispitanici su bili dobrovoljni davaoci krvi iz Instituta za transfuziologiju i hemobiologiju Vojnomedicinske akademije. Za obradu podataka korišćen je SPSS (*Statistical Package for Social Sciences*) softverski program. **Rezultati**. U grupi od 851 osobe bilo je 728 muškaraca i 123 žene. Srednje životno doba ispitanika bilo je 39,1 ± 11,6 godina. Za sve ispitanike određena je aktivnost AChE, dok je aktivnost butirilholinesteraze određena kod 205 ispitanika (169 muškaraca i 36 žena). Srednja vrednost aktivnosti acetilholinesteraze iznosila je 8 090,6 ± 1 976,7 IJ/L, a za butirilholinesterazu 14 556.6 ± 4 078,1 U/L. Odsustvo normalne distribucije podataka kod muškaraca uslovilo je da referentne vrednosti enzima odredimo kao 2,5-ti i 97,5-ti percentil. **Zaključak**. Rezultati ove pilot studije o aktivnosti holinesteraza u zdravoj populaciji u Republici Srbiji, koja je sada urađena prvi put, ukazuju na potrebu da se pri proceni težine trovanja uzmu u obzir širi rasponi aktivnosti holinesteraza. Preporučuje se proširivanje studije za BuChE, sa uključenjem većeg broja ispitanika ženskog pola i podataka o telesnoj masi, u cilju dobijanja preciznijih referentnih vrednosti.

Ključne reči:
holinesteraze; butirilholinesteraze; holinesteraza, inhibitori; insekticidi; trovanje; referentne vrednosti; srbija.

Correspondence to: Milica Zlatković, Military Medical Academy, National Poison Control Center, Crnotravska 17, 11 000 Belgrade, Serbia. Phone: +381 11 3609 198, E-mail: zmilica5@gmail.com

Introduction

Acetylcholinesterase (E.C. 3.1.1.7) (AChE) and butyrylcholinesterase (E.C. 3.1.1.8) (BChE or BuChE) are enzymes from the group of hydrolases, which catalyze the hydrolytic reaction of choline esters in the presence of water molecules [1–3]. The difference between the two enzymes is based on their different affinity for certain choline esters. AChE hydrolyzes acetylcholine (ACh) and acetyl-β-methyl choline, but unlike BuChE it does not hydrolyze benzoylcholine. Also AChE rate of hydrolysis decreases with the longer length of carbohydrate chain from ACh, than propionyl chloride to butyrylcholine. One molecule of AChE can hydrolyse 25,000 molecules of ACh *per* second, terminating its effect on muscarinic and nicotinic receptors and impulse transmission. This enzyme is found in erythrocytes, neuromuscular junctions, lungs, spleen and in all compartments of the brain. Inhibition of enzyme leads to accumulation of ACh in central and peripheral nervous system and cholinergic hyperstimulation, manifested as cholinergic crisis. Determination of the activity of AChE (true cholinesterase), along with the clinical picture, is therefore used to confirm cholinesterase inhibitors [(organophosphates (OP) and carbamates, nerve agents)] poisoning [3–6].

BuChE was known as plasmatic cholinesterase or pseudocholinesterase, and it is named according to its preference for the artificial substrate butyrylcholine. BuChE is also able to hydrolyze succinylcholine, adipoylcholine, benzoylcholine and propionylcholine [5]. The physiological role of this enzyme is still not known, but the clinical practice has shown that the use of suxamethonium in cases of genetic or acquired BuChE deficit may lead to neuromuscular block [7]. Although still under research, BuChE becomes increasingly promising therapeutic agent for detoxification of organophosphorus nerve agents and in cocaine abuse [6, 7]. BuChE is produced by the liver and is secreted into the circulation. BuChE is present in almost all tissues and in blood [6–12]. Reduced activity of this enzyme follows certain pathological conditions of the body (liver disease, malnutrition, acute infection, cancer, chronic anemia) [8]. Poisoning by OP pesticides can also cause a reduction of enzyme activity, as well as certain drugs (cimetidine, procainamide, androgens, estrogens, oral contraceptives, contrast agents, etc.) [9]. There are 2 types of pseudocholinesterases, normal (typical) and atypical. BuChE activity changes with age, with the lowest levels found in the newborn (about 60% of levels in healthy adults). According to some authors, a significant reduction of up to 30% BuChE activity occurs during pregnancy [11]. However, other authors have found that besides the genetic polymorphism, the difference in BuChE levels was not influenced by age, but only by body weight and height [12].

According to the World Health Organization, more than 3 million OPs poisonings, of which over 300,000 ends fatally, are registered annually. OPs irreversibly inhibit the enzyme AChE. The accumulation of the neurotransmitter ACh in the cholinergic synapses leads to the onset of symptoms and signs of acute poisoning. Besides reaction with AChE, OPs inhibit BuChE and other esterases [11].

Determination of cholinesterase activity is of great importance to confirm or rule out poisoning by OPs and as an indicator of the condition of patients and the success of the applied therapy. The degree of the enzyme inhibition, among other criteria (clinical picture, the level of OPs in blood, dose of atropine applied), determines the severity of poisoning [8, 11].

Methods for determination of cholinesterases activity in preventive and clinical diagnostics should be simple, rapid, reliable, sensitive and specific. There are different methods for their determination but most often spectrometric method and enzyme immunoassay tests are used [13–19].

The estimation of the level of cholinesterase inhibition, corresponding to the severity of OP poisoning, depends on the normal values which show biological variations among different populations, mainly due to genetic polymorphism [20–23]. Thus, it is important to determine their values in Serbian population, which has not been done so far. Therefore, the aim of this study was to determine the reference values for AChE and BuChE activities in a healthy population of adults in the Republic of Serbia.

Additionally, it was also intriguing to compare our results with the findings of the authors from other countries, and also to check the reliability of the previous estimation of ChE activity in our patients poisoned by OPs performed according to their findings.

Methods

The study was conducted on 851 volunteers, in order to determine the reference values of cholinesterases (AChE and BuChE) activity in the Republic of Serbia. The study was conducted from 31 March 2014 to 10 February 2015 under the project of the National Poison Control Centre, Military Medical Academy ("Assessment of the Efficacy of Standard Antidotes and Adjuvant Therapy in Acute Organophosphorus Insecticide Poisoning, MF/MMA 20/12-15).

Chemicals and Reagents for determination of the enzymes activity were Disodium phosphate, p.a. (Merck, Darmstadt, Germany); monopotassium phosphate p.a. [(Merck, Darmstadt, Germany); 5,5′-Dithiobis (2-nitrobenzoic acid, (Sigma-Aldrich, St. Louis Missouri, U.S.)]; acetylthiocholine iodide (Sigma-Aldrich, St. Louis Missouri, U.S); ethopropazine (10- [2-diethylaminopropyl] phenothiazine) hydrochloride (Sigma-Aldrich, St. Louis Missouri, U.S.); analytical standard human acetylcholinesterase (Sigma-Aldrich, St. Louis Missouri, U.S.) enzyme activity – 2,624 units / mg protein; Sigma, Flex® reagent cartridge, (Siemens, Munich, Germany).

Determination of AChE

As Reagents we used: Indicator erythrocyte cholinesterase (DTNB, 5 mM); the substrate for erythrocyte cholinesterase (Acetylthiocholine iodide, 0.34 M) Phosphate buffer (Na_2HPO_4/KH_2PO_4, 0.1 M – pH 7.4); Ethopropazine (6 mM).

Working conditions in the spectrophotometer GBC Cintra 10e: λ= 412 nm; recording speed range: 60 nm/min; slot width: 1.5 nm; total recording time: 180 s; the sample is taken via flow cuvette (cuvette length 10 mm).

The method of sample preparation

Not coagulated blood sample (anticoagulant K_2EDTA) was centrifuged for 10 min at 3,000 rev/min. After centrifugation, the upper layer was rejected (plasma). The test tube was charged with 1 mL of hemolysate (6 mL distilled water and 10 ml of washed erythrocytes in phosphate buffer), 0.8 mL of phosphate buffer, 0.1 mL of indicators, 10 uL ethopropazine and finally 0.1 mL of substrate. Measurement of erythrocyte cholinesterase activity was carried out at 412 nm.

Analytical measurement range of this method was 700–12000 IU/L, and reference values were 4 000–8 000 IU/L.

The method for determining the activity of AChE [18] was validated in the Department for Toxicological Chemistry, Military Medical Academy. The method was accredited (validated documented method number 43).

Determination of BuChE

BChE activity in plasma was determined by the integrated chemical system (Dimension RxLMax) with ready-made reagent cartridge for analysis [19].

Range of measurement was from 0 to 14 U/mL (0–14000 U/L); reference values: 7,000 to 19,000 U/ L [24].

SPSS, U.18 (USA), software programme was used for statistical analysis. Normality of data distribution was evaluated by using Kolmogorov-Smirnov test. Mann-Whitney test and Kruskal-Wallis test were used for comparison between groups.

Results

In the group of 851 examinees, there were 728 males and 123 females of different ages. For all of them, the erythrocyte cholinesterase activity was determined. Due to technical reasons, BuChE activity was determined in 205 subjects (169 males and 36 females).

Table 1 shows basic demographic characteristics of the examinees (gender, age) regarding AChE and BuChE determination. Although the examinees were predominantly males, there were no differences in relation to age structure and mean age for both sexes.

The basic parameters of descriptive statistics (mean, median, standard deviation, the minimum and maximum value of the selected variables with their difference - the scope of distribution) for AChE in both sexes are presented in Table 2. Kolmogorov-Smirnov test revealed that there was no normal distribution for data in the group of male examinees. Therefore, application of nonparametric statistics was necessary in further statistical analysis. Mann-Whitney test showed that there was no significant difference in average values of AChE activity between sexes, thus both gender groups were conjoined for further evaluation.

In accordance with the found data for AChE activity levels, the percentile distribution of AChE activity was calculated, and activity levels of 4,037.7 and 11,733.8 IU/L, corresponding to the position of 2.5 and 97.5 percentiles, respectively may be used for determination of lower and upper reference limits in a healthy population (Table 3).

Descriptive statistics for BuChE in both sexes was similar as the one for AChE, as Kolmogorov-Smirnov test revealed that there was no normal distribution for data in males, and nonparametric statistics showed that there was no significant difference in average activity levels of BuChE between genders, so the groups were further evaluated as one (Table 4).

The percentile distribution of BuChE activity levels of examinees was calculated. The activity of 9,053.7 U/L corresponding to the position of 2.5 percentiles, and 23,671.8 U/L corresponding to the position of 97.5 percentiles may be used for determination of lower and upper reference limits of plasma BuChE activity in a healthy population (Table 5).

The activity levels of AChE and BuChE of examinees of different age categories were also analyzed.

Kruskal-Wallis test did not reveal significant changes among age category regarding AChE ($\chi^2 = 1.13$; $p = 0.76$) as well as BuChE activity ($\chi^2 = 3.62$; $p = 0.30$) (Figure 1).

Table 1

Basic demographic characteristics of the examinees regarding with AChE and BuChE determination

Patients' characteristics	AChE	BuChE	Total
Gender, n (%)			
male	728 (85.5)	169 (82.4)	851 (100.0)
female	123 (14.5)	36 (17.6)	205 (100.0)
Age (years), $\bar{x} \pm$ SD (range)			
male	39.07 ± 11.28 (17–65)	39.10 ± 10.64 (17–65)	
female	39.63 ± 13.43 (16–63)	38.75 ± 14.92 (21–65)	
Total	39.15 ± 11.61 (16–65)	39.04 ± 11.46 (17–65)	

AChE – acetylcholinesterase; BuChE – butyrylcholinesterase; $\bar{x}$ – arithmetic mean; SD – standard deviation.

Table 2

Descriptive data of AChE (IU/L) samples (n = 851)

AChE activity (IU/L)	Gender		Total	Statistics (Mann-Whitney)
	male	female		
Mean	8,075.95	81,77.29[ns]	8,090.60	$z = 0.75$
Standar deviation	1,966.93	2,040.03	1,976.76	$p = 0.44$
Median	8,164.50	8,324.00	8,188.00	
Minimum	942.0	3,922.0	942.0	
Maximum	13,890.0	12,202.0	13,890.0	

Table 3

Percentiles distribution of acetylcholinesterase (AChE) activity levels of examinees

Activity of AChE (IU/L)	Percentiles
4,037.7	2.5
4,663.6	5
5,412.8	10
6,758.0	25
8,188.0	50
9,501.0	75
10,580.2	90
11,029.8	95
11,733.8	97.5

Table 4

Descriptive data of butyrylcholinesterase (BuChE) samples (n = 205)

BuChE activity (U/L)	Gender		Total	Statistics (Mann-Whitney)
	male	female		
Mean	14,938.15	12,765.56^{ns}	14,556.62	$z = 1.62$
Standard deviation	4,259.06	2,421.12	4,078.10	$p = 0.10$
Median	14,622.00	12,363.00	14,229.00	
Minimum	8,788.0	7,866.0	7,866.0	
Maximum	46,228.0	18,220.0	46,228.0	

Table 5

Percentiles distribution of butyrylcholinesterase (BuChE) values

Activity of BuChE (U/L)	Percentiles
9,053.7	2.5
9,605.8	5
10,374.6	10
12,201.0	25
14,229.0	50
16,045.0	75
17,816.6	90
20,308.4	95
23,671.8	97.5

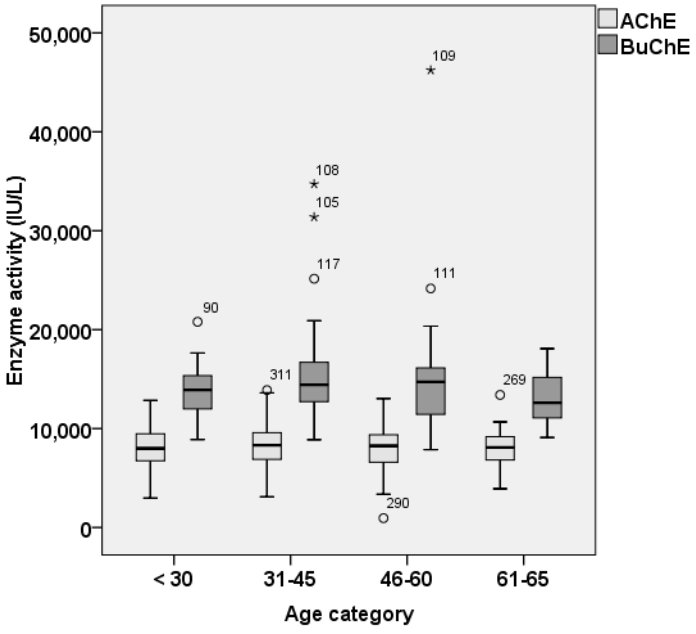

Fig. 1 – Mean values of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity in relation to age categories.

Based on this results, it seems reasonable to assume that unique reference intervals of AChE and BuChE activities are applicable for healthy subjects, regardless of their age.

Compared to reference values for AChE and BuChE activities used so far in our everyday practice, this pilot study has shown wider ranges for AChE (4,037.7–11,733.8 IU/L) and BuChE (9,053.7–23,671.8 U/L) activity.

Disscusion

Inhibition of AChE and BuChE is one of the pertinent diagnostic criteria in acute anticholinesterases poisoning. Whilst the role of AChE is thoroughly investigated, the precise role of BuChE still remains unknown despite extensive scientific research. Besides being a sensitive indicator for confirmation of exposure to anticholinesterases and hepatic biosynthetic capacity, there are new trends of BuChE use as a biomarker and detoxifying agent in OP nerve agents poisoning. Except in occupationally exposed workers who should have predetermined baseline activity levels of cholinesterases, in acute poisonings whether accidental or suicidal, they are not readily available. Thus, the degree of poisoning has to be estimated according to the reference values, that may differ among laboratories due to different methods and techniques, and different populations. There are also inter-individual and intra-individual variations caused by genetic polymorphism, age, body weight, sex, height [12]. Considering the reported biological varitions of cholinesterase activity levels in different populations, it was important to determine their values in our population, which so far have not been done.

Determining the reference values is extensive work that involves selection of suitable reference persons, preparing them for standardized sampling, analyzing of samples, statistical data analysis and presentation of the results [25–27]. The Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommends that the establishment of reference intervals requires a minimum of 120 individuals in each subgroup [28]. The IFCC recommends estimating a confidence range of 95% for each limit of the reference interval in both Gaussian and non-Gaussian distribution [26, 29]. Manufacturers of biochemical reagents recommend that each laboratory determines its own reference ranges, because of characteristics of the population covered by certain laboratories [24].

According to the number of subjects (851 and 205) our study meets the criteria with relevant standards, as compared to other studies [20, 24, 28]. Examinees were voluntary blood donors who have denied that they have any disease, and who had not taken any medicine that affects cholinesterase activity. The mean value of acetylcholinesterase activity was 8,090.6 ± 1,976.7 IU/L, and of butyrylcholinesterase activity was 14,556.6 ± 4,078.1 U/L. In this presented small pilot study of examinees in whom AChE and BuChE activity levels were determined, there were no differences according to age structure and ranges for both sexes. This is similar to results of other studies which failed to identify age as a significant factor for biological variations of cholinesterases [20, 30]. Although most of our examinees were males, nonparametric statistics showed no significant difference of average AChE and BuChE activity levels between sexes, so both gender groups were analyzed as one group. By using Kolmogorov-Smirnov test, the lack of normal data distribution was revealed in male (AChE as well as BuChE subgroups). After the percentile distribution of cholinesterases activity levels of our examinees was calculated, activity levels of 4,037.7 and 11,733.8 IU/L for AChE, and 9,053.7 and 23,671.8 U/L for BuChE, corresponding to the positions of 2.5 and 97.5 percentiles, respectively, were proposed to be used for determination of lower and upper reference limits in a healthy population.

Various laboratories in U.S. have different reference ranges for the values of AChE and BuChE activity in a healthy population. Mayo Medical Laboratory has reference range for BuChE activity for males > 18 years from 3,100 to 6,500 U/L and females at the age 18–49 years from 1,800 to 6,600 U/L and for those over 50 years from 2,550 to 6,800 U/L [31]. In Quest Diagnostics Laboratory this range for BuChE for males is 3,342–7,586 U/L, and for females 2,637–6,592 U/L. AChE activity levels for both sexes are in the range of 9,572–15,031 IU/L [32].

Reference values for BuChE activity in a Colombian population for people with different genotypes ranged between 4,796.3 – 10,321.1 U/L and 5,768.2 – 11,180.4 U/L [20]. The biological variations in cholinesterase activities in plasma were determined for a population of 3,372 subjects attending the Center of Preventive Medicine in Vandoeuvre-les-Nancy, France, for health examination in 1982. There were approximately equal numbers of examinees of both genders (1,732 males, 1,640 females), and 29% of the population were children 4–14 years old. The range of enzyme activity was 2,000–12,000 U/L [21].

The recommended normal values for cholinesterase activity vary according to values reported by other researchers, because tests were carried out on different populations (from various geographical areas, with racial, ethnic, nutritional status differences) [7, 10, 26, 29].

According to literature data, the reference values for AchE activity are in the range of 6,000 to 13,000 IU/L, and for BuChE for males 5,400–13,200 U/L and for female 3,700–9,300 U/L [33].

In our study, the activities of AChE and BuChE of examinees of different age categories were also analyzed. Kruskal-Wallis test did not reveal significant changes among age category regarding AChE as well as BuChE activity.

Based on this results, it seems reasonable to assume that unique reference intervals of AChE and BuChE are applicable for healthy subjects, regardless of their age.

With regard to the reference values for both cholinesterases, results of this pilot study are highly suggestive for the need to further evaluate the reference values for AChE and BuChE activity, expanding the study with other relevant parameters (including body weight) and a larger number of female patients for BuChE.

Conclusion

AChE and BuChE are important biomarkers of exposure to organophosphorus and carbamate insecticides. Inhibition of cholinesterase activity can also indicate the severity or course of poisoning and represents the useful gui-

de for the therapy. Due to this, it is very important to provide the correct reference values. The results of this small pilot study of these enzymes activities in healthy population in the Republic of Serbia which has now been done for the first time indicate the need for considering their higher ranges when estimating the severity of poisoning. However, further studies for BuChE with the inclusion of a larger number of females and data for the weight of the examinees, in order to get more precise reference limits, are necessary.

The limitation of this pilot study is a small number of female examinees and the fact that other parameters, such as body weight, have not been taken into account, so further study with larger number of female examinees and body weight, is recommended.

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Predictors and outcomes of new-onset atrial fibrillation in patients with acute myocardial infarction

Prediktori i ishod novonastale atrijumske fibrilacije kod bolesnika sa akutnim infarktom miokarda

Mihailo Vukmirović*, Aneta Bošković*, Zoran Bukumirić†, Irena Tomašević Vukmirović‡, Filip Vukmirović§

Clinical Centre of Montenegro, *Department of Cardiology, ‡Department of Radiology, §Department of Pathology, Podgorica, Montenegro; University of Belgrade, Faculty of Medicine, †Institute of Medical Statistics and Informatics, Belgrade Serbia

Abstract

Background/Aim. The onset of atrial fibrillation (AF) in the acute phase of myocardial infarction (MI) may be a predictor of poor prognosis. The aim of our study was to examine this relationship. **Methods.** Six hundred patients were enrolled in the study and divided into two groups. The first group included 48 patients with new-onset AF and the second group of 552 patients without this arrhythmia. Patients with previously registered AF were excluded from the study. We investigated the correlation between new-onset AF and intra-hospital mortality as well as mortality during the follow-up period of 48 months. We also analyzed predictors of this arrhythmia. **Results.** New-onset AF was registered in 48 (8%) patients. The independent predictors of this arrhythmia were older age, particularly more than 70 years [odds ratio 2.37; 95% confidence interval (CI) 1.23–4.58) and increased body mass index (odds ratio 1.17; 95% CI 1.04–1.33). Patients with new-onset AF had a higher mortality during the hospital course than patients without AF, but this difference was not statistically significant (10.4% *vs* 5.6%, p = 0.179). Patients with this arrhythmia had also a higher mortality after follow-up period of 48 months than patients without AF (33.3 % *vs* 17.8%, p = 0.009). Major adverse cardiac and cardiovascular events (MACCE) defined as death, recurrent MI, revascularization, and stroke were more after registered in patients with new-onset AF than in those with no this arrhythmia after follow-up period of 48 months (52.1% *vs* 33.9%, p = 0.011). However, multivariate Cox's regression analysis demonstrated that new-onset AF was not an independent predictor of mortality during the follow-up period of 48 months (HR 0.68; 95% CI 0.38–1.20; p = 0.182). **Conclusion.** New-onset AF in patients with MI was associated with a higher mortality as well as MACCE after the follow-up period of 48 months but was not an independent predictor of mortality during this period.

Key words:
myocardial infarction; atrial fibrillation; risk factors; aged; obesity; prognosis; mortality; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Pojava atrijalne fibrilacije (AF) u akutnoj fazi infarkta miokarda (MI) može biti prediktor loše prognoze. Cilj naše studije bio je da ispitamo ovu povezanost. **Metode.** Ukupno, 600 bolesnika je uključeno u istraživanje i podijeljeno u dve grupe. Prva grupa je obuhvatila bolesnike sa novonastalom AF, dok je druga grupa obuhvatila bolesnika bez ove aritmije. Bolesnici sa ranije registrovanom AF isključeni bili su iz istraživanja. Ispitivana je korelacija između novonastale AF i intrahospitalnog odnosno mortaliteta u toku perioda praćenja od 48 mjeseci. Takođe, analizirani su prediktori novonastale AF. **Rezultati.** Novonastala AF registrovana je kod 48 (8%) bolesnika. Nezavisni prediktor novonastale AF bilo je životno doba, naročito preko 70 godina [*odds ratio* 2,37; confidence interval (CI) 1,23–4,58), a potom i povišeni indeks telesne mase (*odds ratio* 1,17; 95% CI 1,04–1,33). Bolesnici sa novonastalom AF imali su povišeni intrahospitalni mortalitet u odnosu na one bez tog poremećaja srčanog ritma, ali ova razlika nije bila statistički značajna (10,4% *vs* 5,6%; p = 0,179. Bolesnici sa novonastalom AF imali su povišeni mortalitet nakon perioda praćenja od 48 meseci (33,3 % *vs* 17,8%; p = 0,009). Veliki neželjeni kardiovaskularni događaji (*major adverse cardiac and cardiovascular events* – MACCE) koji obuhvataju smrt, ponovni MI, odnosno revaskularizaciju i šlog, bili su češće prisutni kod bolesnika sa novonastalom AF (52,1% *vs* 33,9%; p = 0,011) tokom perioda praćenja od 48 meseci. Međutim, u multivarijantnom Cox regresionom modelu novonastala AF nije identifikovana kao nezavisni prediktor mortaliteta tokom perioda praćenja od 48 meseci (HR 0.68; 95% CI 0.38–1.20; p = 0.182). **Zaključak.** Novonastala AF kod bolesnika sa MI bila je povezana sa povišenim mortalitetom odnosno MACCE tokom perioda praćenja od 48 meseci, ali nije bila nezavisni prediktor mortaliteta tokom ovog perioda.

Ključne reči:
infarkt miokarda; fibrilacija pretkomora; faktori rizika; stare osobe; gojaznost; prognoza; mortalitet; osetljivost i specifičnost.

Correspondence to: Mihailo Vukmirović, Clinical Centre of Montenegro, Department of Cardiology, Ljubljanska b.b. 81 000 Podgorica, Montenegro. E-mail: mihailovukmirovic@t-com.me

Introduction

New-onset atrial fibrillation (AF) frequently complicates acute phase of myocardial infarction (MI) with the incidence of 6–21% [1, 2].

The large epidemiological studies demonstrated that new-onset AF is associated with high mortality and adverse events in patients with MI [1–7]. However, the outcome of this association is still unclear. Thromboembolic complications are one of the known mechanisms [1–7]. Patients with new-onset AF are older as well as with higher rate of hypertension (HTA) and heart failure (HF) which may contribute to worse outcome [1–7]. AF may precipitate the occurrence of severe ventricular arrhythmias which may lead to sudden death in these patients [8]. A large number of research have been done in patients with ST elevation myocardial infarction (STEMI), but some studies have also included patients with non-ST elevation myocardial infarction (NSTEMI) [3, 7, 9]. However, there are a small number of studies that examined association between new-onset AF and clinical outcomes among patients with both STEMI and NSTEMI [2].

Furthermore, some studies showed a higher mortality in patients with new-onset AF, but this arrhythmia was not an independent predictor of mortality [10–12]. This was the reason why we performed this research.

Its aim was to assess the impact of new-onset AF on mortality during the hospital period as well as mortality after a follow-up of 48 months in patients with MI, both STEMI, and NSTEMI, as well as predictors of new-onset AF.

Methods

This prospective study enrolled 600 patients with both STEMI and NSTEMI admitted to the Coronary Care Unit (CCU) of the Department of Cardiology, Clinical Center of Montenegro, between January 2009 to December 2010, after the approval by the local Ethics Committee.

Inclusion criteria involved patients aged 18 or older with MI both STEMI and NSTEMI, and in sinus rhythm on admission. Patients were divided into two groups: the first group which included patients with new-onset AF, i.e. developed during the hospital period, and the second group which included patients without AF registered previously as well as during the hospital period.

Permanent AF on admission or AF registered before, age < 18 years, congenital cardiac disease, severe valvular disease and healed endocarditis were exclusion criteria. Diagnosis of acute MI was determined according to the European Society of Cardiology Clinical Practical Guidelines for STEMI and NSTEMI [13, 14].

The irregular rhythm on electrocardiography (ECG) with the lack of discernible P waves and duration more than 30 seconds not presented at hospital admission defined AF. All patients were continuously monitored by ECG during the whole period in the CCU. In patients with palpitations after the CCU period, permanent ECG monitoring was performed to confirm or exclude AF.

Echocardiography also was performed but with a delay of least 5 days of admission due to minimizing the impact of myocardial stunning [15–17]. Simpson's method was used to assess left ventricular ejection fraction (LV-EF). Mitral regurgitation (MR) was estimated as mild when the jet area was under than 20%, moderate in patients in whom the jet area was between 20–40% and severe with the jet area more than 40% of the left atrial (LA) area [18]. LA diameter was determined by parasternal long axis view using a systolic frame in M-mode imaging.

Thrombolytic therapy was applied or primary percutaneous coronary intervention (PCI) was performed within 24 hours of the onset of symptoms in patients with STEMI as well as other therapy such as aspirin, heparin, angiotensin converting enzyme (ACE) inhibitors, ß-blockade, and statins which was also performed in NSTEMI patients.

Patients were followed-up 48 months after being discharged from the hospital. The assessment was made 1 month after discharge and thereafter every 6 months until the study was completed.

Follow-up data were obtained for 99% of patients.

Statistical analysis

Continuous variables were presented as either means (± SD) or median values and categorical variables as numbers or percentages. Unpaired *t*-test was used for comparing continuous variables, and χ^2 and Fisher's and Mann-Whitney's test for categorical variables of baseline characteristics. The relationship between patient's variables and new-onset AF was determined by univariate and multivariate logistic analysis. The crude cumulative incidence of mortality according to the AF status was illustrated by Kaplan-Meier plot and survival rate was assessed by Log Rank test. The prognostic effect of new-onset AF on mortality during the follow-up period of 48 months was examined using Cox's proportional hazards models. *P* value < 0.05 was considered as significant. Statistical analysis was performed using IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA).

Results

A total of 600 patients with MI were enrolled in this study. AF was registered in 48 (8%) patients during the hospital course. The baseline characteristics of patients in regards to the presence or absence of new-onset AF are listed in Table 1.

During the hospital course, 212 (73.1%) patients with STEMI as well as 140 (45.2%) patients with NSTEMI underwent PCI ($p < 0.001$).

Predictors of new-onset atrial fibrillation during the hospital course

The strongest predictors of new-onset AF during the hospital course were older patients, particularly more than 70 years, and with increased body mass index (BMI) (Table 2). The other parameters such as heart rate above more than 80 bpm on admission and Killip class after adjustment by logistic analysis were not independent.

Table 1

Baseline characteristics in regards to the presence or absence of new-onset atrial fibrillation (AF)

Characteristics	AF group n = 48	No AF group n = 552	*p*
Age (years), $\bar{x} \pm SD$	69.9 ± 9.4	63.1 ± 11.4	< 0.001
Gender, n (%)			
male	32 (66.7)	393 (71.2)	0.508
female	16 (33.3)	159 (28.8)	
MI, n (%)			
STEMI	26 (54.2)	264 (47.8)	0.399
NSTEMI	22 (45.8)	288 (52.2)	
Previous MI, n (%)	14 (29.2)	120 (21.7)	0.236
Previous CABG, n (%)	4 (8.3)	48 (8.7)	1.000
Killip class, n (%)			
I	35 (72.9)	486 (88.0)	
II	9 (18.8)	55 (10.0)	0.002
III	3 (6.3)	7 (1.3)	
IV	1 (2.1)	4 (0.7)	
Previous HF, n (%)	6 (12.5)	33 (6.0)	0.116
Diabetes mellitus, n (%)	15 (31.3)	152 (27.5)	0.582
Diabetic neuropathy, n (%)	15 (31.3)	119 (21.6)	0.122
COPD, n (%)	14 (29.2)	166 (30.1)	0.889
CKD, n (%)	17 (35.4)	170 (30.8)	0.507
BMI (kg/m^2), $\bar{x} \pm SD$	28.0 ± 2.6	26.7 ± 2.6	0.001
Dyslipidemia, n (%)	17 (35.4)	180 (32.6)	0.691
Smoking, n (%)	23 (47.9)	273 (49.5)	0.838
Previous CVI, n (%)	3 (6.3)	24 (4.3)	0.469
HTA, n (%)	26 (54.2)	268 (48.6)	0.455
LV-EF (%),$\bar{x} \pm SD$	41.7 ± 4.6	43.9 ± 4.9	0.003
LA (mm), $\bar{x} \pm SD$	43.6 ± 3.9	40.4 ± 3.6	< 0.001
MR, n (%)			
none	8 (16.7)	303 (55.0)	< 0.001
mild	28 (58.3)	208 (37.7)	
moderate-severe	12 (25.0)	40 (7.3)	
Heart rate on admission (bpm), median (range)	85.5 (55.0–122.0)	77.0 (43.0–125.0)	< 0.001
HTA on admission (mmHg), median (range)			
systolic blood pressure	158 (201–85)	154 (194–87)	0.657
diastolic blood pressure	81 (132–47)	80 (128–50)	
Localization of STEMI, n (%)			
anterior	14 (53.8)	113 (42.8)	0.279
inferior	12 (46.2)	151 (57.2)	
PCI during the hospital course, n (%)	30 (62.5)	322 (58.3)	0.574
Thrombolytic therapy, n (%)	17 (65.4)	192 (72.7)	0.930
Primary PCI, n (%)	4 (15.4)	49 (18.6)	0.720
VT during the hospital course, n (%)	9 (18.8)	42 (7.6)	0.014

MI – myocardial infarction; STEMI – ST elevation myocardial infarction, NSTEMI – Non-ST elevation myocardial infarction; CABG – coronary artery bypass graft; HF – heart failure; COPD – chronic obstructive pulmonary disease; CKD – chronic kidney disease; BMI – body mass index; CVI – cerebrovascular insult; HTA – hypertensio arterialis; LV-EF – left ventricle ejection fraction; LA – left atrium; MR – mitral regurgitation; PCI – percutaneous coronary intervention; VT – ventricular tachycardia.

Table 2

The predictors of new-onset atrial fibrillation (AF) and echo parameters in patients with myocardial infection (MI)

Independent variable	Univariate logistic regression		Multivariate logistic regression	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Age (more than 70 years)	3.32 (1.82–6.04)	< 0.001*	2.37 (1.23–4.58)	0.010*
Body mass index	1.22 (1.09–1.37)	0.001*	1.17 (1.04–1.33)	0.012*
Heart rate on admission (bpm)				
up to 80	reference category		reference category	
81–100	2.33 (1.21–4.50)	0.012*	0.70 (0.28–1.72)	0.438
more than 100	6.37 (2.60–15.60)	< 0.001*	1.71 (0.40–7.29)	0.469
Killip class	1.97 (1.27–3.06)	0.003*	0.72 (0.34–1.51)	0.386
LV-EF	0.92 (0.87–0.97)	0.003*	1.06 (0.97–1.17)	0.205
Diameter of LA	1.26 (1.16–1.37)	< 0.001*	1.18 (1.03–1.33)	0.015*
MR				
none	reference category		reference category	
mild	5.10 (2.28–11.41)	< 0.001*	3.56 (1.25–10.32)	0.018*
moderate to severe	11.36 (4.38–29.48)	< 0.001*	3.32 (0.72–15.365)	0.124

***statistically significant predictors of new-onset AF; LV-EF – left verticle ejection fraction; LA – left atrium; MR – mitral regurgitation; OR – odds ratio; CI – confidence interval.**

predictors of new-onset AF. Echo parameters such as the enlarged diameter of LA as well as presentation of MR significantly correlated with new-onset AF, but LV-EF did not (Table 2). Nevertheless, the other parameters such as gender, STEMI, localization of MI, thrombolytic therapy, PCI as well as CABG during the initial hospital period, previous MI, HF and CVI, diabetes mellitus, diabetic neuropathy, COBP, CKD, dyslipidemia, smoking and HTA were not included in the multivariate logistic regression analyses because univariate logistic regression analyses showed no statistical significance.

A total of 43 (89.6%) patients with new-onset AF were recovered to sinus rhythm during the hospital period. Recurrent AF was registered in 37.5% of patients with new-onset AF during the follow-up period of 48 months.

Impact of atrial fibrillation on mortality during the hospital period

A total of 36 (6.0%) patients died during the hospital course. A total of 5 patients (10.4%) with AF died during the hospital course as well as 31 patients (5.6%) without AF, but this difference was not statistically significant (p = 0.179). A total of 3 (11.5%) patients with STEMI and AF died during the hospital course as well as 2 (9.1%) patients with NSTEMI, but with no statistically significant difference ($p > 0.05$).

Impact of atrial fibrillation on mortality during the follow-up period of 48 month

A total of 486 (81.0%) patients survived after the follow-up period of 48 months. A total of 16 patients with new-onset AF died after this follow-up period, 8 (30.8%) patients with STEMI and 8 (36.4%) patients with NSTEMI ($p > 0.05$). A total of 16 (33.3%) patients with AF developed during the hospital period as well as 98 (17.8%) those without AF died after the follow-up period of 48 months ($p = 0.009$) (Figure 1).

The correlation between mortality and new-onset AF was assessed using unadjusted and adjusted Cox's proportional hazards model (Table 3).

Correlation between new-onset AF and major adverse cardiac and cardiovascular events after follow-up period of 48 months

MACCE defined as death, recurrent MI, revascularization and stroke were registered more often in patients with new-onset AF during the follow-up period of 48 months (Table 4 and Figure 2).

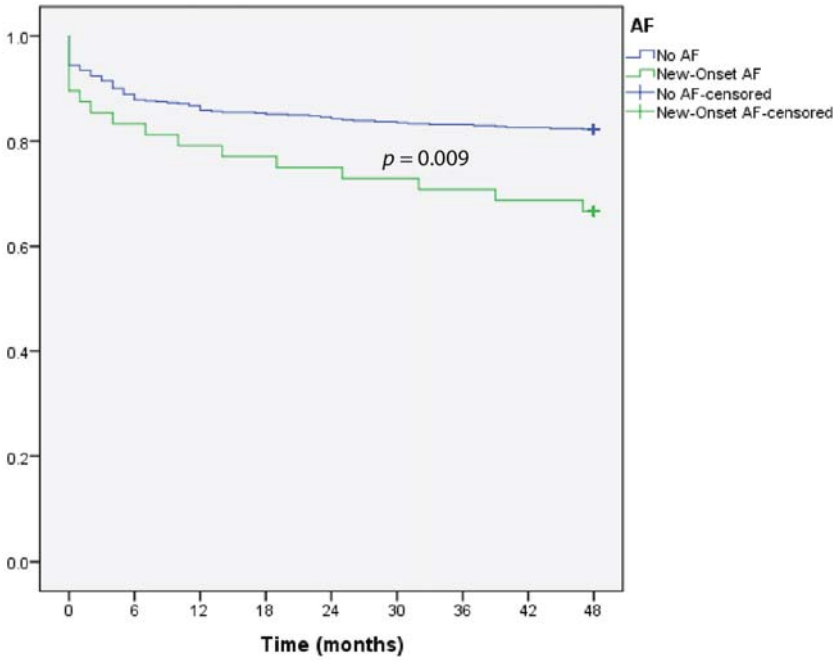

Fig. 1 – Crude cumulative incidence of mortality during the follow-up period of 48 months presented by Kaplan-Meier plots. AF – atrial fibrillation

Table 3

Cox's proportional hazard models for mortality predictors during the follow-up period of 48 months

Predictors	Univariate Cox's regression model		Multivariate Cox's regression model	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Age (more than 70 years)	2.06 (1.42–2.98)	< 0.001*	1.42 (0.96–2.08)	0.078
Killip class	3.71 (3.00–4.59)	< 0.001*	2.77 (2.13–3.61)	< 0.001*
Body mass index	1.45 (1.36–1.56)	< 0.001*	1.35 (1.26–1.45)	< 0.001*
Atrial fibrillation	1.97 (1.16–3.34)	0.012*	0.68 (0.38–1.20)	0.182

HR – hazard ratio; *statistically significant predictors; CI – confidence interval.

Table 4

Recurrent cardiovascular events after follow-up period of 48 months

Recurrent cardiovascular events	AF group n (%)	No AF group n (%)	*p*
MI	8 (16.7)	67 (12.1)	0.363
CABG	5 (10.4)	39 (7.1)	0.384
PCI	7 (14.6)	70 (12.7)	0.705
CVI	7 (14.6)	41 (7.4)	0.093
MACCE	25 (52.1)	187 (33.9)	0.011

AF – atrial fibrillation; MI – myocardial infarction; CABG – coronary artery bypass graft; PCI – percutaneous coronary intervention; CVI – cerebrovascular insult; MACCE – major adverse cardiac and cardiovascular events.

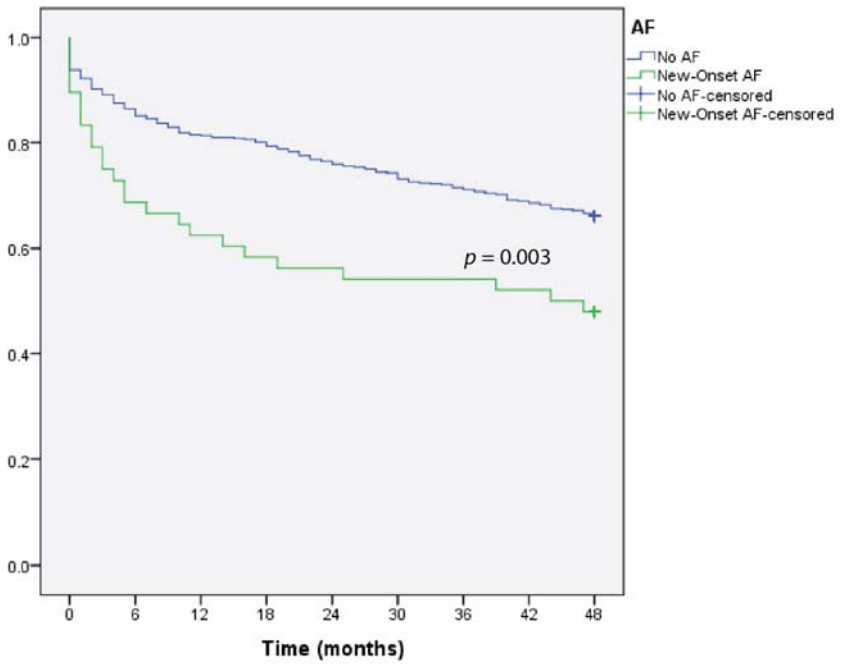

Fig. 2 – Composite end-point of death, recurrent myocardial infarction, revascularization and stroke during the follow-up period of 48 months presented by Kaplan-Meier plots, *p* = 0.003.
AF – atrial fibrillation.

Discussion

In our study, we presented the incidence of new-onset AF in STEMI and NSTEMI patients. In accordance with other previous studies, new-onset AF was more frequent in the STEMI group than in the NSTEMI one, but this difference was not statistically significant [1, 2]. The reason of higher incidence of AF in the STEMI population is still undetermined. The incidence of AF in MI with and without ST-segment elevation was also compared and published RICO study, but the result was also without statistical significance (7.6 *vs* 7.7%; $p = 0.334$) [15].

We identified the several important baseline predictors of new-onset AF in the setting of MI. Namely, except for age, this study is one of the first which emphasized that the obesity is an independent predictor of new-onset AF in patients with both STEMI and NSTEMI. The correlation between obesity and new-onset AF in a patient with MI remains unclear. However, according to data from large German AF registry, obesity was present in 25% of patients with AF with BMI of 27.5 kg/m^2 [2, 16]. Recent data from a Danish cohort indicates that BMI is incrementally associated with the volume of left atrium which leads to more pronounced trigger activity provoked by a more profound stretching of the pulmonary veins [17]. The enlarged volume of left atrium also may lead to prolongation of ectopic signals with the easier perpetuation of AF [17, 18]. Higher BMI is associated with inflammation which is supported by a recent study demonstrating that gene coding for the interleukin-6 receptor polymorphism is related to AF [19]. Obesity is a major risk factor for obstructive sleep apnea which also may predispose to AF [20]. MR in MI may also lead to both acute overload and enlargement volume of left atrium which through the described mechanisms may initiate and perpetuate AF [17, 21–24]. Unlike the previous study, we did not observe a positive association between MR severity and new-onset AF [25].

In our study we also presented the incidence of new-onset AF in STEMI patients according to the reperfusion regimens. In accordance with a recently published study, there

were no significant differences in the development of new-onset AF according to the reperfusion regimens (primary PCI *vs* thrombolysis) [26, 27].

In the present study we demonstrated a positive association between new-onset AF in patients with MI and complications developed during the hospital course such as HF and cardiogenic shock, but after adjustment for clinical and echo variables the risk associated with AF was attenuated. New-onset AF also was not an independent predictor of mortality during the hospital course. This finding was observed in both STEMI and NSTEMI patients for all of the studied outcomes. In spite of previous studies, there were significant differences in mortality during the hospital period according to the reperfusion regimens (primary PCI *vs* thrombolysis) [28–32].

New-onset AF was correlated with higher mortality after a follow-up period of 48 months. Furthermore, MACCE were more often registered in patients with new-onset AF after a follow-up period of 48 months. This finding was observed in both STEMI and NSTEMI groups. However, after multivariate Cox's regression analysis new-onset AF was not an independent predictor of mortality during the follow-up period of 48 months. This finding is in accordance with data of the study which included 4,108 patients hospitalized due to MI in 16 hospitals [10]. Namely, this study showed that patients with new-onset AF had higher long-term mortality than patients without this arrhythmia, but independent effect of AF on long-term prognosis was not confirmed by using a multivariate analysis [10].

Conclusion

New-onset AF was common in both patients with STEMI and those with NSTEMI and difference in its incidence between these two groups was not statistically significant. The strongest predictors of new-onset AF were older age and increased BMI. We also registered that echo parameters such as the enlarged diameter of left atrium as well as the presentation of MR were at the significant correlation with new-onset AF. There were no significant differences in mortality during the hospital period between MI patients with and without new-onset AF according to the reperfusion regimens. New-onset AF was associated with higher mortality as well as MACCE during the follow-up period of 48 months but was not an independent predictor of mortality during this period.

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Quality of life of hemodialysis patients waiting for kidney transplant

Kvalitet života bolesnika na hemodijalizi predviđenih za transplantaciju

Gordana Dedić*†, Nenad Milojković‡, Zoran Čukić§, Dubravko Bokonjić†‖

Military Medical Academy, *Clinic for Psychiatry, §Clinic for Nephrology, ‖National Poison Control Center, Belgrade, Serbia; University of Defence, †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; ‡Military Medical Center New Belgrade, Belgrade, Serbia

Abstract

Background/Aim. Dialysis and kidney transplantation are treatments that can be applied to patients with the end-stage renal disease. There is a lack of information on the quality of life (QOL) among hemodialysis (HD) patients on the waiting list for a kidney transplant, a group that is increasing all over the world. The aim of this study was to investigate the quality of life of patients on HD waiting for a kidney transplant. **Methods.** In the clinical comparative 12-month study, QOL level was compared between consecutively recruited patients waiting for a kidney transplant (WT patients) (N = 24) and patients not waiting for a kidney transplant (non-WT patients) (N = 52). All patients were older than 18 years and were on HD at least three months. To measure QOL, the short Form Health Survey (SD-36) was used. **Results.** WT patients were younger (43.50 ± 12.64 *vs* 63.58 ± 13.88 years; $p < 0.001$), they had started dialysis in the younger age (32.38 ± 14.50 *vs* 57.12 ± 15.79 years; $p < 0.001$) and spent more time on dialysis (112.04 ± 82.48 *vs* 72.40 ± 81.31 months; $p < 0.05$) than non-WT patients. Non-WT patients had more comorbidities than WT patients ($p < 0.01$). In laboratory parameters, there were statistically significant differences in values of serum creatinine ($p < 0.01$), phosphorus ($p < 0.05$) and number used to quantyfy hemodialysis treatment adequacy (Kt/V index: K – dialyzer clearance of urea; t – dialysis time; V – volume of distribution of urea approx equal to patients' total body water) ($p < 0.05$). Mean scores were higher among WT patients compared to non-WT patients in four dimensions of QOL: Physical Function (PF) (83.33 ± 10.59 *vs* 66.53 ± 27.87; respectively $p > 0.05$), Role Physical (RP) (58.66 ± 21.39 *vs* 46.90 ± 23.73; respectively $p > 0.05$), General health (GH) (45.00 ± 14.81 *vs* 37.98 ± 12.88; respectively $p > 0.05$); Social Functioning (SF) (93.66 ± 16.10 *vs* 78.30 ± 29.80; respectively $p > 0.05$) including Physical Component Summary (PCS) scores (64.16 ± 13.77 *vs* 52.38 ± 19.53; respectively $p > 0.05$). **Conclusion.** Patients waiting for a kidney transplant were younger, had started dialysis in the younger age and spent longer on dialysis compared with patients not eligible for transplantation. Low comorbidity, better laboratory parameters interferes in all domains with higher values of QOL in patients waiting for a kidney transplant, especially in general health, physical conditions and social functioning.

Key words:
renal dialysis; kidney transplantation; quality of life; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Dijaliza i transplantacija bubrega primenjuju se u lečenju bolesnike u terminalnoj fazi bubrežne insuficijencije. Postoji malo informacija o kvalitetu života bolesnika na dijalizi predviđenih za transplantaciju, grupi bolesnika koja se povećava u celom svetu. Cilj istraživanja bio je procena kvaliteta života bolesnika na dijalizi u terminalnoj fazi bubrežne insuficijencije, predviđenih za transplantaciju bubrega. **Metode.** U kliničkoj komparativnoj jednogodišnjoj studiji, poređene su vrednosti kvaliteta života bolesnika na dijalizi predviđenih za transplantaciju (N = 24) i bolesnika koji nisu predviđeni za transplantaciju (N = 52) bubrega. U istraživanje su bili uključeni samo bolesnici stariji od 18 godina, koji su bili na dijalizi najmanje tri meseca. Za merenje kvaliteta života je korištena kratka forma Upitnika kvaliteta života (SF-36). **Rezultati.** Bolesnici predviđeni za transplantaciju bubrega bili su mlađi (43,50 ± 12,64 *vs* 63,58 ± 13,88 godina; $p < 0,001$), dijalizu su započeli u mlađem životnom dobu (32,38 ± 14,50 *vs* 57,12 ± 15,79 godine; ($p < 0,001$) i na dijalizi su duže od bolesnika koji nisu bili predviđeni za transplantaciju (112,04 ± 82,48 *vs* 72,40 ± 81,31 meseci; $p < 0,05$). Komorbiditet je bio veći kod bolesnika koji nisu bili predviđeni za transplantaciju $p < 0,01$). U laboratorijskim parametrima postojala je statistički značajna razlika za vrednosti kreatinina ($p < 0,01$) i fosfora ($p < 0,05$) u serumu i broja koji kvantifikuje adekvatnost hemodijalize (Kt/V index: K – dijalizni klirens uree; t – vreme dijalize; V – volumen distribucije ureee približno jednak ukupnoj telesnoj vodi bolesnika) (1,36 ± 0,12 *vs* 1,29 ± 0,19; $p < 0,05$). Na Upitniku kvaliteta života, bolesnici koji su bili predviđeni za

Correspondence to: Gordana Dedić, Military Medical Academy, Clinic for Psychiatry, Crnotravska 17, 11 000 Belgrade, Serbia.
E-mail: sdedic@sezampro.rs

transplantaciju u odnosu na one koji nisu bili predviđeni za transplantaciju imali su više srednje vrednosti za: Fizičko funkcionisanje (PF) (83,33 ± 10,59 *vs* 66,53 ± 27,87; $p > 0,05$), Ograničenje zbog fizičkih teškoća (RP) (58,66 ± 21,39 *vs* 46,90 ± 23,73; $p > 0,05$), Percepciju opšteg zdravlja (GH) (45,00 ± 14,81 *vs* 37,98 ± 12,88; $p > 0,05$); Socijalno funkcionisanje (SF) (93,66 ± 16,10 *vs* 78,30 ± 29,80; $p > 0,05$), kao i za domen Fizičko zdravlje (PCS) (64,16 ± 13,77 *vs* 52,38 ± 19,53; $p > 0,05$). **Zaključak.** Bolesnici predviđeni za transplantaciju bili su mlađeg životnog doba, dijalizu su počeli u mlađim godinama života, na dijalizi su bili duže od bolesnika koji nisu bili predviđeni za transplantaciju. Niži komorbiditet, bolje laboratorijske vrednosti bili su u saglasnosti sa višim skorom na svim domenima kvaliteta života bolesnika predviđenih za transplantaciju, posebno u vezi sa njihovim boljim opštim zdravstvenim stanjem, fizičkom sposobnosti i socijalnim funkcionisanjem.

Ključne reči:
bubreg, dijaliza; transplantacija bubrega; kvalitet života; ankete i upitnici.

Introduction

Dialysis and kidney transplantation are treatments that can be applied to patients with the end-stage renal disease (ESRD) and represent a replacement for kidney function. Dialysis and kidney transplantation occur usually after several months or even years after the diagnosis of chronic kidney disease. The need for dialysis or transplantation is generally considered the best treatment for patients developing ESRD, both in the quality of life (QOL), long-term outcomes and financial burden on the society and patient. The importance of a successful kidney transplantation and survival is in reducing the risk of death among people treated by dialysis. People undergoing kidney transplantation save their time on daily dialysis too [1].

The choice between dialysis and transplantation is a complex problem. Patients must find the best solution together with their doctors, and frequently in consultation with their family members after careful consideration of all other factors. Many patients who are candidates for kidney transplantation are on waiting lists, but due to lack of transplantation organs, they need dialysis until a suitable organ for kidney transplantation is found. On the other hand, some people with kidney failure could not be candidates for transplantation. For patients with severe heart and vascular disease or for the elderly patients, treatment by dialysis is safer than kidney transplantation [2–4].

The quality of life is a multidimensional concept used to measure satisfaction or society to social and economic outcomes. However, the concept of QOL relates to a deeper meaning of an individual's experience of life and health. Healthcare researchers have demonstrated that the QOL has emerged as an important parameter for evaluating the quality of healthcare for patients with chronic diseases, because a chronic disease, with its physical and psychosocial characteristics, affects patients QOL. The concept of health-related QOL covers the patient's perceptions of his or her physical, emotional, cognitive and social functions and, importantly, disease symptoms and side effects of a treatment [5].

Comparing with a general population, it is a fact that patients with chronic kidney diseases have a worse QOL [6]. Assessment of QOL in patients with ESRD on dialysis treatment especially attracts an attention of researchers because it is a complex phenomenon which represents a complex interaction of the negative consequences of primary renal disease and the positive aspects of dialysis treatment [7].

There are some investigations how QOL is changed in the transition from dialysis to renal transplantation [8–10] and few data about the QOL level among patients undergoing hemodialysis (HD) and not eligible for kidney transplantation [11–14]. But there is a lack of information on QOL among the group of HD patients waiting for a kidney transplant, a group that is increasing all over the world [15].

Knowing the predictors of waiting for a kidney transplant, quality of life can improve patient's quality of work and the treatment outcome. Accordingly, the aim of our study was to estimate QOL of patients with ESRD undergoing dialysis and waiting for a kidney transplant.

Methods

We conducted the investigation in patients treated at the Department for Dialysis, the Clinic for Nephrology, Military Medical Academy in Belgrade, Serbia, in the period from February 1, 2014 till March 3, 2014. We also collected data about lethal outcomes and receiving a kidney transplant in the following 12-month study period.

Department for Dialysis of the Clinic for Nephrology in the Military Medical Academy in Belgrade, Serbia, is a tertiary care referral centre for kidney diseases that performs more than 20,000 procedures on dialysis (hemodialysis, hemofiltration, hemodiafiltration and continuous dialysis procedures) *per* year. A multidisciplinary team of nephrologists, nurses and technicians is engaged there to ensure optimal outcomes for dialysis patients. For more than 30 years (from 1983) this institution has delivered a range of dialysis therapies supporting and facilitating patients who suffer from the severe renal failure. Innovative use of the latest technologies ensures the highest quality dialysis care, accessible and comfortable with significantly better outcomes.

Patients

During a period of 12 months, a total of 108 patients on HD were asked to participate in the study if they met the following inclusion criteria: age over 18 years, and HD treatment for at least three months. All patients on HD were previosly assessed by nephrologists. Thirty two patients were

excluded from the study. Some of them did not meet the inclusion criteria (less than three months on dialysis), or had serious somatic (cancer) and mental illnesses (demention), and 10 of them refused to sign the informed consent.

Ultimately, the sample group consisted of 76 ESRD patients undergoing HD (48 males and 28 females). They were divided into two groups. The first group included 24 (31.57%) patients waiting for a transplant (WT patients) and the second group included 52 (68,43 %) patients not waiting for transplant (non-WT patients).

In all patients, we also estimated the efficacy of the hemodialysis treatment, complications in terms of undernutrition, anemia and secondary parathyreoidism.

Questionnaires

In the study, we used Short Form Health Survey, 36-Item Quality of life Questionnaire (SF-36) and Sociodemographic and clinical questionnaire.

SF-36 is an internationally accepted generic measure of the QOL, which has been translated and adapted for the use in Serbian. It covers aspects of physical, psychological and social functioning. SF-36 includes one multi-item scale that assesses eight health status dimensions: 1) Physical functioning (PF): limitations in physical activities because of health problems; 2) Social functioning (SF): limitations in social activities because of physical or emotional problems; 3) Role physical (RP): limitations in usual role activities because of physical health problems; 4) Bodily pain (BP); 5) Mental health (MH): general mental health (psychological distress and well-being); 6) Role emotional (RE): limitations in usual role activities because of emotional problems; 7) Vitality (VT): feeling of energy and fatigue; and 8) General health (GH): general health perceptions. For each of the eight dimensions item scores were recorded, summed up, and transformed using a scoring algorithm into a scale ranging from 0 (worst) to 100 (best), with higher scores representing better results in view of the subjective perception of physical and mental health.

Sociodemographic characteristics of patients waiting and not waiting for a kidney transplant were obtained from semi-structured questionnaire, which was specifically designed for this study. Clinical characteristics, including the laboratory parameters routinely measured in HD patients, were obtained from the medical protocol in the Department for Dialysis.

Monthly patients' incomes were divided into three categories 1) less than 300 Euro *per* month (unfavorable); 2) 300 to 500 Euro *per* month (satisfying); 3) more than 500 Euro *per* month (favorable).

The kidney disease was classified by clinical criteria [Intenational Classification of Diseases – 10th revision (ICD-10)] and based on the National Registry of patients on chronic regularly repeated hemodialysis treatment.

Each patient was assigned to a low, medium or high-risk index based on presence of comorbidities as described by Khan et al. [16] (comorbidity index takes into consideration age in three classes and nine comorbidities: diabetes, myocardial infarction, angina pectoris, congestive heart failure, liver cirrhosis, obstructive pulmonary disease, systemic collagen disease, pulmonary fibrosis, and visceral malignancies.

We also took into account a length of dialysis as an independent risk factor for complications, which is a direct consequence of some of these comorbid conditions, mainly cardiovascular, cerebrovascular, mineral-bone and hematologic ones.

All questionnaires were administrated by two qualified psychiatrists, that did not belong to the dialysis unit team.

This study was approved by the Ethics Committee of the Military Medical Academy in Belgrade. Written informed consent was obtained from all patients prior to their inclusion in the study. Confidentiality of the response was assured. The participation was completely voluntary, with neither financial nor other motivation.

Statistical analyses

Data analysis was carried out using Statistical Package for the Social Sciences (IBM SPSS) software version 20.0.

Following statistical tests were used: Student's *t*-test, χ^2-test, and Mann-Whitney test. Variables regarding sample characteristics including QOL scores were compared between patients waiting and those that were not waiting for a kidney transplant using Cronbach's alpha. In this research, QOL applied on our sample, had good internal consistency: Physical Component Summary (PCS) ($\alpha = 0.779$) and Mental Component Summary (MCS) (alpha $\alpha = 0.846$).

Differences were considered statistically significant when the *p*-value was < 0.05.

Results

Sociodemographic characteristics of patients included in the study are presented in Table 1. The mean age of patients was 57.24 ± 16.37 years (full sample), 43.50 ± 12.64 years (WT patients) and 63.58 ± 13.88 (non-WT patients). There were statistically significant differences among groups in age ($p < 0.001$) and in marital status ($p < 0.01$). As shown in Table 1, there were no statistically significant differences among groups in education, sex and monthly incomes.

Observing primary kidney diagnosis, WT patients more frequently suffered from glomerulonephritis and polycystic kidney. Graft failure was present in 41.7% WT patients. Non-WT patients more often suffered from Glomerulonephritis, hypertension, diabetes and obstructive uropathy (Figure 1).

Table 2 shows clinical characteristics of patients included in the study. There were statistically significant differences in all domains. WT patients were more frequently undergoing hemodiafiltration (54.2%), but non-WT patients were more frequently undergoing hemodialysis (88.5%) ($p < 0.001$). WT patients compared to non-WT patients started dialysis in the younger age (32.38 ± 14.50 *vs* 57.12 ± 15.79 years respectively; $p < 0.001$) and spent more time on dialysis (112.04 ± 82.48 *vs* 72.40 ± 81.31 months respectively; $p < 0.05$). Non-WT patients had higher

Table 1

Sociodemographic characteristics of patients waiting (WT) and non-waiting transplantation (non-WT)

Variable	Full sample	WT	non-WT	*p*
Age of patients (years), $\bar{x}$ ± SD	57.24 ± 16.37	43.50 ± 12.64	63.58 ± 13.88	0.001
(range)		(26–67)	(20–82)	
Education (years), $\bar{x}$ ± SD	13.50 ± 3.31	12.79 ± 2.89	13.83 ± 3.47	0.176
Sex, n (%)				0.861
male	48 (63.2)	16 (66.7)	32 (61.5)	
female	28 (36.8)	8 (33.3)	20 (38.5)	
Monthly income, n (%)				0.271
unfavorable	7 (9.2)	4 (16.7)	3 (5.8)	
satisfying	31 (40.8)	10 (41.7)	21 (40.4)	
favorable	38 (50.0)	10 (41.7)	28 (53.8)	
Marital status, n (%)				0.01
married	50 (65.8)	12 (50.0)	38 (73.1)	
divorced	2 (2.6)	0	2 (3.8)	
widowed	8 (10.5)	1 (4.2)	7 (13.5)	
single	16 (21.1)	11 (45.8)	5 (9.6)	

$\bar{x}$ – arithmetic mean; SD – standard deviation.

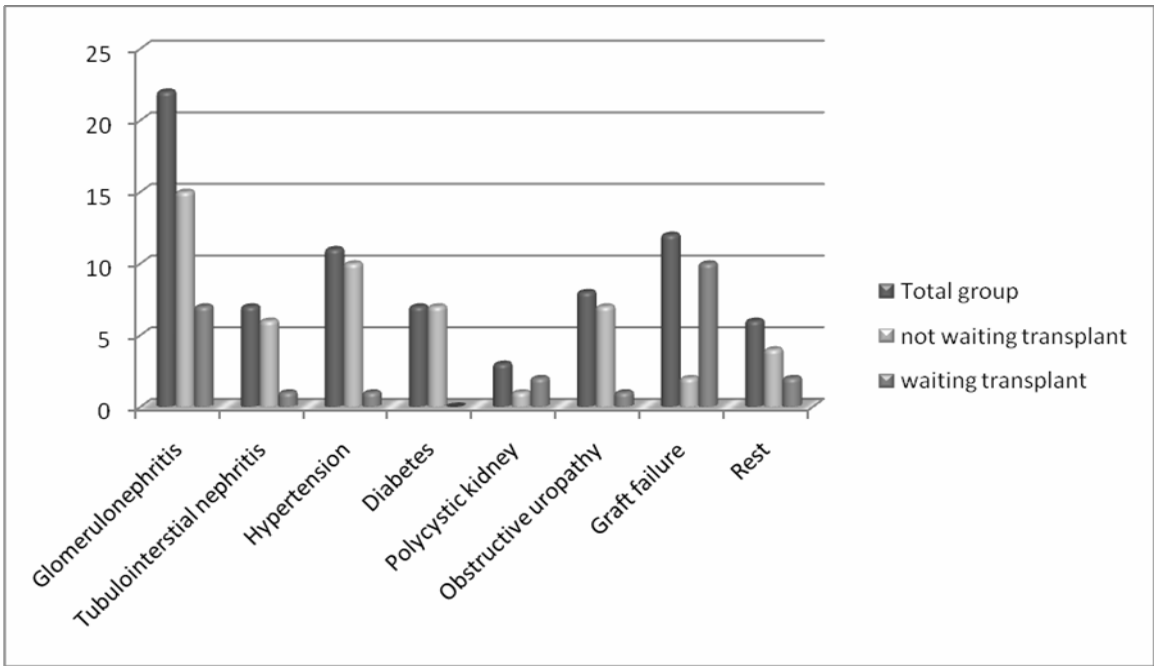

Fig. 1 – Primary kidney disease of patients included in the study.

Table 2

Clinical characteristics of patients waiting (WT) and not waiting transplantation (non-WT)

Variable	Full sample	WT	non-WT	*p*
Dialysis, n (%)				0.001
hemodiafiltration	19 (25.0)	13 (54.2)	6 (11.5)	
hemodialysis	57 (75.0)	11 (45.8)	46 (88.5)	
Dialysis beginning (years), $\bar{x}$ ± SD (range)	49.30 ± 19.19	32.38 ± 14.50	57.12 ± 15.79	0.001
	(10–80)	(10–61)	(12–80)	
Duration of dialysis (months), $\bar{x}$ ± SD (range)	84.92 ± 83.22	112.04 ± 82.48	72.40 ± 81.31	0.05
	(3–360)	(7–334)	(3–360)	
Death (follow-up 12 months), n (%)	5 (6.57)	1 (1.31)	4 (5.26)	0.001
Transplanted (follow-up 12 months), n (%)		3 (3.95)		
Comorbidity, n (%)				0.01
low	12 (15.8)	8 (33.3)	4 (7.7)	
medium	32 (42.1)	11 (45.8)	21 (40.4)	
high	32 (42.1)	5 (20.8)	27 (51.9)	

$\bar{x}$ – arithmetic mean; SD – standard deviation.

comorbidity than WT patients ($p < 0.01$). In the follow-up 12-month period non-WT patients died more often than WT patients (5.26% *vs* 1.31%; $p < 0.001$). In the same period, three (3.95 %) patients received transplantation.

In laboratory parameters all values were higher in WT patients than in non-WT patients. There were statistically differences between groups in values of serum creatinine ($p < 0.01$), phosphorus ($p < 0.05$) and (Kt/V index: K – dialyzer clearance of urea; t – dialysis time; V – volume of distribution of urea approx equal to patients total body water), $p < 0.05$, (Table 3).

Table 4 shows the scores of the domains of SF-36 in studied groups. All scores were higher in WT patients than in non-WT patients. Significant differences between groups were found in four dimensions: PF ($p < 0.05$), RP ($p < 0.05$), GH ($p < 0.05$) and SF ($p < 0.05$), including PCS domain ($p < 0.05$).

Aiming to identify the factors that may have an adverse effect on the outcome, candidates for renal transplantation undergo an extensive pretransplantation evaluation. Every patient must be assessed for a degree of eligibility for the kidney transplantation procedure. Basic principles of eligibility assessment include: medical risk assessment, evaluation of psychosocial status and the level of family support. Assessment of patient's motivation level for a kidney transplantation is a very important factor, too.

Medical risk assessment involves establishing the etiology of the primary kidney disease, cardiovascular status assessment, risk assessment for renal graft thrombosis, screening for early malignancy detection, assessment of mineral metabolism and bone tissue disorders, immunological risk assessment and viral status assessment. The main reasons for refusing kidney transplantation are the unpredictability of

Table 3

Laboratory parameters of patients waiting (WT) and not waiting transplantation (non-WT)

Laboratory parameters	WT $\bar{x} \pm SD$	non-WT $\bar{x} \pm SD$	References ranges	*p*
Creatinine (μmol/L)	881.05 ± 254.55	766.02 ± 162.38	62–115	0.01
Hemoglobin (g/L)	115.59 ± 19.77	107.70 ± 16.70	130–180	0.076
Albumin (mol/L)	38.05 ± 6.059	37.15 ± 3.69	32–50	0.620
Calcium (mol/L)	2.30 ± 0.23	2.27 ± 0.19	2.15–2.60	0.136
Phosphorus (mol/L)	1.95 ± 0.51	1.66 ± 0.42	0.78–1.65	0.05
PTH pg/mL	103.95 ± 137.68	88.71 ± 113.83	120 ± 300	0.804
CRP mg/L	6.03 ± 7.13	12.14 ± 22.79	< 5	0.321
Kt/V index	1.36 ± 0.12	1.29 ± 0.19	> 1.2	0.05
Virus, n (%)				0.586
none	41 (78.8)	18 (75.0)		
HBV	3 (5.8)	3 (12.5)		
HCV	8 (15.4)	3 (12.5)		

PTH – parathiroid hormone; CRP – C reactive protein; Kt/V: K – dialyzer clearance of urea; t – dialysis time; V – volume of distribution of urea approx equal to patients total body water; HBV – hepatitis B virus; HBC – hepatitis C virus; $\bar{x}$ – arithmetic mean; SD – standard deviation.

Table 4

36-Item Short Form Quality of life Questionnaire (SF-36) scores in patients waiting (WT) and not waiting transplantation (non-WT)

Health status domain	Full sample ($\bar{x} \pm SD$)	WT ($\bar{x} \pm SD$)	non-WT ($\bar{x} \pm SD$)	*p*
PF	71.84 ± 24.98	83.33 ± 10.59	66.53 ± 27.87	**0.05**
RP	50.34 ± 23.34	58.66 ± 21.39	46.90 ± 23.73	**0.05**
BP	63.97 ± 34.03	71.29 ± 26.58	60.59 ± 36.70	0.299
GH	40.19 ± 13.81	45.00 ± 14.81	37.98 ± 12.88	**0.05**
PCS	56.11 ± 18.65	64.16 ± 13.77	52.38 ± 19.53	**0.05**
VT	52.59 ± 21.49	58.12 ± 17.75	50.03 ± 22.71	0.200
SF	83.27 ± 27.28	93.66 ± 16.10	78.30 ± 29.80	**0.05**
RE	63.69 ± 27.42	63.91 ± 26.53	63.59 ± 28.06	0.901
MH	64.14 ± 18.54	67.50 ± 17.10	62.59 ± 19.12	0.353
MCS	64.26 ± 20.12	70.37 ± 15.73	61.44 ± 21.40	0.125

PF – physical functioning; RP – role-physical; BP – bodily pain; GH – general health; VT – vitality; SF – social functioning; RE – role-emotional; MH – mental health; PCS – Physical Component Summary; MCS – Mental Component Summary.
$\bar{x}$ – arithmetic mean; SD – standard deviation.

Discussion

For many patients with chronic renal failure, kidney transplantation is considered the treatment of choice. Sometimes, is the best alternative to dialysis in terms of quality of life, cost-effectiveness and survival [11, 12].

transplantation outcome, the side-effects of immunosuppressive therapy and unfavorable outcomes in fellow patients [13]. On the other side, identification of patients with the highest degree of kidney transplantation eligibility will improve the quality of life in those patients and decrease morbidity and mortality in the same time [14].

In our investigation, we formed two groups from the sample consisted of 76 ESRD patients. Observing primary kidney diagnosis, patients from both groups more frequently suffered from glomerulonephritis, but non-WT patients more often had more comorbid diseases like diabetes, hypertension, obstructive uropathy and polycystic kidney. Glomerulonephritis, as the leading cause of terminal renal failure in both groups of patients, although not the leading cause of terminal renal failure according to the relevant epidemiological studies, has emerged as the most common in both groups of our patients, only due to the structure of the patients included in our study.

There were 10 (41.7%) patients undergoing hemodialysis with previous transplantation who were included in this study because terminal renal graft represents a condition equal to ESRD. Also, clinical estimation was that previous transplant could have significance for the patients quality of life by giving them hope that the re-transplant will be again successful.

In our study, more than one half non-WT patients had high comorbidity index that is in accordance with other similar investigations, indicating it as an important contributing factor to clinical outcomes and quality of life [8].

WT patients began HD on the average about 25 years earlier than non-WT patients and spent on HD more than three years longer than non-WT patients, as expected, because WT patients, besides being younger, started dialysis in the younger age compared to non-WT patients, and the duration of receiving dialysis treatment was longer.

We analyzed a wide spectrum of sociodemographic and clinical characteristics of patients and their influence on different aspects of QOL in order to reduce differences between groups that could have an influence on QOL.

The differences in socio-demographic characteristics between WT and non-WT patients were predictable. Sex, education level, and monthly income are not factors that are important for the assessment of patients for transplantation selection.

But, differences in age and marital status were expected. We can explain them with the fact that WT patients were on the average about 20 years younger than non-WT patients, and even five times more frequently single. On the other side, not-WT patients were older and more frequently married. Our results are in accordance with results reported by [16].

Some investigations suggested that elderly patients were a rapidly growing subset of the kidney transplantation waiting list, what our group of WT patients where the oldest had 67 years confirmed [16, 17].

In laboratory parameters, all values were higher in WT patients than in non-WT patients, and our findings are in accordance with the results of some other studies [15]. Statistically, significant differences were found in serum creatinine and phosphorus levels. A higher dose of dialysis estimated by Kt/V index in the WT patients is in accordance with mentioned study, too [15]. Laboratory differences between two groups of patients are related to conditions precluding transplantation. Non-WT patients had lower creatinine, because of malnutrition and inflammation, and were submitted to a lower dose of dialysis, estimated by lower Kt/V index.

In the 12-months study period, 3.95% of the patients on the waiting list received transplantation and we consider it to be a good result. In the same period (6.58%) patients died. This is in accordance with other studies in which non-WT patients died more often than WT patients [16].

In our study, we found the connection between age, clinical characteristics, laboratory parameters and QOL of HD patients. Younger patients, who began HD earlier and spent longer on HD, with low comorbidity index and better laboratory parameters including serum creatinine and phosphorus levels, and lower Kt/V index, had higher values in all domains of QOL. On the other side, those precluded from transplantation were frequently older, with advanced comorbidities that decrease their QOL.

Analysing the SF-36, our study showed that patients undergoing HD and waiting for a kidney transplant had generally higher QOL scores in all domains compared with patients not eligible for transplantation. The lowest values were observed in both groups of patients in general health GH (less than 50%) and in RP, which was expected, taking into account difficult health condition of such patients.

There were statisticaly significant differences between groups in four of the eight dimensions. Three dimensions PF, RP, GH belong to Physical Function Summary (PF) domain and the fourth SF belongs to MCS domain.

PF domain measures the impact of physical health on life. Poor physical performance and poor outcome of renal disease are associated with significantly increased atrophy in the muscle and non-contractile tissue in all patients on hemodialysis. Physical QOL impairment increases the risk of graft failure and mortality too [18]. Prior to renal transplantation, increased controlled physical activity is highly recommended to all patients with chronic renal failure. Physical rehabilitation programs, could improve muscular strength, increase the ability for daily activities and encourage independent living. Accordingly, patients not eligible for transplantations are at higher risk of poor QOL level, mainly regarding PF and RP aspects and due to this, special attention could be paid to this group of patients. In this respect, physical rehabilitation programs can be valuable for all patients on dialysis [19–21].

It will be important to find out the main factors of such poor SF scores in non-WT patients. Both groups of patients were dialyzed in the Department for Dialysis, where they felt comfortable and friendly to hospital staff, which positively affected their mood. But WT patients were more optimistic which could have an influence on their answers in SF-36. We consider that besides clinical aspects, such as associated diseases and old age, the main factor of such a poor SF in the non-WT patients might be hopelessness because of no perspective for transplantation

Some investigations confirmed the close relationship between physical disorders, mental suffering, reduced vitality and lack of socialization. There are data on anxiety and depression among HD patients that are waiting for transplants. For them, the main stressors are psychological:

uncertainty of organ availability, mistrust, and anger when other candidates receive an organ, the possible adverse outcome of the transplantation, fear of being overlooked by the transplantation staff, etc. [19, 20].

On the other hand, in not-WT patients, not suitable candidates for kidney transplantation, total worse health condition, personal preferences and bad situations in their home are only part of the factors that must be taken into account. Some patients have no family support, and in specific, affective and painful conditions, they do not have to share pain, suffering, and grief with someone.

Our findings indicate a general need for psychosocial support for both groups of patients on dialysis. The psychiatrist and psychologist could help them improve their quality of life by providing new coping strategies for each member of the family, occupational and social network [22–24].

Conclusion

Patients waiting for kidney transplant compared with patients not eligible for transplantation are younger, started dialysis in the younger age and spent longer time on dialysis. They have fewer comorbidities and better laboratory parameters (serum creatinine, and phosphorus) including lower Kt/V index. They have higher values in all domains of QOL especially in general health, physical condition and social functioning.

Althgouh our study offer important and useful information on factors that influence QOL of the patients waiting for kidney transplantation, more research is needed in this field to confirm our findings.

Potential limitations of the study are a small sample of patients and the cross-sectional study design, which makes it impossible to know about changes in QOL over time. Finally, specific factors related to non-WT patients involved in the low QOL were not completely identified.

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Biochemical liver function tests parameters do not indicate any difference in the degree of hepatotoxicity in patients with metastatic colorectal carcinoma treated with conventional anticancer drugs regardless the use of bevacizumab

Biohemijski parametri funkcije jetre ne ukazuju na razliku u stepenu hepatotoksičnog efekta konvencionalnih citostatika bez obzira na korišćenje bevacizumaba kod bolesnika sa metastatskim kolorektalnim karcinomom

Kristina Denić*, Dino Tarabar[†‡], Slobodan Obradović[‡§], Nemanja Stanić[‖], Jelena Spasić[‖], Nenad Ugrešić[¶]

Military Medical Academy, *National Poison Control Centre, [†]Clinic for Gastroenterology and Hepatology, [§]Clinic for Emergency Medicine, Belgrade, Serbia; University of Defence, [‡]Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; [‖]Institute for Oncology and Radiology, Belgrade, Serbia; University of Belgrade, [¶]Faculty of Pharmacy, Belgrade, Serbia

Abstract

Background/Aim. Colorectal carcinoma (CRC) is one the most frequent malignant disease with early liver metastasis. It requires the timely use of anticancer drugs. Current treatment of metastatic CRC consists of conventional anticancer drugs use, but they cause liver damage which is manifested by disorder in biochemical liver function parameters. The addition of one of monoclonal antibodies, e.g. bevacizumab improves their therapeutic effect, but its influence on caused biochemical disturbances is not completely known. Therefore the aim of this study was to compare the level of liver function test parameters in patients treated with conventional anticancer drugs with parameters in patients additionally treated with bevacizumab. **Methods**. The study was performed on the two groups of adult patients with liver metastatic CRC assigned according to the treatment protocol. One group of the patients (n = 44) was treated with FOLFOX4 (the group 1), and the other one (n = 52) with bevacizumab added to FOLFOX4 treatment protocol (the group 2). Depending on the response of patients, the duration of treatment varied from 2 to 6 months. Standard liver function tests were performed before and after the completion of the treatment. **Results**. Initial values of some biochemical function test parameters [alkaline phosphatase (ALP) in the group 1 of patients, gamma-glutamyl transferase (GGT) and lactate dehydrogenase (LDH) in both groups] were increased in relation to the normal reference values, with some intergroup differences ($p = 0.001$). Biochemical disturbances of liver function tests in the group of patients treated with conventional anticancer drugs were due to not only their metastases but also due to the hepatotoxic effect of drugs used. After the treatment, significant differences in biochemical liver tests parameters were found in aspartate aminotransferase (AST), alanine aminotransferase (ALP), GGT and LDH, being lower in the group 2 (patients additionally treated by bevacizumab) (p values were: 0.002 for AST; 0.001 for ALP and GGT; 0.000 for LDH). The levels of the other studied parameters, alanine aminotransferase (ALT) bilirubin, and proteins did not differ significantly between groups both pre- or post-treatment. **Conclusion**. Both, metastatic CRC and treatment with the conventional anticancer drugs induce significant disturbances of several liver function parameters. The addition of bevacizumab to the conventional anticancer drugs did not affect these disturbances.

Keywords:
colorectal neoplasms; neoplasm metastases; antineoplastic combined chemotherapy protocols; antibodies, monoclonal; bevacizumab; liver function tests.

Apstrakt

Uvod/Cilj. Kolorektalni karcinom (CRC) jedno je od najčešćih malignih oboljenja. U trenutku dijagnostikovanja kod većine obolelih otkrivaju metastaze na jetri. Zbog toga je pravovremena upotreba hemioterapeutika od velikog značaja za njihovo lečenje. Lečenje metastatskog CRC zasniva se na upotrebi konvencionalnih citostatika, koji oštećuju tkivo jetre što se manifestuje poremećajem vrednosti biohemijskih parametra kojima se prati funkcija jetre. Dodatak nekog od monoklonskih antitela, npr. bevacizumaba, konvencionalnim citostaticima, poboljšava njihov terapijski efekat, dok je njegov uticaj na prouzrokovani poremećaj vrednosti biohemijskih parametara nepoznat. Shodno tome, cilj istraživanja bio je da se ispita kako dodavanje bevacizumaba konvencionalnim ci-

Correspondence to: Kristina Denić, Military Medical Academy, National Poison Control Centre, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: kristinadenic@yahoo.com

tostaticima utiče na vrednost biohemijskih parametara korišćenih za praćenje funkcije jetre. **Metode**. U istraživanje su bili uključeni odrasli bolesnici sa metastatskim CRC podeljenu u dve grupe na osnovu hemioterapijskog protokola koji su primali. Jedna grupa bolesnika (n = 44) lečena je po FOLFOX4 protokolu, dok je druga grupa (n = 52) lečena kombinacijom FOLFOX4 protokola i bevacizumaba. U zavisnosti od efekta primenjene terapije, bolesnici su lečeni od 2 do 6 meseci. Pre i posle završenog lečenja rađeni su kompletni testovi funkcije jetre. **Rezultati**. Početne vrednosti određenih biohemijskih parametara [alkalne fosfataze (ALP) u grupi 1, a gama-glutamil transferaze (GGT) i laktat dehidrogenaze (LDH) u obe grupe bolesnika bile su iznad gornje granice referentnih vrednosti, uz postojanje statistički značajne razlike između grupa (p = 0.001)]. Poremećaj vrednosti biohemijskih parametara kod bolesnika koji su lečeni konvencionalnim citostaticima posledica je ne samo metastatskih promena već i toksičnog efekta hemioterapije. Nakon sprovedenog lečenja, statistički značajno su se razlikovale vrednosti aspartat aminotransferaze (AST), ALP, GGT i LDH (p vrednosti bile su redom: 0.002 za AST; 0.001 za ALP i GGT; 0.000 za LDH). Vrednosti pomenutih parametara bile su niže kod bolesnika u grupi 2 koja je uz konvencionalne citostatike primala i bevacizumab. Suprotno tome, poređenjem vrednosti alanin aminotransferaze (ALT), ukupnog bilirubina i ukupnih proteina na početku i kraju lečenja nije utvrđena statistički značajna razlika između grupa. **Zaključak**. I metastatski CRC i lečenje konvencionalnim citostaticima dovode do značajnih poremećaja vrednosti nekih od biohemijskih parametara kojima se prati funkcija jetre. Dodatak bevacizumaba konvencionalnim citostaticima ne utiče na ove poremećaje.

Ključne reči:
kolorektalne neoplazme; neoplazme, metastaze; lečenje kombinovanjem antineoplastika, protokoli; antitela, monoklonska; bevacizumab; jetra, funkcijski testovi.

Introduction

Colorectal carcinoma (CRC), with an annual incidence of one million cases worldwide, is the third most frequent one among all malignant tumors. After lung and prostatic carcinomas in men and breast carcinoma in women, CRC is the most frequent cause of death [1, 2]. The primary site of CRC hematogenic metastases is liver [2]. At diagnosis, liver metastases are present in about 25% of patients (synchronous metastases), while in the one third of patients metastases are developed during the course of follow-up (metachronous metastases) [3, 4]. Aside this, liver metastases in patients died from CRC are found in 70% of cases, being thus considered as the main cause of mortality in these patients [3]. Because of that, the treatment of liver metastatic CRC (mCRC) is of the great importance and its success is dependent on their resectability.

Metastases are fully resectable in less than 10% of patients and can be removed surgically without prior use of chemotherapy [5]. However, there are far more patients with potential resectable or nonresectable metastases in which surgical removement is possible only after the use of neoadjuvant treatment.

The data show that the use of such treatment leads to the improvement of resectability in about 30% of patients with nonresectable liver metastasis, and thus to the increase of expected 5-year survival of these patients up to 25% [5].

In patients with mCRC, liver function is impaired not only as a consequence of the impact of metastases on healthy liver tissue but also as a result of the direct hepatotoxic effect of conventional anticancer drugs used for their treatment [6–8].

There are only a few papers dealing with the changes of liver biochemical parameters in oncologic patients. In one of them, Field et al. [7] presented only qualitative, but not quantitative results of these changes. In another one, King et al. [6] described the hepatotoxic effect of chemotherapy depending on the treatment protocol manifested by increased values of some liver biochemical tests.

In both mentioned papers, it was also pointed out that there are many comorbid factors (e.g. obesity, personal history of viral hepatitis, sex and age) which contributed to these disturbances.

The results of several clinical studies have shown that the addition of bevacizumab to conventional anticancer drugs [5-fluorouracil (5-FU), oxaliplatin, irinotecan, capecitabine)] greatly contributed to their therapeutic effect [9–16]. However, there were no data on its influence on the increased values of liver function tests parameters.

Methods

The research was designed as a retrospective study in which 96 patients with liver mCRC treated with FOLFOX4/FOLFOX4+Bevacizumab [FOLFOX: FOL – folinic acid (leucovorin; F – fluorouracil (5-FU); OX – oxaliplatin] as a neoadjuvant chemotherapy protocol were enrolled. Treatment was conducted at the Institute for Radiology and Oncology in Belgrade, Serbia, in the period from January 2009 to December 2014. Data were collected from patients' medical history documents.

According to the treatment protocol patients were divided into two groups: the group 1 (n = 44) was treated with FOLFOX-4, and the group 2 (n = 52) with bevacizumab added to FOLFOX-4 treatment protocol. Used chemotherapy protocols were in accordance with National Comprehensive Cancer Network recommendations for colorectal carcinoma treatment (NCCN) [17].

The group 1 of patients received FOLFOX4, which consisted of a 2-hour infusion of leucovorin (20 mg/m^2) followed by 5-FU *iv* bolus (400 mg/m^2) and 22-hour infusion (600 mg/m^2) for 2 consecutive days, with oxaliplatin (135 mg/m^2) as a 2-hour infusion on day 1. Besides this, patients from the group 2 additionally received bevacizumab on the first day of the therapy in a dose of 5 mg/kg. The duration of bevacizumab treatment was determined by a physician. The treatment was conducted on every two weeks, except in a case of high grade of toxicity when it was postponed until patient's recovery. Patients from both groups received at least four cycles of chemotherapy. Treatment response was determined after every fourth cycle until the end of the therapy. Patients response to therapy was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 as a complete or partial response,

stable disease, and progressive disease. The evaluation was performed by a surgeon, oncologist, pathologist and radiologist who did not take part in the study.

Preoperatively, in order to confirm the diagnosis, examinations such as clinical, endoscopic and radiologic ones [abdominal ultrasound, chest x-ray, multislice computerized tomography (MSCT) of the abdomen] were performed in all the patients.

Only the patients who received FOLFOX4/ FOLFOX4 + bevacizumab for potentially resectable liver metastases as a first line treatment protocol were included in the study. Other inclusion criteria were: Eastern Cooperative Oncology Group (ECOG) performance status score 0-2, age 18 to 80 years, normal function of the bone marrow [white blood cells (WBC) > 4×10^9/L; platelet count > 100×10^9/L)], liver (upper limit of the normal range < 1.5 × ULN), and kidney (serum creatinine concentration < 1.5 × upper limit of the normal range (ULN) function, no previous other malignant disease except cervical carcinoma *in situ* and no contraindications for the drugs administration. Patients were followed-up until the end of the treatment or until the disease progression and switch to another treatment protocol.

The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. Approval of the protocol was obtained from institutional Ethics Committee.

The biochemical parameters of liver function [aspartate aminotransferase (AST); alanine aminotransferase (ALT); alkaline phosphatase (ALP); gamma glutamyl transferase (GGT); Lactate dehydrogenase (LDH) relevant to determine the chemotherapy hepatotoxic effects were determined before and after the completion of the treatment. Parameters were measured in serum utilizing commercial biochemical tests on the biochemical analyzer Advia 1800.

The intent-to-treat (ITT) patient population included all patients who participated in the study. The usual descriptive statistic parameters were used in statistical analysis of the obtained results (median with interquartile range 25–75 percentiles). Values of the analyzed parameters had no normal distribution. For dependent or independent non-parametric characteristics Wilcoxon test and Mann-Whitney *U*-test were performed. Commercially available statistical software package SPSS version 17.0, 2008 was used for statistical analysis.

Results

After completed treatment, a complete response was accomplished in only 3 (3.13%) patients, partial response in 38 (39.58%), stable disease in 23 (23.96%) and progression of the disease was observed in 32 (33.33%) patients. Complete response was accomplished only in the group 2 of patients. Out of 38 patients with partial response, 30 (78.95%) patients belonged to the group 2. Stabilization of the disease was almost equally represented in both groups of patients while progression of the disease was more common for patients in the group 1.

Results of studied biochemical parameters are presented in Table 1 and Figures 1–6. The results in both groups are given before and after the treatment.

In the group 1 initial values of ALP, GGT and LDH were above ULN in 53.5%, 45% and 33% of patients respectively (not shown). At the same time, in the group 2 differences in values of GGT and LDH were found in 24% and 21.1% of patients, respectively (not shown).

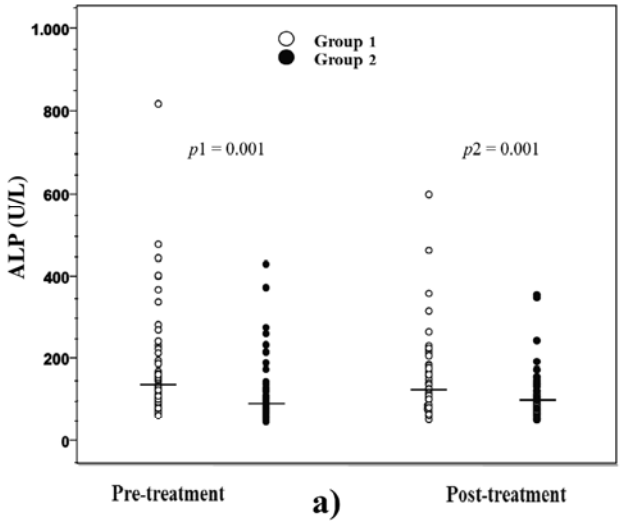
The intragroup pre- and post-treatment values of tested biochemical parameters found in the group 1 showed that conventional anticancer agents led to the statistically significant increase in serum levels of AST ($p = 0.002$) and bilirubin ($p = 0.001$).

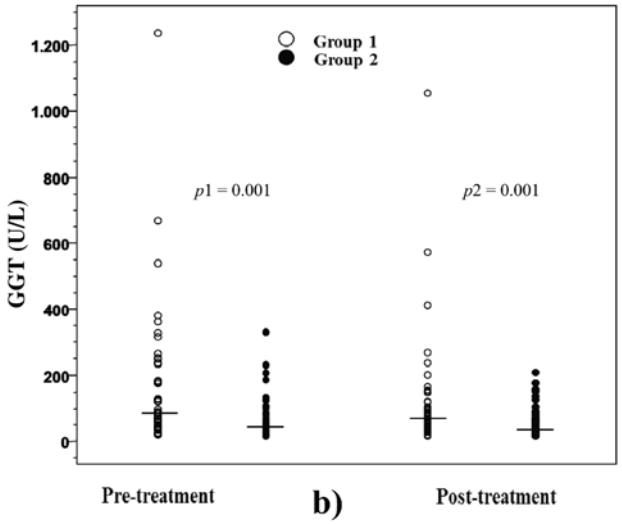
Table 1

Pre- and post-treatment values of biochemical parameters of liver function tests in patients treated with conventional anticancer (the group 1) and with their combination with bevacizumab (the group 2)

Parameters	ULN	Pre- treatment, median (IQR)		Post-treatment, median (IQR)	
		Group 1	Group 2	Group 1	Group 2
AST (U/L)	40	24.00	21.00	33.00	25.00**
		17.25–35.75	17.25–26	25.25–46.75	20.25–34.25
ALT (U/L)	40	23.00	20.00	26.00	25.00
		17.25–37.25	14.00–28.75	21.00–33.25	18.00–38
ALP (U/L)	141	134.00	94.50**	129.00	99.00**
		93.25–228.00	72.00–126.25	105.00–187.50	76.50–133.50
GGT (U/L)	60	87.00	47.00***	76.00	38.50**
		44.25–236.75	30.00–97.00	47.00–149.00	23.25–70.25
LDH (U/L)	460	449.00	350.50***	475.50	380.50***
		347.00–955.25	287.75–490.25	394.25–724	327.25–439.00
Bil (μmol/L)	20.5	8.75	8.00	10.70	10.10
		7.00–11.55	6.82–11.17	8.67–15.17	7.57–13.60
Prot (U/L)	82	73.00	73.50	71	72.00
		70.00–76.75	72.00–77.00	68.00–73.75	70.00–74.00

***$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ intergroup pre- and post-treatment comparison; ULN – upper limit of normal; IQR – interquartile range; AST – aspartate aminotransferase; ALT – alanine aminotransferase; ALP – alkaline phosphatase; GGT – gamma glutamyl transferase; LDH – lactate dehydrogenase; Bil – bilirubin; Prot – proteins.**





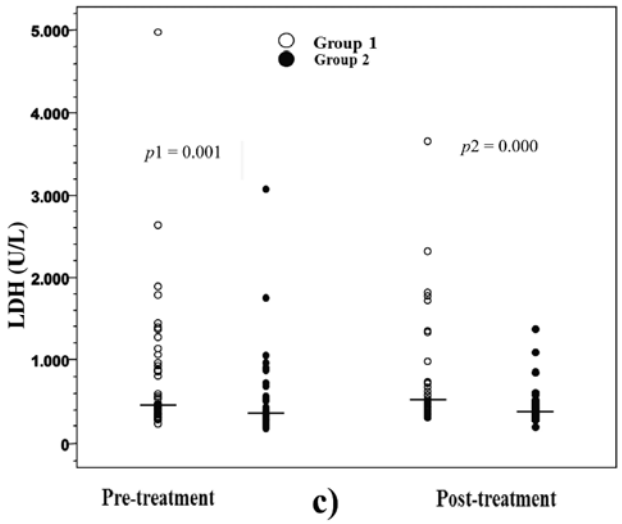
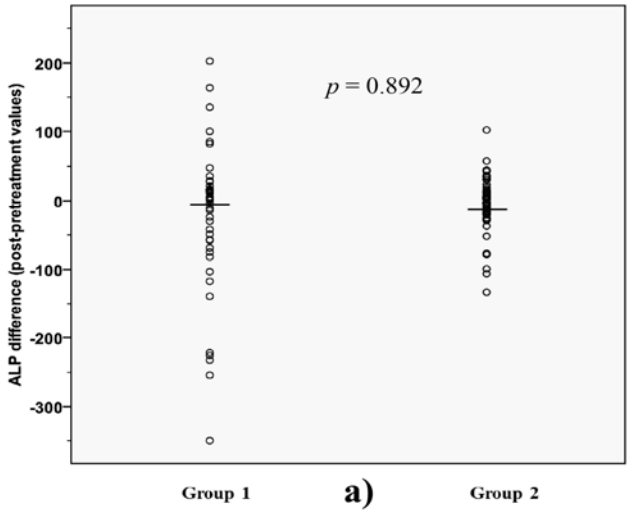
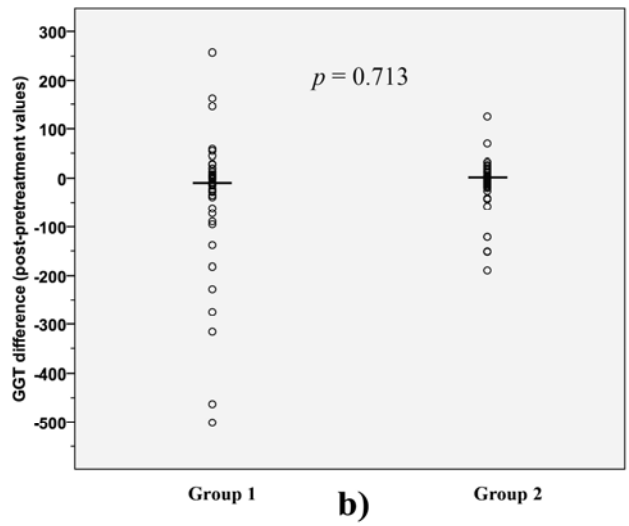


Fig. 1 – Intergroup comparison of pre- and post-treatment values: a) alkaline phosphatase (ALP); b) gama glutamyl transferase (GGT); c) lactate dehydrogenase (LDH). Group 1 – treatment with conventional anticancer drugs (FOLFOX4 protocol) alone; group 2 – treatment with combination of conventional anticancer drugs (FOLFOX4 protocol) and bevacizumab.





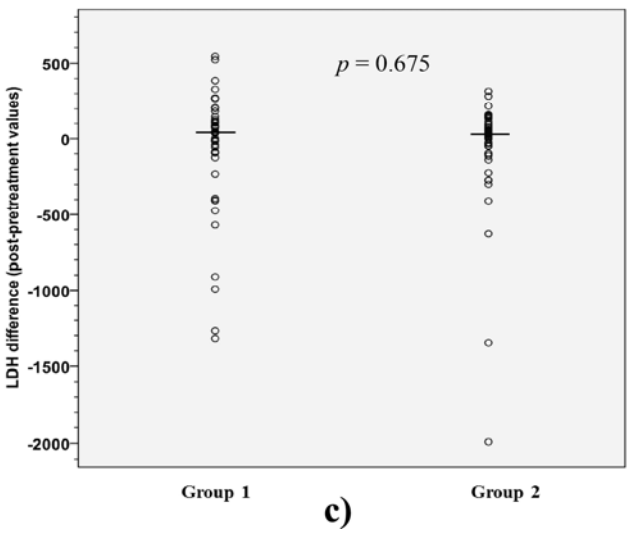


Fig. 2 – Intergroup comparison post- and pre-treatment value difference in: a) alkaline phosphatase (ALP); b) gama glutamyl transferase (GGT); c) lactate dehydrogenase (LDH). Group 1 – treatment with conventional anticancer drugs (FOLFOX4 protocol) alone; group 2 – treatment with combination of conventional anticancer drugs (FOLFOX4 protocol) and bevacizumab.

In the group 2 of patients, statistically significant increase in serum levels after the treatment was found in three parameters: AST, ALT and bilirubin ($p = 0.001$; $p = 0.001$; $p = 0.006$ respectively).

In both groups of patients amount of serum proteins after the treatment was statistically significantly decreased (p values for the group 1 and 2 were 0.043 and 0.005).

The analysis of intergroup (group 1 : group 2) pre-treatment results showed statistically significant difference ($p = 0.001$) in serum levels of ALP, GGT and LDH with the higher initial values registered in the group 1 (Table 1).

Comparison of post-treatment results in the group 1 in relation to the results in the group 2, showed statistically significantly lower values of some parameters in the group 2: AST ($p = 0.002$); ALP ($p = 0.001$); GGT ($p = 0.001$) and LDH ($p = 0.000$) (Table 1).

Results of the analysis of post and pre-treatment value difference between groups showed no statistically significant difference in values of the eight tested biochemical parameters.

In order to be easy-comprehended, the absolute values of statistically significant results obtained after intergroup comparison of pre- and post-treatment values are shown in Figures 1 a–c.

Intergroup comparisons of post- and pre-treatment value difference are shown in Figures 2 a–c.

Figures 1 a–c show that pre- and post-treatment ALP, GGT and LDH statistically significantly differ between two groups of patients, with a pronounced variability of values in the group 1 of patients.

Figures 2 a–c show no statistically significant difference when post- and pre-treatment value differences in serum levels of ALP, GGT and LDH were compared between two groups of patients.

Discussion

The results of the study showed that the treatment of mCRC patients with conventional anticancer agents led to the increase of values of several liver function tests parameters. As a consequence of the disease, these results were also initially increased in relation to the ULN. At the same time, the addition of bevacizumab to conventional anticancer treatment did not lead to a statistically significant decrease of those values.

The influence of mCRC on liver function tests

The unfavorable effects of liver colorectal metastasis on its biochemical parameters are dual: space occupying and energy consumption. The occupied space compresses nearby liver tissue with simultaneous "steal" of the energy required for normal liver function. This is so more pronounced because malignant cells are fast divided and thus consume a large amount of nutritional compounds. This leads to the disturbances of ion pumps followed by the leak of intracellular enzymes and increase of their blood values [18, 19].

Results of the study showed that the pre-treatment values of standard liver function tests parameters, such as ALP, GGT and LDH were significantly increased in relation to their ULN. This itself points out that liver metastases of CRC exert a hepatotoxic effect on liver cells.

The influence of conventional anticancer drugs on liver function tests

Many anticancer drugs, including 5-FU and oxaliplatin exert a direct hepatotoxic effect at least partly by producing free radicals [20–24]. They in turn damage lipoprotein membranes of liver cells which release and thus increase the values of corresponding enzymes in systemic circulation [5]. As a result of anticancer treatment, a hepatotoxic effect and mCRC as a basic disease, liver function tests parameters values were further additionally aggravated. If one compares the therapeutic and hepatotoxic effect of anticancer used drugs the conclusion is that there is a disparity between those two effects. Namely, 5-FU and oxaliplatin lead to temporarily stabilization of the disease but at the same time to the further increase of liver function tests parameters values (Table 1).

The influence of bevacizumab on conventional anticancer agents hepatotoxic effect

Bevacizumab is one of the newer monoclonal antibodies used as an additional treatment to current anticancer drugs in patients with mCRC. The results of so far clinical studies show that bevacizumab increased the therapeutic effect of 5-FU and oxaliplatin leading to the significant clinical improvement [9–16]. These findings were confirmed in our study, where the largest number of patients with complete and partial responses belong to the group of patients additionally treated with bevacizumab. However, there were no data about its influence on disturbances of liver function tests parameters induced by conventional anticancer drugs.

In this respect, results of our study showed that bevacizumab added to conventional anticancer treatment did not remarkably decrease the disturbed values of biochemical liver function tests parameters caused by these drugs (Table 1). These results do not correlate to the clinical improvement in patients treated with combined use of these drugs which provides more complete and partial remissions compared with those achieved with conventional anticancer drugs given alone. In other words, used as an addition to conventional anticancer treatment, bevacizumab leads only to the significant clinical improvement but not to the decrease of the hepatotoxic effect of these drugs.

Conclusion

The results of the study showed that out of seven tested biochemical liver function tests parameters, liver metastases of CRC led to the significant increase in serum values of ALP, GGT and LDH. Conventional anticancer drugs (5-FU and oxaliplatin) exerted the hepatotoxic effect in these patients, leading to the further significant increase of serum values of the mentioned parameters. The addition of bevacizumab to conventional anticancer drugs did not abate their hepatotoxic effect, in term of decreasing the values of monitored biochemical parameters.

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The impact of silicone hydrogel contact lenses on the measurement of intraocular pressure using non-contact tonometry

Uticaj silikon hidrogel kontaktnih sočiva na merenje intraokularnog pritiska metodom nekontaktne tonometrije

Snežana Pešić*, Svetlana Jovanović*†, Miloš Mitrašević‡, Biljana Vuletić*§, Milena Jovanović‖, Zorica Jovanović¶

University of Kragujevac, *Faculty of Medical Sciences, Kragujevac, Serbia; Clinical Center "Kragujevac", †Department of Ophthalmology, ‡Department for Hospital Organization, Planning, Evaluation and Hospital Healthcare Information Technology, §Department of Pediatrics, Kragujevac, Serbia; University of Belgrade, ‖Faculty of Medicine, Belgrade, Serbia; University of Kragujevac, Faculty of Medical Sciences, ¶Department of Pathophysiology, Kragujevac, Serbia

Abstract

Background/Aim. Measurement of intraocular pressure (IOP) over therapeutic silicone hydrogel soft contact lenses by a non-contact method of tonometry could be applied in ophthalmologic practice but the results obtained are still controversial. The aim of this study was to evaluate the effect of spherically designed silicone hydrogel soft contact lenses and their power on values of IOP measured by using a non-contact tonometry method. **Methods.** We measured IOP with and without spherical silicone hydrogel soft contact lenses on 143 eyes of 80 subjects who did not have any ocular or systemic diseases. **Results.** The Wilcoxon statistical analysis test for ranking average values of IOP measured on 143 eyes over a spherical silicone hydrogel soft contact lenses showed significantly higher values compared to those measured with no contact lenses (15.81 ± 3.46 mm Hg *vs* 14.54 ± 3.19 mm Hg; respectively; Z = -5.224, p = 0.001). Refractive power analysis of the contact lenses of -9.00D to +6.00 D showed a significant difference of IOP in the range from 0.00D to -6.00D. **Conclusion.** Non-contact tonometry is not an accurate method of IOP measuring over spherical silicone hydrogel soft contact lenses which belong to therapeutic contact lenses.

Keywords:
intraocular pressure; tonometry, ocular; diagnostic errors; contact lenses, hydrophylic.

Apstrakt

Uvod/Cilj. Mada se u oftalmološkoj praksi može primetiti metod merenja intraokularnog pritiska preko terapijskih mekih kontaktnih sočiva od silicon hidrogel materijala, dobijeni rezultati još uvek su kontroverzni. Cilj rada bio je ispitivanje uticaja mekih kontaktnih sočiva od silikon hidrogel materijala sfernog dizajna i njihove refraktivne jačine na izmerene vrednosti intraokularnog pritiska metodom nekontaktne tonometrije. **Metode.** Intraokularni pritisak je meren bez i sa silikon hidrogel kontaktnim sočivima sfernog dizajna na 143 oka kod 80 osoba koje nisu imale očne ili sistemske bolesti. **Rezultati.** Analiza srednjih vrednosti intraokularnog pritiska Vilkoksonovim testom rangova pokazala je statistički značajno više vrednosti preko silikon hidrogel mekih kontaktnih sočiva sfernog dizajna nego bez kontaktnih sočiva (15,81 ± 3,46 mmHg *vs* 14,54 ± 3,19 mmHg; Z = -5,224, p = 0,001). Analiza refraktivne jačine kontaktnih sočiva od -9,00D do +6,00D pokazala je značajnu razliku intraokularnog pritiska u rangu od 0,00D do -6,00D. **Zaključak.** Nekontaktna tonometrija nije statitički pouzdana metoda merenja intraokularnog pritiska preko silikon hidrogel kontaktnih sočiva sfernog dizajna, kojima pripada i terapijsko kontaktno sočivo.

Ključne reči:
intraokularni pritisak; tonometrija, očna; dijagnostičke greške; kontaktna sočiva, hidrofilna.

Introduction

Measurement of intraocular pressure (IOP) over therapeutic silicone hydrogel soft contact lenses using a non-contact method of tonometry could be applied in ophthalmologic practice, particularly in patients with corneal decompensation and subsequent bullous keratopathy, post-surgical sutures or exposed suture knots other important conditions

Correspondence to: Svetlana Jovanović, Clinical Center "Kragujevac", Clinic of Ophthalmology, Zmaj Jovina 30, 34 000 Kragujevac, Serbia. E-mail: drsvetlanajovanovic@yahoo.com

with corneal pain, and for facilitating healing [1]. The detecting of increased IOP and applying adequate treatment may help reduce the incidence and prevalence of glaucoma in these patients. The therapeutic silicone hydrogel contact lenses also may aid in sealing leaky wounds after cataract, penetrating keratoplasty or glaucoma filtering surgery [2–4].

Sugimoto-Takeuchi et al. [5] suggest the possibility of precise measurements of IOP over therapeutic soft contact lenses using a non-contact tonometry method. There are also studies that suggest the negligible effect of therapeutic soft contact lenses and soft contact lenses of low power on the value of IOP measured through them [6–11]. These studies suggest that changes of measured IOP depend on the refractive power and central thickness of soft contact lenses, although the results are still controversial.

The aim of this study was to analyze the effect of spherical silicone hydrogel soft contact lens and their refractive power (myopic and hyperopic) on IOP measured with a non-contact tonometer.

Methods

Subjects

This study included 143 eyes of 80 subjects (male and female), aged 25.41 ± 7.11 (15–47) years, tested in contact lens practice in 2013 and 2014. The subjects had no ocular and systemic disease, no corneal astigmatism greater than 1.50 D cylinder or no contraindication to wearing soft contact lenses. Exclusion criteria were: corneal pathology before and after surgery. Patients with glaucoma were excluded. We measured IOP of each subject using non-contact tonometry with and without soft contact lenses. All subjects were evaluated by slit lamp examination and corneal topography. Informed consent was obtained from each subject after explanation of the procedure. The study was conducted by the ethical standards of the Declaration of Helsinki.

Materials

The group involving 143 eyes were fitted with monthly replacement silicone hydrogel soft contact lenses (Ciba Vision, Bausch + Lomb, CooperVision) with the same modalities of wearing.

All tonometry measurements were carried out with a non-contact tonometer (Topcon CT – 80A Computerized Tonometer Topcon, Tokyo, Japan).

We used a corneal topography and a biomicroscopy for anterior segment evaluation (CA100 Topcon, Sl8 Z Topcon, Tokyo, Japan).

Procedures

All IOP measurements were performed on contact lenses in the 143 eyes using non – contact tonometry before the inserting of spherical silicone hydrogel soft and seven days after the wearing them.

In order to prevent the possible effect of multiple consecutive measurements of IOP in a non-contact tonometer, IOP was measured three times at 2-min intervals and the mean values were calculated for each recorded IOP [12].

Five different ranks of refractive power were used: rank 1 [from 0.00 diopters (D) to-3.00D, n = 83], rank 2 [from -3.25D to -6.00, n = 48], rank 3 [from -6.25D to -9.00D, n = 3], rank 4 [from +0.25D to+3.00D, n = 3] and rank 5 [from +3.25D to +6.00D, n = 6].

Statistics

Statistical analysis was based on SPSS 20.0. In the descriptive statistics, measures of central tendencies were used. A test of normality was performed with the Shapiro-Wilk test. For comparative statistical procedures ring, we used non-parametric tests: the Spearman's rank correlation test and the Wilcoxon Signed Rank test. For comparing the effect size, we used Cohen`s (1988) criterion.

The Shapiro-Wilk test did not confirm normal distribution for all analyzed variables and because of that nonparametric tests in the study were used.

Results

The mean value of the measured IOP in 143 eyes of 80 subjects before inserting spherical silicone hydrogel soft contact lenses was 14.54 ± 3.19 mm Hg (min = 7 mmHg, max = 23 mmHg). The measured mean IOP with the spherical silicone hydrogel soft contact lenses was 15.81 ± 3.46 mmHg (min = 8 mmHg, max = 24 mmHg) (Table 1). The non-parametric Wilcoxon Signed Rank test was used to compare IOP values between these groups, and it was found that there was a highly significant difference (p=0.001). The size effect between variables compared using Cohen's (1988) criterion was r = 0.2.

The Spearman's rank correlation test of non-parametric statistics was used for repeated measurements and for comparing IOP values at five different powers. To compare groups, they were divided into five ranks and in each rank the IOP with and without spherical silicone hydrogel soft contact lenses was measured. In the first rank from 0.00D to -3.00D (n = 83) there was a statistically significant difference (p = 0.001). The average IOP was significantly greater in this rank after the wearing of spherical silicone hydrogel soft contact lenses. The average IOP measured in the second rank

Table 1

Descriptive statistics of intraocular pressure (IOP) measurements on 143 eyes with and without soft contact lenses

IOP without contact lenses (mmHg)		IOP with contact lenses (mmHg)		*p*-values
mean ± SD	min−max	mean ± SD	min−max	
14.54 ± 3.19	7−23	15.81 ± 3.46	8−24	0.001

SD − standard deviation.

of -3.25D to -6.00D (n = 48) was significantly higher after wearing the spherical silicone hydrogel soft contact lenses than before wearing them, $p = 0.001$ (Table 2). The size effect between variables which was compared using Cohen's (1988) criterion was $r = 0.67$ (a big effect).

Statistical analysis of the third rank of -6.25D to -9.00D (n = 3), the fourth rank of +0.25D to +3.00D (n = 3), and the fifth rank of +3.25D to +6.00D (n = 6) showed no statistically significant differences in IOP measurements with and without the spherical silicone hydrogel soft contact lenses (Table 2). The number of subjects in these ranks was insufficient for performing the reliable statistical evaluation.

Change in measured IOP (ΔIOP) as a function of lens power (x) of silicone hydrogel contact lenses had the following characteristics: in 1st rank ΔIOP was + 1.16 mmHg, in 2nd rank ΔIOP was +1.31 mmHg, in 3rd rank ΔIOP is + 2.00 mmHg, in 4th rank ΔIOP was +1.00 mmHg, and in 5th rank ΔIOP was +2.34 mmHg (Table 3). Relationships between ΔIOP and lens power revealed the algorithm by which we can predict the real IOP.

We found that a silicone hydrogel contact lens significantly influences IOP measurements using non-contact tonometry ($p = 0.001$). Analysis of the impact of the refractive power ranking of silicone hydrogel soft contact lenses on IOP values, measured with a non-contact tonometer showed significantly higher IOP values for lens power 0.00D ≤ -6.00D in comparison to IOP values measured before inserting lenses.

Firat et al. [13] conclude that silicone hydrogel soft contact lens use does not significantly affect IOP values measured with a non-contact tonometer, but it affects IOP values measured with Pascal dynamic contour tonometry in 0.00D power. In our study, we did not measure IOP over silicone contact lenses with 0.00D power. However, we found that silicone hydrogel contact lenses in ranks 4 and 5 from +0.25D to +6.00D did not significantly influence the IOP measurements using non-contact tonometry, while in ranks 1 and 2 from 0.25D to -6.00D they influenced statistically the values of IOP. We did not test the 0.00D power of the contact lens because we included healthy patients, with only refractive error and without any ocular disease. Recent studies have shown that IOP measurements over hydrogel soft contact lenses with non-contact tonometry depend

Table 2

Comparison of the impact of intraocular pressure (IOP) within the same rank, without and with contact lense

Ranks	D level			*p*-values
	from	to	Z	
1	0.00	-3.00	2.743	0.001
2	-3.25	-6.00	4.833	0.001
3	-6.25	-9.00	0.546	0.585
4	+0.25	+3.00	1.129	0.259
5	+3.25	+6.00	0.060	0.952

D − diopters.

Table 3

Change in measured intraocular pressure (IOP), (ΔIOP) as a function of lens power (x) of silicone hydrogel contact lenses (Si Hy CL)

Range (D)	n	Mean IOP without Si Hy CL	Mean IOP with Si Hy CL	**ΔIOP**	*p*-values
1 0.00 to -3.00	86	14.57	15.73	+1.16	0.001
2 -3.25 to -6.00	48	14.66	15.91	+1.31	0.001
3 -6.25 to -9.00	3	15.00	17.00	+2.00	0.585
4 +0.25 to +3.00	3	11.66	12.66	+1.00	0.259
5 +3.25 to +6.00	6	14.66	17.00	+2.34	0.952

D – diopters.

Discussion

Silicone hydrogel contact lenses due to their high oxygen permeability materials are now much more used than conventional hydrogel contact lenses. We tested the effect of silicone hydrogel contact lenses on the value of IOP by non-contact tonometry for glaucoma screening and its potential applicability in contact lens practice.

on the lens power [10, 11, 14, 15]. Liu et al. [11] compared the IOP using non-contact tonometry taken without a contact lens and with different myopic contact lens power from -3.00D to -12.00D. They found a statistically significant difference in IOP values in lens power from -6.00D and below.

The different results found in the present studies may be attributed to different study designs. In our study, we obtained IOP measurements after the insertion of the silicone hydrogel

contact lenses which are different to the hydrogel soft contact lenses. Silicone hydrogel material has low water content, lower modulus of elasticity and a relatively high modulus of rigidity and it differs from hydrogel material which has high water content and relatively low modulus of rigidity. We measured IOP seven days after contact lenses had been worn according to the daily regimen of wear, while in other studies the IOP was measured 30 min after insertion of soft contact lenses [13]. In other studies, IOP was measured at baseline, immediately after contact lens removal or displacement, and 5 minutes thereafter [16, 17].

Zeri et al. [15] consider the possibility that the tonometry result can be influenced by central corneal resistance. Corneal resistance is influenced by corneal thickness, corneal curvature, and corneal biomechanical factors. IOP value will be overestimated in eyes with thick corneas, a steep corneal curvature, and high corneal hysteresis. When a soft contact lens is fitted, the "new" body composed of cornea and contact lens has a greater central thickness than the cornea alone, a possible different external surface curvature depending on the contact lenses power and, presumably, different biomechanical characteristics, depending on the lens material mechanical property as in Young's modulus [15].

Conclusion

According to the results of our study, the spherical silicone hydrogel contact lenses of power from 0.00D to -6.00D significantly affect intraocular pressure values measured using non-contact tonometry. For intraocular pressure measurement over the silicone contact lenses with power from 0.00D to – 6.00D non-contact tonometry is not a reliable method. We can advise accurate measurement of IOP over silicone hydrogel contact lenses in contact lens practice, eventually making a tentative assessment of IOP adding ΔP to given rank.

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Conflict of interest

The authors declare no conflict of interest.

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GENERAL REVIEW

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The use of collagen membranes in guided tissue regeneration

Primena kolagenih membrana u vođenoj regeneraciji tkiva

Marija Bubalo*, Zoran Lazić*†, Zoran Tatić*†, Radomir Milović*, Marko Magić*

Military Medical Academy, *Dentistry Clinic, Belgrade, Serbia; University of Defence, †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Key words:
dental implants; guided tissue regeneration; bone regeneration; membranes, artificial; collagen.

Ključne reči:
implanti, stomatološki; tkivo, vođena regeneracija; kost, regeneracija; membrane, veštačke; kolagen.

Introduction

Dental implants' rehabilitation success is defined by sufficient quantity of the jaw bone. Filling of bone defects with bone substitutes is a procedure of choice in order to maintain bone, but ingrowth of the connective tissue from mucoperiosteal part can compromise the coalescence process of the bone substitute with bone defects walls. Usage of membrane as a barrier is indicated as a solution for this problem [1, 2].

Guided bone regeneration (GBR), a method which originates from guided tissue regeneration (GTR), is based on a concept of dividing bone from soft tissue, i.e. preventing apical migration of the gingival epithelial and connective tissue inside the defect with a membrane as a barrier which favorites proliferation of regeneration-potent cells and their differentiation in the desired tissue type [3].

Five surgical objectives should be reached in order to achieve the goal of guided bone regeneration. This implies the following: using the appropriate membrane; reaching primary soft tissue healing; creating and maintaining a location protected by the membrane; adapting and stabilizing membrane with the surrounding bone; and enabling a sufficiently long healing period [4, 5].

According to Hardwick et al. [6], the main purpose of the membrane's barrier function is creating suitable surroundings in which the natural biological potential for functional regeneration is pushed to the maximum. Creating and preserving the location where a blood coagulum is placed, preventing inflammation which can occur as a result of bacteria penetration, isolating regeneration space from unwanted tissue, and ensuring mechanical stability and compactness of the organized coagulum are just a few of the most important factors for creation of a suitable place for regeneration. Their role in preventing permeation of epithelial cells in solid bone substitute applied, has to be mentioned, and also better fixation of the applied bone substitute [6, 7].

Membrane's features for GBR have been described by several authors [7–9].

They include: biocompatibility; appropriate barrier potency (mechanical prevention of soft tissue proliferation); tissue integration; immunological inertness; preservation of the location for new alveolar bone; and application simplicity.

Membrane must resist chewing force and cut tissue tension, and prevent collapsing of the soft tissue and narrowing the wound space. The ability to integrate into the tissue secures wound stabilization and epithelial migration inhibition [10].

Based on clinical and histological researches of different barrier materials so far, neither of them showed as an ideal one for every clinical situation, because each has its specific characteristics, advantages and limitations.

Depending on the reaction to their biological surroundings, membranes can be grouped as non-resorbable and resorbable.

Non-resorbable membranes keep structure and shape in tissues, and it is necessary to have another surgery in order to remove it; this increases trauma to the patient, the wound healing process, costs and duration of the whole treatment.

Resorbable membranes are not needed to be removed after placement, which reduces the inconvenience and cost of the treatment, and also the risk of surgical complications. Due to the resorbable membrane's nature, it is not possible to precisely determine duration of their degradation. The process of degradation begins immediately after the placement. Data from the literature regarding the desirable duration of membrane persistence *in vivo* show that it varies from 4 weeks

Correspondence to: Marija Bubalo, Dentistry Clinic, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia.
Phone: +381 64 140 44 48. E-mail: bubalo.marija@yahoo.com

to a few months [11]. Due to biological degradation, resorbable membranes induce tissue response, which may negatively impact wound healing and disturb regeneration.

The ideal bioresorbable membrane for GBR has the following characteristics: biocompatibility, the absence of inflammatory reaction, total resorption, degradation and elimination. It should be easy to handle, cut, contour and adapt, maintain desired shape and configuration, be easily secured in place, reliably exclude non-osseous tissues from defect, be resistant to bacterial attachment and colonization, have a predictable resorption time, compatible with bone formation [12, 13].

Two materials are mainly used to manufacture resorbable membranes: synthetic aliphatic polyester and collagen, derived from different animal sources, including bovine tendon, bovine dermis, calf skin, or porcine dermis [14–16].

Collagen membranes

The ability of collagen to stimulate adhesion, chemotaxis and physiological degradation of progenitor cells, together with the possibility of its own degradation, makes it an ideal material for building the membrane. Collagen is an insoluble fibrous protein that is an essential component of the connective tissue stroma. There are at least 16 types of collagen found in interstitial tissues, matrix of bone, cartilage, epithelial and blood vessel basement membrane and the vitreous of the eye, among others. Types I, II and III collagen constitute 80– 90% of the body`s collagen. Commercially available collagen products are composed mainly of types I and III collagen [17, 18].

Collagen has weak immunogenicity, induces hemostasis, and can augment tissue thickness; during healing of the wound occurs, an interaction between collagen and different types of cells [19, 20]. Collagen is made from animal skin, tendons or offal. First, it is isolated and purified with enzymes and chemicals, then processed in different forms. Most common chemical modification of collagen creates transverse connections, usually with exposure to aldehyde, which decreases absorption of water, influences ability to melt, degradation rate and increases firmness [21].

Collagen membranes are degraded by macrophages and polymorphonuclear neutrophils, and the absorption rate is different, based on the collagen source and modifications. Collagen membrane resorption begins with the activity of collagenase enzyme (matrix-metalloproteinase), which divides collagen molecule on specific position. Created parts are denatured and transformed into gelatin, which is then degraded to amino acids by gelatinase and other proteinase [22].

During enzymatic degradation, it will incorporate with in the flap to support new connective tissue attachment [20]. This may result in augmenting tissue/flap thickness to protect further bone formation.

Cross-linking of collagen: pro and contra

Structure stability increased by cross-linking slows down the degradation process. Cross-linking of collagen is achieved by ultraviolet and gamma rays, hexamethylene glutaraldehyde, diphenyl phosphorylase and ribose. Cross-linking is controlled and reduces *in vivo* the rate of collagen material resorption and increases mechanical characteristics. The essence of the process is building different mutual connections between specific amino-acids, and between aminoacids and carboxylate groups, under chemical and physical agents' influence [23].

There is a controversy arising from whether to apply cross-linked or non-cross-linked membranes in GBR. Although many studies have proved that with cross-linking the biodegradation of the collagen membrane is being expanded, and that they have shown positive, but limited effect on GBR in different types of experimental defect models [24 25], other studies have shown that their application associated with the initial reaction of foreign body, reduces tissue integration and with compromised trans membrane vascularization [26, 27]. Despite all the disagreements, it has been shown that membrane vascularization is being improved in 2 weeks after its submucosal implantation in rats by using certain procedures of cross linking. [28]. This is probably because the initial hyperemia is being caused in the neighboring tissue, which directs angiogenesis toward experimental membrane. In 2006 Schwarz et al. [28] examined the model of angiogenesis in natural and crossed-linked collagen membranes, because previous tests have shown that vascularization is weaker in cross-linked membranes. The conclusion was that angiogenesis in different types of membranes is without statistical significance.

In two studies done in the Military Medical Academy, defects covered with cross-linked collagen membranes showed a better level of vascularization in comparison with defects with non-cross-linked membrane or with empty defects [29, 30].

In 2012, Thoma et al. [31] studied the differences in cross-linked collagen, but instead of collagen membranes they used high and low degree collagen patterns, which have been chemically cross-linked and they have put them in the soft tissue of mice. Histopathologic and histomorphometric researches were performed 3 and 6 months after surgical intervention, and referred to the presence of tissue integration, collagen biodegradation and formation of new blood vessels. The results have shown that the level of crosslinking was in negative correlation with observed parameters, because collagen with lower level of crosslinking has shown better tissue integration, stability and angiogenesis.

All of these studies showed the importance of crosslinking. Despite few negative characteristics, many authors suggested that the use of cross-linked collagen membranes brought many benefits to GBR.

Exposure of collagen membranes

Several periodontal pathogens are capable to produce collagenase, an enzyme which can lead to premature membrane degradation. These are *Porphyromonas gingivalis* and *Bacteroides melaninogenicus* [32]. Bacterial colonization may lead to early degradation of the collagen membranes, which can compromise the procedure. Both cross-linked and non-cross-linked membranes are being equally exposed to lysis

under the influence of bacterial proteases, although some studies have shown that cross-linked membranes are more resistant to proteolysis [32, 33]. Therapeutic concentrations of antibacterial and antibiotic agent, such as chlorhexidine, minocycline and doxycycline, partially inhibit the enzymatic membrane degradation.

Collagen membranes differ in their microarchitecture (space between collagen molecules, fibers, beams and layers within the membrane) and crosslinking.

Microarchitecture and cross-linking define membrane characteristics, such as tension power, easy manipulation, flexibility, tissue integration, biodegradation.

Membranes with a higher level of crosslinking remain intact for a longer period [34]. The studies have shown that premature membrane resorption or its removal can lead to incomplete bone healing, so it is advised that the membranes applied in GBR should have degradation period between 3 to 9 months, the time needed to achieve bone formation [4].

Biodegradation of collagen membranes

Rothamel et al. [26] studied biodegradation over time, the reaction to tissue, tissue integration and the vascularization of commercially available collagen membranes as well as those experimental, after being placed subcutaneously in 40 rats. Histological and histometric researches were performed 2, 4, 8, 16 and 24 weeks after placing the membrane. The conclusion was that the cross-linked collagens types I and III of bovine and porcine origin extend biodegradation, but reduce tissue integration and vascularization, and foreign body reaction appears which is characteristic for cross-linked membranes. This study shows the abovementioned differences between cross-linked and non-cross-linked membranes, proving that the non-cross-linked membranes have better vascularization and tissue integration, longer-lasting barrier role, slower degradation, but also that the cross-linked membranes have weaker tissue integration. The absorption rate directly correlated to the crosslinking degree – higher level of connection, longer resorption rate [27].

In 2006 and 2008, Tal et al. [15, 35] studied clinically and histologically the barrier function duration and biointegrity in places that have been treated by cross-linked and non-cross-linked collagen membranes. Special attention was given to the spontaneous mucosal perforations through the barrier membranes. It was shown that cross-linked membranes were more resistant to tissue degradation and that they maintained integrity in the longer period. Neither type of membranes was resistant to tissue degradation when being exposed. Exposure occurred more frequently in cross-linked membranes. However, a complete primary closure is essential to prevent early exposure.

The impact of membrane thickness on bone regeneration

So far, there has not been a lot of published researches on the impact of the resorbable membrane thickness on bone regeneration. The attempt of applying a thicker membrane was published in 2005 by Busenlechner and et al. [36]. The purpose of their study was to question the possibility of slowly resorbable prototype 3-layer membrane in bone regeneration during augmentation of the alveolar ridge after the extraction of the first and second molars in the lower jaw of a monkey, and after making the cavity three months after extraction. Experimental animals were sacrificed after 9 months. The study supports implementation of the slowly resorbable three-layer membrane, because the best achieved bone regeneration was made using this membrane and bone graft. The membrane was made by adding a polylactide layer between two layers of collagen in order to increase the degradation time and also the barrier's function. Polylactide fragments were found in histological examinations even after 9 months. The 3-layer membranes' design can be the important step in improving membrane stability with a specific exposure rate. In this study, it amounted 8.33%, which is extremely low compared to 43.75% recorded in the study done by Sculean et al. [37].

The same 3-layer membrane prototype was examined by von Arx et al. [38]. The aim of their study was to examine the three-layer membrane prototype in combination with a variety of materials for augmentation. Patterns were analyzed histopathologically and histomorphometrically after four and a half months. The 3-layer membrane prototype in combination with autograft showed the best bone regeneration.

In 2009, Kozlovsky et al. [39] made histological comparison of Bio-Gide® membrane biodegradation (non cross-linked collagen membrane) placed in one and two layers in mechanical defects created on rat's calvarias. Application of the second layer of Bio-Gide® membrane (double layer technique) resulted in a significantly greater residual amount of collagen, at least up to 9 weeks following surgery in rats. Also there was much more barrier material left in the bilayer membrane tissue, which indicates a longer-term barrier role of membranes, but also that monolayer membrane could not achieve barrier function in the long period of time. Therefore the bilayer membrane made better bone regeneration and defects ossification. It should be noted that the second layer achieves a reduction of micro movements and improves its stability. Transmembranous vascularization of the membrane was manifested histologically already 4 weeks following implantation and become well-defined through all layers of membrane 9 weeks following implantation. In spite of the difference in the thickness of 2-membrane preparations, similar degradation rate of 80% for both membranes was measured at 9 weeks. Since the transmembranous formation of blood vessels is essential for collagen resorption [28], it seems that vascularization of the double layer membrane was not impaired by its increased thickness. It has been claimed that increasing the density of cross links between collagen molecules has a negative effect on membrane biocompatibility [29, 40], membrane to tissue integration and vascularization, and inhibits attachment and proliferation of PDL fibroblasts and osteoblasts [5, 40]. Using a second layer of resorbable cross-linked membrane avoids these disadvantages, while extending membrane longevity. In the double layer 9 weeks membrane specimens, central intramembrane neo-ossification was clearly identified with collagen fibers embedded in the osteoid [41, 42].

The efficiency of bilayer membranes in bone grafts application, in terms of bone resorption, has been analyzed in a study on rabbits [43]. Bone blocks of parietal bones were taken from one side and placed on the other, and covered with membranes. Histological and histomorphometrical analysis were performed 2, 4 and 6 months after surgery. The results of the study show that the double membrane application decreases bone resorption of the graft significantly more in respect to the single layer one [41].

A study done in the Military Medical Academy examined the impact of collagen membranes of different origin and thickness on post-extraction ridge preservation. The results show that the best outcome was reached with application of thicker membranes [30].

The results of these few studies regarding the thickness of the membranes, show that membranes of greater thickness, whether they are arranged in several layers or are thicker, show greater barrier ability and remain for longer time in tissue, because they decompose slowly and enable better bone defect ossification. While this finding has never been fully understood, it may be speculated that the significant increase in membrane thickness and longevity results in increasing angiogenesis and cellular population of collagen matrix, leading to cell proliferation, differentiation and ossification.

Collagen membranes of human origin

Special attention should be given to resorbable collagen membrane of human origin. The role of the resorbable human demineralized membrane in GBR and GTRhas been insufficiently studied. There are a few experimental studies done in the Military Medical Academy, Belgrade. The authors examined the impact of thickness and origin of human resorbable membranes on bone regeneration. The resorbable human demineralized membrane (RHDM) was prepared by the combination of physical and chemical methods (demineralization of cortical bone with successive removal of lipoproteins) from human cadaver in calvarium region. These studies showed that RHDM proved a greater degree of bone regeneration compared to other membranes, especially the thicker one [43–46].

Disadvantages of collagen membranes

Compared to non-resorbable membranes, collagen membranes lack space-making ability. The use of bone graft material to preserve space tends to improve the outcome of GBR. Alveolar ridge augmentation can be expected only if the space under the collagen membrane is created and preserved in an appropriate period while the new bone is being formed. It is therefore advisable to use materials which will provide support as to prevent collapse of the barrier due to pressure of overlay issue or due to chewing forces [47].

These membranes are often used with tenting or supporting materials (different bone grafts or bone fillers) to prevent space collapse. When grafting materials are used with bioresorbable membranes, the results of GBR procedures are generally favorable and even comparable to the results achieved with non-resorbable barriers, especially in management of localized alveolar horizontal ridge defects [48–52]. Grafting materials alone seems to be less effective than the combination of a supporting material and a barrier. Combination of bioresorbable membranes and non-resorbable membranes with grafting material can achieve good results in treating vertical alveolar ridge defects because one of the main disadvantages of collagen membrane is disability to achieve vertical height of bone. In order to solve this problem, the mentioned combination was used. Membranes, in these cases, needed an extra-stabilization with mini screws and tacks [52–54].

Combination of membranes and growth factors

Lately, the incorporation of growth factors and differentiation in the membrane has also been explored. There is sufficient evidence that certain growth factors and similar mediators can influence regeneration of many tissues, among others, regeneration of bones. An example is the development of combined membranes, which would control release of transforming growth factor (TGF-β). The local delivery of a wide variety of growth factors, such as platelet-derived growth factors (PDGF) and bone morphogenetic proteins that are both osteoinductive growth factors, have been utilized in dentistry possessing capability to further stimulate cell recruitment, proliferation and differentiation. Numerous *in vitro*, animal and clinical trials have demonstrated the advantages of these growth factors in combination with membranes [55–62]. Such combinations could lead to major changes in the outcome of GBR.

Conclusion

This paper reviews the basic principles in membranes utilized in guided bone regeneration. Much advancement has been made since the original non-resorbable polytetraflouroethylene (e-PTFE) membrane was used. Synthetic and natural biomaterials have now been utilized in dentistry with great clinical success for over 20 years, and improvements are continuously being made regarding their mechanical properties and degradation rates.

The next generation of membranes is expected to combine more functional biomolecules projected to increase the success of GBR therapy.

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Non-plug technique of bilayer patch device insertion for indirect inguinal hernia repair

Neutiskujuća tehnika ubacivanja dvolisne mrežice u reparaciji indirektne preponske kile

Miroslav Ilić*†, Srdjan Putnik†, Ivan Kuhajda*†, Dejan Ivanov†‡

Institute for Lung Diseases of Vojvodina, *Clinic for Thoracic Surgery, Sremska Kamenica, Serbia; University of Novi Sad, †Faculty of Medicine, Novi Sad, Serbia; Clinical Center of Vojvodina, ‡Clinic for Abdominal, Endocrine and Transplant Surgery, Novi Sad, Serbia

Abstract

Background/Aim. Despite a huge success in decrease rate of recurrences of inguinal hernia in mesh and "plug in" techniques, a new problem appears such as chronic pain and other complications. The aim of this paper was to present the original modification of bilayer patch device (Prolene Hernia System®, Ethicon) insertion in "non-plugged" fashion and 11-year experience with this open technique for the indirect hernia repair in a male. **Methods.** This retrospective study included 96 male patients with 103 indirect unilateral and bilateral inguinal hernias, operated due to a primary or recurrent hernia in an 11-year interval (2004–2015). In all operation an extended Prolene Hernia System® (PHS) bilayer patch device was inserted medially of inferior epigastric vessels through a small incision in Hasselbach's triangle, thus avoiding plug component of device connector into the internal ring. All data were taken from the medical records, operative protocols, and telephone questionnaire. **Results.** Non-plugged technique of bilayer patch device insertion was presented in details. Mean age of patients was 59 years. There were 3 (2.91%) patients with procedure-related complications, two patients with postoperative seroma and one with scrotal ecchymosis. There were 86 (89.6%) patients who answered on the questionnaire. During 11 years of following, recurrence of a hernia occurred in 1 patient, one had funiculocele and only one had chronic pain during 6 months. Almost all patients (97.68%) were satisfied with the procedure and results of hernia surgery. **Conclusion.** Non-plugged insertion of bilayer patch device is a safe technique for solving the primary and recurrent indirect inguinal hernias. The low incidence of the recurrence and chronic pain many years after the operation justifies this technique even in hospitals not specialized for the hernioplasty.

Key words:
hernia, inguinal; surgical procedures, operative; surgical mesh; treatment outcome; pain, postoperative; surveys and questionnaires.

Apstrakt

Uvod /Cilj. Uprkos ogromnom smanjenju stope recidiva ingvinalne hernije primenom tehnike mrežice i „plug in" tehnike, nastaju novi problemi, kao što je hroničan bol i druge komplikacije. Cilj rada bio je da se predstavi originalna modifikacija postavljanja dvolisne mrežice (*Prolene Hernia System*®, PHS, *Ethicon*) neutiskujućom tehnikom i 11-godišnje iskustvo sa tom otvorenom tehnikom u reparaciji indirektnih preponskih kila kod muškaraca. **Metode.** Retrospektivnom studijom obuhvaćeno je 96 bolesnika muškog pola sa 103 jednostrane odnosno obostrane preponske kile, operisane kao primarne ili recidivne, u 11-godišnjem intervalu (2004–2015). U svim operacijama korišćena je proširena *Prolene Hernia System*® (PHS) dvolisna mrežica koja je postavljana medijalno od donjih epigastričnih sudova kroz inciziju u Haselbahovom trouglu, kako bi se izbeglo ubacivanje konektora u unutrašnji ingvinalni otvor. Svi podaci dobijeni su iz medicinske dokumentacije, operativnih protokola i telefonskog upitnika. **Rezultati.** Detaljno je prikazana neutiskajuća tehnika umetanja dvolisne mrežice (PHS) u hirurgiji indirektnih hernija. Prosečna starost bolesnika bila je 59 godina. Kod tri (2,91%) bolesnika registrovane su komplikacije povezane sa tehnikom, dva bolesnika imala su postoperativni serom, a jedan ekhimozu skrotuma. Na upitnik je odgovorilo 86 (89,6%) bolesnika. Tokom 11 godina praćenja recidiv kile razvio se kod jednog bolesnika, zabeležena je jedna funikulokela, a jedan bolesnik imao je hronični bol u periodu od šest meseci. Zadovoljstvo procedurom i

Correspondence to: Miroslav Ilić, Institute for Lung Diseases of Vojvodina, Clinic for Thoracic Surgery, Put dr Goldmana 4, 21 230 Sremska Kamenica, Serbia. Phone: +381 21 480 5253. E-mil: drmiroslavilic@gmail.com

rezultatima operacije kile bilo je prisutno kod gotovo svih pacijenata (97,68%). **Zaključak.** Neutiskajuća tehnika postavljanja dvolisne prolenske mrežice u toku reparacije indirektne preponske kile sigurna je i bezbedna metoda kojom se mogu rešiti i primarne i recidivantne hernije. Niska stopa recidiva i hroničnog bola utvrđena dugotrajnim postoperativnim praćenjem opravdavaju široku primenu ove tehniku i u centrima koji nisu usko specijalizovani za hernioplastike.

Ključne reči:
hernija, ingvinalna; hirurgija, operativne procedure; hirurška mrežica; lečenje, ishod; bol, postoperativni; ankete i upitnici.

Introduction

The evolution of an inguinal hernia′s repair has gone through the phase of simple reposition, tension techniques based on Bassini's technique, and mesh techniques, open or laparoscopic [transabdominal preperitoneal (TAPP), totally extraperitoneal (TEP)] [1–4]. "Plugged in" techniques are based on the insertion of synthetic, non absorbable, semi absorbable or absorbable materials into the internal inguinal hiatus, the weak point where the indirect inguinal hernia appears [5, 6]. Although there has been a huge success in decrease rate of recurrences in mesh and "plug in" techniques, a new problem appears such as chronic pain, erosive complications of intraperitoneal organs and rejection of artificial material [7–11]. An ideal technique for an indirect hernia′s repair is still not established.

Bilayer patch device (Ethicon, Prolene Hernia System® (PHS) Extended) made of polypropylene is three-dimensional mesh, with two patch layers (underlay and onlay) attached to the connector in order to keep both patches stable. It has been in use for the last 20 years. Although it was created as a two-layer patch (underlay and onlay) this device has a plug component connector. After the preparation of hernia′s sac and reposition in preperitoneal space, the connector is placed through the internal inguinal hiatus and underlay patch is placed in preperitoneal space. In original technique, the spermatic cord is located close to the connector and it can be compressed above through the internal inguinal hiatus, so the space for the spermatic cord is created laterally through the narrowest side of onlay mesh. Onlay mesh has to cover the space between internal oblique's muscle and inguinal ligament [6, 12].

"Plugged in" component has been accused of the late complications in an indirect hernia′s repair with this technique in some papers [8, 9].

The aim of this paper was to explain in details the original modification of bilayer patch device insertion in non-plugged fashion and 11-year clinical experience with this technique, as well as early, midterm and late results for the male indirect hernia repair in the tertiary surgical institution not specialized for hernia surgery.

Methods

This retrospective study included 96 male patients operated due to indirect primary or recurrent inguinal hernias between November 2004 and December 2014. Among them, 7 patients had bilateral inguinal hernias, in total numbers of 103 indirect hernias. All the operations were done by one general surgeon in the tertiary surgical institution specialized for thoracic and esophageal surgery. All the patients received preoperatively one dose of antibiotics. All hernioplasty procedures were done under general anesthesia. In all operations, an extended PHS mesh was used with non-plugged insertion technique with bilayer patch device (PHS®, Ethicon). All patients were discharged from the hospital on first postoperative day.

All data were taken from the medical records and operative protocols. For late complications and quality of life, a subject-related questionnaire was made and fulfilled during the telephone call. The focus was intolerance of patch, chronic pain, recurrent hernias or other complications on intraperitoneal organs.

Operative technique

With the skin incision of about 4 cm above the inguinal canal, the fascia of abdominal external oblique muscle was opened from the spermatic cord to internal inguinal ring. After the identification of spermatic cord structures and nerves of inguinal region, the hernia sac was dissected, pulling back into preperitoneal space without resection. Original modification of the technique did not include a preperitoneal space for underlay patch through internal ring, but medially from the inferior epigastric blood vessels in Hesselbach's triangle, by making a small incision of about 15 mm on transversal fascia, parallelly with the inferior epigastric blood vessels (Figure 1a), where the PHS connector were placed.

In preperitoneal space (space of Borgos), a place for the underlay patch was made with moist gauze covering the internal hernia hiatus from the back side (Figure 1b). Lateral and lower end of the underlay patch was set above the femoral blood vessels. In this fashion, the connector of the bilayer patch device PHS was positioned on the spot where a direct hernia could hypothetically appear. The underlay patch covered internal hernia hiatus and all the other weak points of preperitoneal space of inguinofemoral region.

The small Y incision was made on the longest diameter of the onlay patch on about 15 mm from the connector and the spermatic cord was placed through it (Figure 1c). Above the spermatic cord, the transected part of the onlay mesh was approximated with Prolene 2.0 stitches except for the Y incision.

The onlay mesh has to cover the pubic tubercle for 2 cm and to be sutured with Prolene 2.0 for the inguinal ligament, and for the internal oblique muscle, but avoiding iliohypogastric nerve. The upper part of the onlay mesh was pushed beneath the external oblique muscle aponeurosis and

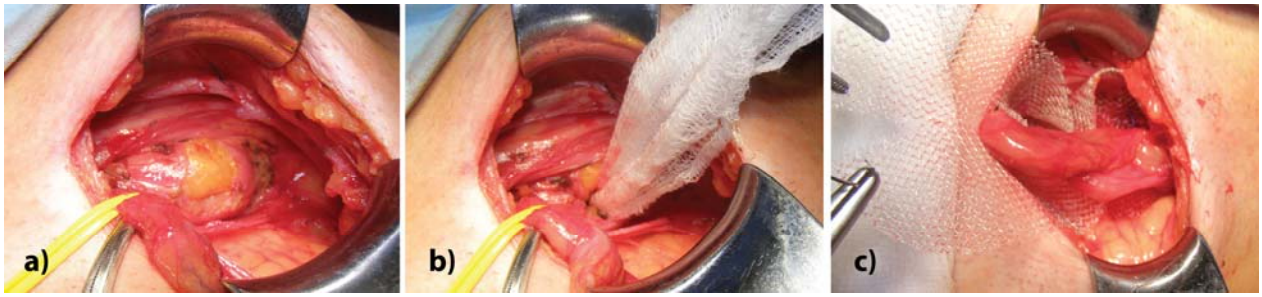

Fig. 1 – Original non plug technique of bilayer patch device insertion for reparing of indirect inquinal hernia: a) medial approach from the inferior epigastric blood vessels in Hesselbach's triangle through small incision of about 15 mm; b) application of underlay patch with moist gauze in preperitoneal space (space of Borgos); c) the small Y incision on the longest diameter on the onlay patch on about 15 mm from the connector and the spermatic cord placed through it.

sutured with usually six Prolene 2.0 stitches. The operation was finished with the skin sutured.

Results

A total of 96 male patients with 103 indirect inguinal hernias were operated by non-plugged insertion of bilayer patch device technique. The mean age of patients was 59.26 (range, 27–91 years). In 49 (51%) patients the hernia was on the right side, in 40 (41.7%) patients on the left side, while 7 (7.3%) had bilateral hernias. The primary indirect inguinal hernia was seen in 96 (93.2%) and the recurrent indirect inquinal hernia in 7 (6.8%) patients.

In patients with bilateral indirect hernias we did the same procedure on both sides using the same original modification. There were 7 (6.8%) patients with recurrent indirect hernias, where operation lasted for a longer time and where the identification of inguinal anatomy was more difficult, but the same procedure of mesh insertion was done.

Figure 2 shows data about a number of operated patients *per* year. Our institution is a low-volume center for hernioplasty with 3 to 17 operations *per* year.

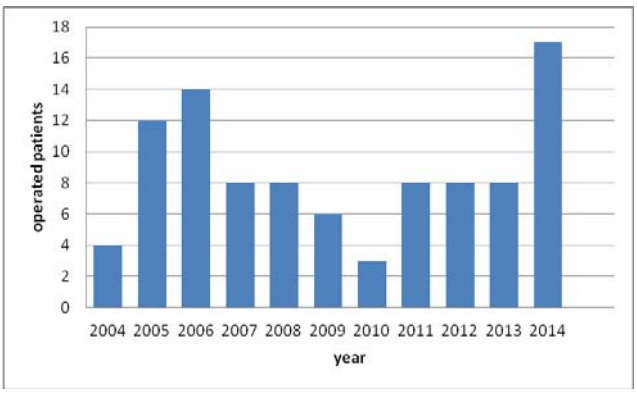

Fig. 2 – Number of patients operated because of the hernia *per* year.

Among 96 operated patients, 86 (89.6%) patients answered all questions and 10 (10.4%) patients died in the meantime, so the information was given by their families.

After the modification of non-plugged PHS mesh insertion, there were 3 relating problems regarding the procedure, of which two (1.94%) lesions of epigastric vessels and one (0.97%) accidental damage of hernias bag. There were 3 patients with early postoperative complications: two (1.94%) patients with seromas and one (0.97%) patient with scrotal wall ecchymosis. Late postoperative complications appeared in 3 patients, chronic pain in one patient, funiculocele in other patient and one recurrence hernia (0.97%).

The patient with recurrence of a hernia had complicated pelvic fracture due to the traffic accident a year before the primary operation. He appeared 4 years after the PHS operation, with a contralateral new inguinal hernia. That new hernia was operated with the same PHS modification technique, while the recurrent hernia was solved with additional onlay patch, without taking out the previously inserted PHS mesh.

The patient with the funiculocele had the other operation in the other hospital, and the PHS mesh was taken out. However, the previous operator and the other hernia surgeon expert claimed that it was not the relapse.

Among all 86 operated patients, 84 (97.68%) were found to be satisfied with the result of the operation, 1 (1.16%) patient was unsatisfied with the operation.

The unsatisfied patient in this study reported his disappointment with the laparoscopic appendectomy which was performed in the same act.

The follow-up period after the surgery in surveyed patients is shown in Figure 3.

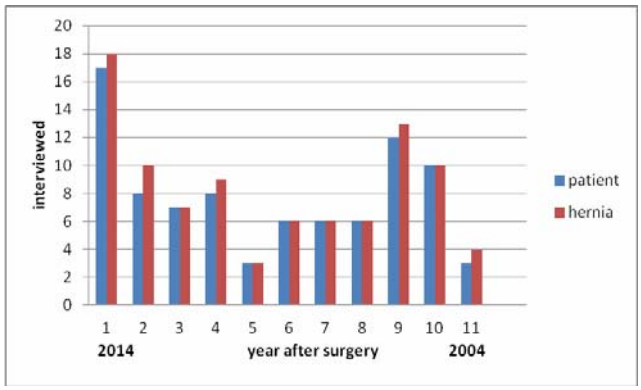

Fig. 3 – The follow-up period after surgery.

The follow-up period of operated patients ranged from 1 to 11 years, with the mean interval of 5 years and 6 months. Up to 4 years of follow-up there were a total of 40 (46. 5%) patients and 44 (47.8%) indirect inguinal hernias.

Over 4 years of follow-up, there were 46 (53. 5%) patients and 48 (52. 2%) indirect hernias.

Discussion

In the original description about the usage of PHS mesh for the indirect hernias, the connector is set as the plug in the internal inguinal ring, and the opening for the spermatic cord is set centrally or laterally, but in both cases, the spermatic cord is in the contact with the connector [12]. In our modification, we decided to place the connector medially of inferior epigastric blood vessels, avoiding the pressure on the spermatic cord providing underlay mesh much easier to place into preperitoneal space. In this position, the connector is not in the contact with the intraperitoneal organs, but only underlay mesh. The onlay mesh is set identically as in the Lichtenstein technique [3]. In this fashion, the onlay mesh sutured with the six Prolene 2.0 stitches prevents the migration of the underlay mesh. With this position of the connector and the both meshes, a double protection of internal inguinal hiatus is achieved and the spermatic cord is not compressed by the connector in the internal inguinal ring.

Potentially weak spot on transversal fascia is protected with the connector and the appearance of the recurrent hernias is prevented.

Positioning of PHS meshes in this fashion is very important in cases of large indirect inguinal hernias or with accidental lesions of hernia sac (one patient in this study), due to medial position of the connector, so there is no risk of hernia sac re-damaging while creating the space for the underlay through the internal inguinal ring.

Using this method, we did not have a complication like the migration of the mesh or damaging of intraperitoneal organs, like it is described in some recent papers about classical PHS technique [9, 10]. We had only one recurrent hernia, four years after the operation, with the expansion of the internal inguinal ring over the underlay and onlay patches. This problem was solved with the additional onlay patch with no need of removing the PHS mesh which was in the primary position. Funiculocele in one patient was solved by taking out the PHS mesh in the other hospital, without any information how the hernia was resolved. Our results with this modification show low rate of the recurrences and late complications. Only one patient had chronic pain, reported also by other authors [13] which lasted up to 6 months.

It is well known that the beginning and the development of the indirect inguinal hernias are connected with the pain in the inguinal region. The pain is caused by nerves of the ilioinguinal region, especially with the genital branch of the genitofemoral nerve, ilioinguinal nerve, sympathetic nerves (testicular plexus) which are included in spermatic cord [14]. The pain during the developing of indirect inguinal hernia is not proportional to the size of the internal inguinal ring defect. The classification of indirect inguinal ring size is more descriptive than the need for the certain type of hernioplasty. Even in recurrent indirect inguinal hernias the pain is very common [7].

We believe that the low incidence of the chronic pain in this modification technique is the result of less manipulation in the region of internal inguinal ring [15], as well as no pressure on the spermatic cord by the connector, with making the Y incision on the onlay patch [16]. The low incidence of the pain is established with the usage of Prolene stitches for onlay mesh fixation and obligatory identification and avoidance of the nerves with the stitches.

Although the telephone survey was very considered of the postoperative pain, as well as for the general satisfaction with the operation, the majority of the patients declared they would have the same operative procedure if necessary [17]. One unsatisfied patient reported his disappointment with the laparoscopic appendectomy which was performed in the same act and not with the inguinal hernia repaired. With obtained data on the satisfaction and the low incidence of postoperative pain, we conclude that this modified technique is very acceptable for all the patients who underwent the operation.

The majority of the patients in this study were elderly patients, unlike some other studies [18], with some other comorbidities, so the antibiotic prophylaxis and general anesthesia were justified. The antibiotic prophylaxis was the reason why we did not have wound infections [19].

The absence of local or regional anesthesia in this study did not have any influence on postoperative pain. This technique could be done in local anesthesia, regarding new trends in herniology with shorter hospital stay, especially in hernia clinics [7].

Non-plugged PHS technique is safer and much easier to learn than some other open techniques, and especially laparoscopic techniques, which are more demanded with much longer learning curves [7, 20].

In this study, we have two lesions of inferior epigastric blood vessels, during the transversal fascia transaction, which were solved by ligations.

With this technique, we solved primary, bilateral and recurrent hernias and showed that this technique is applicable for all indirect inguinal hernias results, despite of some other studies in wich PHS for recurrent hernias were not used [18, 21].

Conclusion

Original modification of Prolene Hernia System® hernioplasty in non-plugged fashion is a safe modification of the original technique for solving the primary and recurrent indirect inguinal hernias, regarding our 11-year experience in tertiary surgical centre. Low incidence of the recurrence and chronic pain many years after the operation justifies this procedure even in hospitals not specialized for the hernioplasty. The major advantage of this modification is the absence of the connector in the internal inguinal ring and its pressure on the spermatic cord and nerves, as well as its position which does not interfere with intraperitoneal organs.

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CASE REPORTS

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The outcome of pregnancy in a kidney transplant patient: a case report and review of the literature

Ishod trudnoće kod bolesnice sa transplantiranim bubregom: prikaz slučaja i pregled literature

Andreja Glišić*, Nevena Divac†, Miroslava Gojnić Dugalić*, Biljana Kastratović Kotlica*, Neven Vavić‡, Nataša Cerovac§, Milica Prostran†

University of Belgrade, Faculty of Medicine, Clinical Center of Serbia, *Clinic for Gynecology and Obstetrics, †Institute of Pharmacology, Clinical Pharmacology and Toxicology, Belgrade, Serbia; Military Medical Academy, ‡Center for Transplantation of Solid Organs, Belgrade, Serbia; §Clinic for Neurology and Psychiatry for Children and Youth, Belgrade, Serbia

Abstract

Introduction. The possibility of a term pregnancy with favorable maternal and neonatal outcome is one of the greatest advances in kidney transplantation, though concerns still exist regarding the safety of the mother, fetus, and graft. The use of immunosuppressive medications during pregnancy is related to possible fetal adverse effects. **Case report.** We report a course of a pregnancy in a patient with a kidney transplant. The patient was treated with immunosuppressive therapy (tacrolimus, azathioprine, and prednisolone) during the pregnancy. The outcome of the pregnancy was without maternal and neonatal complications. Serum creatinine levels were stable and no acute organ rejection occurred during pregnancy. Significant elevation of the D-dimer and coagulant factors II, VII, IX and X were noticed during the third trimester. This could be partially attributed to azathioprine, which was a part of the immunosuppressive regimen. On the other hand, there were no radiological or clinical signs of thromboembolism, but low-molecular-weight heparin prophylaxis was immediately initiated. Cesarean section was performed at the 39th gestational week and a healthy female infant was delivered with a birth weight of 3,150 g and Apgar score 9. **Conclusion.** Pregnancies of kidney transplant recipients are high-risk and require a multidisciplinary approach. Careful clinical follow-up is a prerequisite for favorable outcome.

Key words:
kidney transplantation; pregnancy; fetal development; tacrolimus; azathioprine; prednisolone.

Apstrakt

Uvod. Mogućnost uspešne trudnoće kod žena sa presađenim bubregom smatra se jednim od najvećih uspeha ove vrste lečenja, ali nosi sa sobom i određene probleme u vezi sa bezbednošću majke, fetusa i presađenog organa. Upotreba imunosupresivne terapije tokom trudnoće povezana je sa mnogim neželjenim efektima. **Prikaz slučaja.** Prikazali smo bolesnicu sa presađenim bubregom koja je tokom trudnoće primala imunosupresivnu terapiju (takrolimus, azatioprin i prednizolon). Ishod trudnoće je bio uspešan i po majku i po novorođenče. Nivo serumskog kreatinina majke bio je stabilan sve vreme trudnoće i nije doslo do akutnog odbacivanja presađenog organa. Tokom trećeg trimestra došlo je do neuobičajenog porasta D-dimera i faktora koagulacije II, VII, IX i X, što je retka, ali moguća komplikacija primene azatioprina. Nije bilo kliničkih niti radioloških znakova tromboembolizma, ali je niskomolekularni heparin uveden profilaktički. Trudnoća je završena u 39-oj nedelji gestacije planiranim carskim rezom i rođeno je zdravo žensko dete porođajne težine 3 150 g, ocenjeno Apgar skorom 9. **Zaključak.** Trudnoća kod bolesnice sa presađenim bubregom smatra se visoko rizičnom i zahteva pažljivo planiranje i praćenje.

Ključne reči:
transplantacija bubrega; trudnoća; trudnoća, razvoj fetusa; takrolimus; azatioprin; prednizolon.

Correspondence to: Nevena Divac, University of Belgrade, Faculty of Medicine, Institute of Pharmacology, Clinical Pharmacology and Toxicology, Dr Subotića 1, 11 000 Belgrade, Serbia. Phone: +381 11 616 1746. E-mail: ndivac@med.bg.ac.rs

Introduction

The impaired reproductive function is one of the detrimental consequences of end-stage renal disease (ESRD). The restoration of pituitary-ovarian function and fertility in female kidney transplant recipients is one of the greatest achievements of modern transplantation. The first report of a pregnancy in a kidney transplant recipient was published in 1958. After that, due to the improvement of immunosuppressive therapy and modern perinatal care, series of pregnancies with favorable outcomes have been reported worldwide [1–3]. The pregnancy in a kidney transplant patient is complicated due to previous major abdominal surgery, pre-existing comorbidities such as diabetes, hypertension etc, and the adverse effects of immunosuppressive treatment [3]. Pregnancy complications associated with chronic renal failure, such as hypertension, proteinuria, preeclampsia and preterm delivery, are still possible in kidney transplant recipients. These pregnancies are always considered as high-risk and deserve careful planning and clinical follow-up from the very beginning.

Immunosuppressive medications prevent kidney rejection but also carry significant risks. During pregnancy, these medications are related to many serious fetal adverse effects. Therefore, it is important to carefully evaluate the safety and efficacy of immunosuppressive medication during pregnancy and to provide adequate clinical prenatal care in order to reduce the risk of graft rejection on one side, and unwanted effects on fetal development on the other side.

The present study is a review of a new case of a pregnancy in a kidney transplant recipient treated with tacrolimus, azathioprine, and prednisolone, with successful maternal and fetal outcome. There have been very few reports of such pregnancies in Serbia.

Case report

The patient was a 26 year old pregnant woman with the history of kidney disease. She was diagnosed with ESRD eight years ago. Hemodialysis was initiated three years later. After eight months of dialysis, she was transplanted a living related donor kidney at the Military Medical Academy in Belgrade. The procedure was performed successfully and the patient was discharged on the 23rd post-transplantation day with serum creatinine level of 161 μmol/L. The clinical protocol included immunosuppressive treatment comprising tacrolimus, prednisolone and mycophenolate mofetil. Antihypertensive therapy was prescribed (metoprolol and nifedipine) and on discharge, her blood pressure was within recommended values for kidney transplant patients [4]. The patient was followed-up at regular intervals and the doses of immunosuppressive drugs were gradually tapered to maintenance doses.

Three years later, the patients spontaneously conceived. She was referred to the Clinic of Gynaecology and Obstetrics, Clinical Center of Serbia at 5 weeks gestation. Since pregnancies in transplant patients require a multidisciplinary approach, a close cooperation with nephrologists from the Military Medical Academy was established. Her immunosuppressive regimen was changed to tacrolimus 3.5 mg in the morning and 3.5 mg in the evening, prednisolone 7.5 mg/day and azathioprine 50 mg/day. Mycophenolate mofetil was stopped. Beta blocker was also withdrawn, verapamil was introduced and methyldopa continued. The patient was followed on monthly basis throughout the entire pregnancy. Ultrasonographic examinations showed normal fetal growth and amniotic fluid volume was in the normal range. The laboratory values of serum glucose, electrolytes, serum urea and creatinine, proteins in 24-hour urine collection and protein to creatinine ratio were regularly controlled. The serum creatinine levels throughout the course of pregnancy were stable between 79–110 μmol/L and the day before the cesarean section it was 116 μmol/L. The urine protein to creatinine ratio was stable in range 0.38–0.41. Blood pressure was well controlled with methyldopa and verapamil.

The patient's coagulation status was also closely monitored. During her third trimester, D-dimer and coagulation factors (II, VII, VIII, IX and X) were elevated. D-dimer levels were between 3.13 to 4.38 mg/L. The left-sided leg edema also developed. The lower-extremity venous duplex ultrasound was performed, and she was found to have no signs of deep venous thrombosis (DVT) or superficial thrombophlebitis. However, it was decided that anticoagulant prophylaxis should be started and low molecular weight heparin (fraxiparine) was initiated. Asymptomatic bacteriuria was treated by ceftriaxone 2g /daily i.m. during ten days.

We performed planned caesarean delivery because the transplant kidney was located very low in the pelvis and probably could obstruct the labor.

A healthy female child was born at term by caesarean section. Birth weight was 3,150 g, Apgar score 9 at first minute of life and no congenital malformations were identified. Neurological and clinical status of the baby at birth was normal. During first months of life, the baby achieved age-appropriate developmental milestones. During the early postoperative period, the patient's serum creatinine level showed transient elevation up to 218 μmol/L but reverted to baseline values. We performed intravenous hydration (3,000 mL of fluid *per* a day) during 7 days. There were no signs of acute kidney rejection. The patient was advised against breastfeeding and bromocriptine was administered to terminate lactation.

Discussion

Pregnancy in kidney transplant recipients is burdened with risks and requires careful follow-up. These risks include impaired renal function, graft rejection, spontaneous abortion, preterm delivery, low birth weight and fetal growth retardation [5]. Great concern in such pregnancy is the potential adverse effect of immunosuppressant drugs to the fetus.

The recommended immunosuppressive regimen in pregnant kidney transplant recipients usually comprises a calcineurin inhibitor, corticosteroid and azathioprine [6]. Mycophenolate mofetil and mammalian target of rapamycin inhibitors (mTOR) are associated with increased incidence of spontaneous abortions and congenital malformations in fetuses [7, 8].

Therefore, these two drugs are not recommended during pregnancy and should be discontinued before conception, or, if the pregnancy was not planned, immediately after [7, 9].

Tacrolimus belongs to a class of calcineurin inhibitors, together with cyclosporine. It falls into pregnancy category C by the U.S. Food and Drug Administration (FDA). Tacrolimus is preferred over cyclosporine due to better efficacy regarding graft function and graft survival [10]. Additionally, compared to tacrolimus, cyclosporine in pregnant women is associated with higher incidence of hypertension [11]. Fetal levels of cyclosporine are similar to maternal levels, whereas the umbilical cord concentration of tacrolimus is 19% of maternal unbound plasma concentration. The lower fetal concentration of tacrolimus is attributed to the active efflux of tacrolimus from the fetus toward the mother by placental P-glycoprotein activity [12, 13]. Tacrolimus has been reported to cause hyperkalemia and kidney impairment in neonates, as well as premature delivery and preeclampsia [13, 14]. Teratogenicity in children whose mothers were treated with tacrolimus during pregnancy is not significantly increased compared to general population [15, 16].

Prednisone has been widely used in pregnancy for indications other than solid organ transplantations. It is listed as pregnancy category C by the FDA [17]. Fully developed placenta partially protects the fetus from prednisone exposure by its metabolic activity (enzyme 11-beta-hydroxylase) [18]. The maternal to cord blood ratio of prednisone is 8 : 1 to 10 : 1 [19]. However, the risk of neonatal adrenal insufficiency cannot be ruled out even with low doses of prednisone and careful post-natal monitoring is required.

Azathioprine and its active metabolite, 6-mercaptopurine, are purine analogues which interfere with the synthesis of adenine and guanine ribonucleosides [20]. Azathioprine has been labeled as pregnancy category D by FDA [21]. It crosses the placenta. However, the fetus cannot metabolize it to its active metabolite 6-mercaptopurine due to lack of liver enzymes [22]. Azathioprine can cause hematological disturbances and immunodeficiency in the fetus [11, 23]. The frequency of congenital abnormalities in infants of kidney transplant recipient mothers was between 0.0–11.8% in different case-series studies [20]. The prospective, controlled cohort study by Goldstein et al. [24] which compared pregnancy outcome in women exposed to azathioprine (n = 189) to non-exposed controls (n = 230) showed no difference in terms of teratogenicity. However, the azathioprine exposed group had a higher incidence of low birth weight and prematurity.

Additionally, increased thrombotic complications in kidney transplant recipients have been associated with the use of azathioprine [25]. One of the postulated mechanisms for this is the stimulation the synthesis of coagulation factors induced by azathioprine, especially factors II and X [26]. It could partially explain the elevation of coagulation factors in our patient.

In women with a renal transplant, the cesarean delivery rate approaches 50% [27]. We performed planned caesarean delivery. Transplant kidney was located very low in pelvis near to bladder and a low segment of uterus. We made a medial abdominal incision and medial uterine incision to protect the transplanted kidney. Probably, the transplanted kidney could obstruct the labor.

Our case emphasizes the significance of a multidisciplinary approach to a pregnancy in a kidney transplant recipient. Due to excellent coordination between gynecologists, nephrologists, pediatricians and clinical pharmacologists, our patient had a successful pregnancy with favorable maternal, fetal and graft outcome. The graft function remained stable as well as blood pressure and other clinically significant parameters in the mother. The pregnancy was a full-term without postpartum complications. The baby was born without congenital malformations and with normal neurological status. Undoubtedly, the use of immunosuppressants in pregnancy requires careful planning and monitoring in order to minimize risks and enable favorable outcome.

Conclusion

Pregnancies of kidney transplant recipients are high-risk and require a multidisciplinary approach. Careful clinical follow-up is a prerequisite for a favorable outcome.

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CASE REPORT

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Cataplexy in a patient treated for prolactinoma: Case report

Katapleksija kod bolesnice sa prolaktinomom

Marina Nikolić-Djurović*†, Alberto M. Pereira‡, Zvezdana Jemuović*, Dragan Pavlović†, Dragana Janković*, Milan Petakov*†, Milorad Čivčić*, Olga Vasović§, Svetozar Damjanović*†

Clinical Centre of Serbia, *Clinic of Endocrinology, Diabetes and Metabolic Diseases, Belgrade, Serbia; University of Belgrade, †Faculty of Medicine, Belgrade, Serbia; Leiden University Medical Center, Department of Medicine, ‡Division of Endocrinology, Leiden, The Netherlands; §Institute for Gerontology and Palliative Care, Belgrade, Belgrade, Serbia

Abstract

Introduction. Isolated cataplexy, without the presence of narcolepsy, is a relatively rare condition, and can be regarded as attacks of motor inhibition with loss of muscle tone and areflexia. The diagnosis of cataplexy relies on the clinical presentation and medical history and it is rarely confirmed by video-polygraph. We here described a female patient treated for prolactinoma who developed isolated cataplexy. **Case report.** A 53-year-old female treated with bromocriptine for a macroprolactinoma presented with sudden episodes of weakness and toneless legs leading to falls and injuries on several occasions. Cardiovascular evaluation was completely normal. Psychiatric evaluation showed no psychotic phenomenology or suicidal ideas. Pituitary imaging showed empty sella with a remnant sellar mass with infra- and parasellar extension. Neurological examination revealed mild obstructive sleep hypopnea/apnea. Electroencephalographic monitoring during sleep and awakening did not show appearance of epi potentials. HLA haplotyping was positive for HLA-DR3,16;DR51;DQ1 allele, confirming a diagnosis of isolated cataplexy. Treatment included tricyclic antidepressants and reduction of bromocriptine dosage with resolution of cataplexy. **Conclusion.** We reported the first case of isolated cataplexy most probably associated with dopamine agonist treatment for prolactinoma.

Key words:
prolactinoma; pituitary neoplasms; cataplexy; comorbidity; diagnosis; magnetic resonance imaging; genetics, medical; treatment outcome.

Apstrakt

Uvod. Izolovana katapleksija bez prisustva narkolepsije relativno je retko stanje i može se smatrati napadom motorne inhibicije sa gubitkom mišićnog tonusa i arefleksijom. Dijagnoza katapleksije se oslanja na kliničku prezentaciju i medicinsku dokumentaciju i retko se potvrđuje video-poligrafom. Opisali smo bolesnicu tretiranu zbog prolaktinoma koja je razvila izolovanu katapleksiju. **Prikaz bolesnika.** Kod bolesnice stare 53 godine lečene bromokriptinom zbog makroprolaktinoma ispoljile su se iznenadne, nagle epizode slabosti i nedostatak mišićne snage u nogama, koji su doveli do padova i povreda u nekoliko navrata. Kardiovaskularna evaluacija bila je potpuno normalna. Psihijatrijska procena je pokazala da bolesnica nije imala psihotičnu fenomenologiju ni samoubilačke ideje. Snimci hipofize pokazali su prazno tursko sedlo (*empty sella*) sa ostatkom selarne mase sa infra i paraselarnim širenjem. Neurološki pregled pokazao je blagu opstruktivnu hipopneju/apneju u snu. Elektroencefalogramski nadzor u toku sna i buđenja nije pokazao epi potencijale. HLA halotipizacija bila je pozitivna za HLA-DR3,16, DR51, DK1 alele, potvrđujući dijagnozu izolovane katapleksije. Terapija sa uključenjem tricikličnih antidepresiva i smanjenjem doze bramokriptina dovela je do rezolucije katapleksije. **Zaključak.** Prikazan je prvi slučaj izolovane katapleksije koji je najverovatnije povezan sa terapijom prolaktinoma dopaminskim agonistom.

Ključne reči:
prolaktinom; hipofiza, neoplazme; katapleksija; komorbiditet; dijagnoza; magnetna rezonanca, snimanje; genetika, medicinska; lečenje, ishod.

Correspondence to: Marina Nikolić Djurović, Clinical Centre of Serbia, Clinic of Endocrinology, Diabetes and Diseases of Metabolism, Dr Subotica 13, 11 000 Belgrade, Serbia. E-mail: prof.marina.djurovic@gmail.com

Introduction

Narcolepsy, cataplexy, and emotions constitute a special triad. Cataplexy and sleep paralysis can be regarded as attacks of motor inhibition with loss of muscle tone and areflexia. The diagnosis of cataplexy is thus based on the medical history and on clinical observations and it is rarely confirmed by video-polygraph [1]. Cataplexy is defined as a transition phase from wakefulness directly into an atonic state as seen in rapid eye movement (REM) sleep, that is triggered by emotional stimuli. Supportive therapy includes medication with REM suppressing properties.

Isolated cataplexy should always be considered in the differential diagnosis of a patient with drop attacks. Drop attacks are characterized by spontaneous falls followed by quick recovery [2] as is observed in patients with syncope, associated with transient loss of consciousness. In some patients falls may also result from seizures.

Here, we describe for the first time, a patient with macroprolactinoma and drop attacks that was diagnosed as isolated cataplexy, that completely resolved with antidepressant treatment and with reduction of the dose of bromocriptine.

Case report

A 53-year-old female patient presented with sudden attacks characterized by complete loss of muscle tone with subsequently collaps and injuring herself on several occasions. Occasionally, the attacks were precipitated and triggered by emotional shock such as surprise, excitement or laughing. These events occurred without visual, olfactory or sensory auras, and without motor signs of epilepsy symptoms. She had been treated for macroprolactionoma with dopamine agonists (bromocriptine) for 2 years in a daily dosage of 15 mg. Prolactin levels at the time of diagnosis were high (6,498 mU/L; reference range 151.5–757.5 mU/L). Pituitary imaging showed empty sella configuration but with expansive parasellar mass and infrasellar propagation (Figure 1). While on treatment, prolactin was only mildly elevated (1,102/998/776 mU/L).

The medical history included acute myocardial infarction, hypertension, probable cerebrovascular accident and urinary incontinence after total hysterectomy (performed 30 years ago for uterus myomatosus). In addition, she had suffered from depression. Her family history was negative for any psychiatric or neurologic illnesses, including narcolepsy and cataplexy.

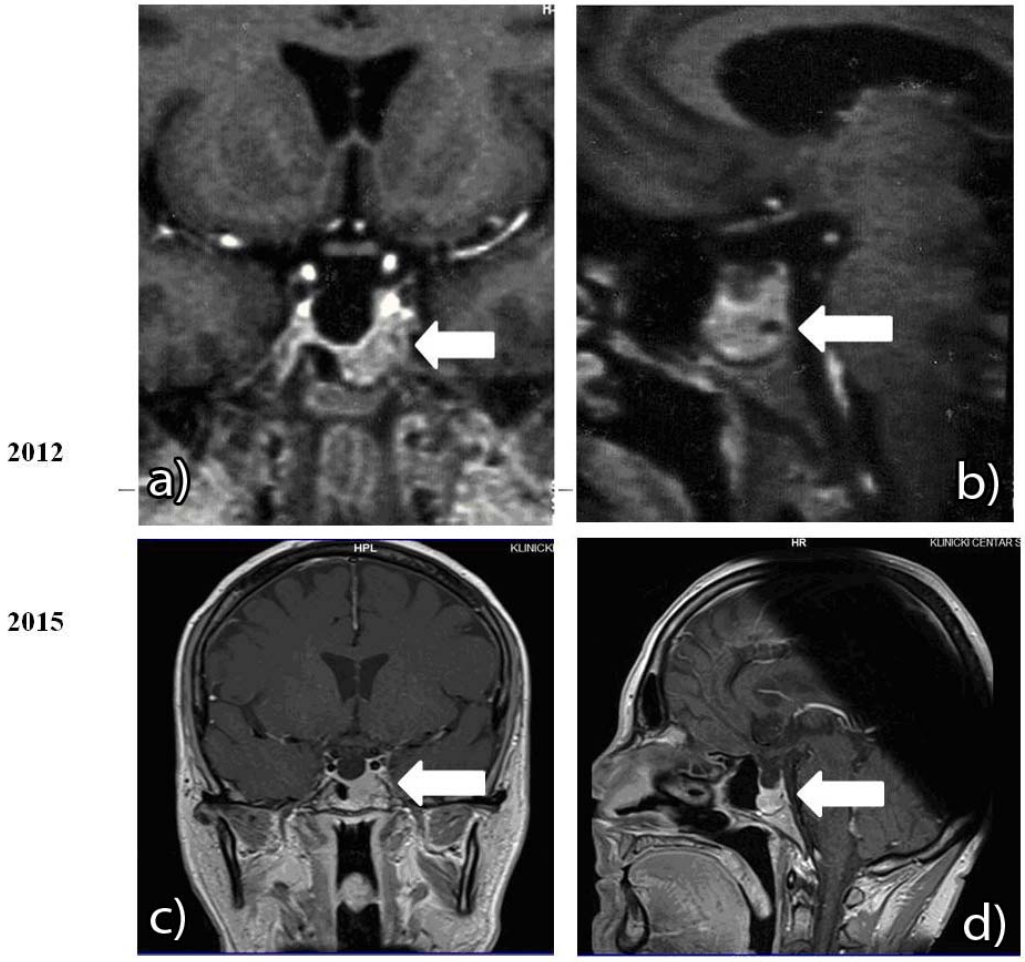

Fig. 1 – Magnetic resonance imaging (MRI) of sellar region shows empty sella configuration but with expansive parasellar mass and infrasellar propagation at the time of diagnosis [a – coronal (frontal), b – sagittal view] and with a slight regression of the changes 3 years later (c – frontal, d – sagittal view).

At the admission, blood pressure and glycaemia levels were normal. In addition, an extensive evaluation of blood count, biochemistry and electrocardiograph (ECG) were all normal. Basal hormonal analyses were within normal range except for elevated prolactin levels.

During hospitalization, an attack was observed, characterized by collaps as a result of a sudden loss of tone in both lower extremities, resulting in a fall on the floor without loosing consciousness. Blood pressure and glucose were normal without vegetative symptoms like sweating and bradycardia. Because a diagnosis of syncope was suspected she was referred for cardiological evaluation, which turned out to be completely normal (including ECG, 24-hour arterial blood pressure monitoring and 24-hour ECG monitoring). Due to prolonged mild depression which became symptomatic few months after the first attack, she was referred to the psychiatrist who concluded that she was not suicidal and advised an expectative approach and treatment with tricyclic anti-depressants. There was no history of insomnia or parasomnia.

Neurological examination confirmed attacks with sudden loss of muscle tone that could be regarded as cataplexy. Additional investigations like electroencephalography (EEG) and carotid ultrasonography were all normal. HLA standardization haplotyping was confirmatory for cataplexy (HLA-DR3,16; DR 51; DQ 1) but negative for narcolepsy (HLA-DQB1 and DQA1 negative). Since cataplexy is often associated with narcolepsy, polysomnography (PSG) was also performed revealing mild obstructive hypopnea/apnea during sleep with dissociation of the architecture and continuity of sleep. The described phenomenology was responsible for temporary patient's somnolence. Also, EEG monitoring during sleep and awakening did not reveal any epi potentials.

Treatment was initiated with tricyclic antidepressants that rapidly reduced the attacks which after few weeks completely disappeared. Thereafter, the dose of bromocriptine was also reduced. During follow-up, she has been free of any attacks since 2012.

Discussion

To the best of our knowledge, this is the first report of isolated cataplexy in a patient with prolactinoma. This is intriguing because both dopamine and serotonin are proposed to play a key role in the pathophysiology of narcolepsy and cataplexy [3], and dopamine agonists and serotonin-reuptake inhibitors are frequently prescribed.

Cataplexy and sleep paralysis can be regarded as attacks of motor inhibition with loss of muscle tone and areflexia, triggered by strong emotions and typically occurring while laughing or joking [1]. Cataplexy, originating from the Greek word *kataplexis* (down-stroke), is considered the main symptom of the narcolepsy syndrome according to the International Classification of Sleep Disorders-2 [4]. Isolated cataplexy without narcolepsy is associated with specific genetic predisposition [5].

Cataplexy is characterized by attacks that may last from a few seconds to several minutes that can be prolonged by emotional stimuli (e.g. by reiteration of the trigger, interventions of helpers) [2, 6]. Long-lasting attacks may evolve into a REM sleep episode, but in rare cases, attacks of cataplexy may occur in tightly packed clusters or be almost continuous, a condition known as "status cataplecticus". This condition may appear at the onset of the disease or may be provoked by antidepressant withdrawal.

Drop attacks are defined as spontaneous falls followed by quick recovery, consequently, syncope and seizures should be excluded. The patient should firstly be clinically screened for cardiovascular causes like orthostatic hypotension, aortic stenosis, and arrhythmias. Since seizures might be the manifestation of epilepsy, brain imaging and EEG should also be performed. In the majority of patients recurrent falls occur without affecting consciousness, and no underlying cause of the drop attacks is found [2, 7].

Cataplexy and sleep paralysis occur only in relation to REM sleep periods, and it may be that they derive from the *nucleus locus coeruleus*. Although cataplexy can result in a complete and often dramatic loss of postural muscle tone with complete paralysis and collapse, the loss of tone in the majority of cases is partial affecting only some muscles [8]. In accordance, the attacks in our patient were provoked by emotional stimuli, such as laughing [4].

The treatment of cataplexy includes norepinephrine and serotonin reuptake inhibitors (tricyclic antidepressants such as amitriptyline) or agents that stimulate the presynaptic release of norepinephrine (amphetamines). Fluoxetine and venlafaxine have also been given to the patients. Sodium oxybutyrate, the sodium salt of γ- hydroxybutyrate (GHB) and a metabolite of gamma amino butyric acid (GABA), was approved in 2002 by the Food and Drug Adminisstration (FDA) for special treatment of cataplexy in patients with narcolepsy. Reduction of cataplectic attacks may be explained with binding specifically to $GABA_B$ and GHB receptors, but the exact mechanisms still remain to be elucidated [2].

It was also shown that obstructive sleep apnea prevalence in patients with prolactinoma, which was found in a very mild form in our patient, is similar to that in the obese subjects and did not change after treatment [9].

Our patient was treated with the dopamine agonist bromocriptine for prolactinoma. It is tempting to speculate that the treatment with a dopamine agonist might have facilitated the manifestation of cataplexy in our patient. In accordance, in a canine model of narcolepsy, the systemic administration of D(2)- dopaminergic agonists increased the frequency of cataplexies [10]. Even more, Burgess et al. [11] showed that a D1 receptor mechanism can suppress sleep attacks and a D2 receptor mechanism can regulate cataplexy. In our patient, the attacks rapidly resolved with antidepressant treatment and she remained free of recurrence with additional reduction of the dose of bromocriptine with stable prolactin concentrations in the high-normal range.

Conclusion

Isolated cataplexy in patients treated for prolactinoma has not been previously reported. The observations in our patient

strengthen the observed effects of dopamine agonists on cataplexy in animal models, and merit further investigations on the role of dopamine agonists in genetically predisposed patients for cataplexy, especially in the presence of depression.

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CASE REPORT

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Flail chest in a polytraumatized patient: management and treatment – case report

Zbrinjavanje politraumatizovanog bolesnika sa torakalnim kapkom

Bojan Milošević*†, Slobodan Milisavljević*†, Nikola Dončić*, Miloš Arsenijević*†, Stanko Mrvić*, Dragan Stojković*, Nebojša Marić‡, Marko Spasić*†

Clinical Center Kragujevac, *Clinic for General and Thoracic Surgery, Kragujevac, Serbia; University of Kragujevac, †Faculty of Medical Sciences, Kragujevac, Serbia; Military Medical Academy, ‡Clinic for Thoracic Surgery, Belgrade, Serbia

Abstract

Introduction. Management of a polytraumatized patient is a problem that requires a multidisciplinary approach, in order to optimise patient's outcome. The purpose of this study was to show the approach in the treatment of a patient with a severe life-threatening polytrauma, including a personalized healthcare approach with the positive outcome after the inadequate initial treatment. **Case report.** We presented a case of a young polytraumatized patient with trauma as a result of road traffic accident. The patient had chest, abdominal and right arm injuries. He was diagnosed of hepatic rupture with conquasation and retroperitoneal hematoma and the patient underwent liver tamponade. Chest trauma due to bilateral serial rib fracture with flail chest was treated by chest drainage. After the adequate multidisciplinary interventions for the patient, the patient was discharged. **Conclusion.** This case report is of great importance since it shows that severe polytraumatized patients with bad initial prognosis can successfully receive a life-saving treatment.

Key words:
multiple trauma; thoracic injuries; liver; humeral fractures; diagnostic techniques and procedures; suction; respiration, artificial.

Apstrakt

Uvod. Zbrinjavanje politraumatizovanog bolesnika je problem kome se mora pristupiti multidisciplinarno u cilju dobijanja najboljeg mogućeg ishoda. Cilj rada bio je da prikaže lečenje bolesnika sa životno ugrožavajućom politraumom, personalizovanim pristupom u lečenju sa uspešnim ishodom posle neadekvatnog inicijalnog lečenja. **Prikaz slučaja.** Prikazan je slučaj mladog politraumatizovanog bolesnika koji je povrede zadobio kao vozač motora. Dominantne povrede bile su grudnog koša, trbuha i desne ruke. Zbog rupture jetre sa konkvasacijom i retroperitonealnim hematomom učinjena je tamponada jetre. Povreda grudnog koša uzrokovana bilateralnom serijskom frakturom rebara i torakalnim kapkom zbrinuta je obostranom grudnom drenažom. Nakon adekvatnog multidisciplinarnog hirurškog lečenja bolesnik je otpušten na kućno lečenje. **Zaključak.** Značaj prikaza ovog slučaja je u tome što pokazuje da se i teška politrauma sa inicijalno visokim trauma skorom i lošom prognozom može brzo i adekvatno lečiti i za rezultat imati očuvanje života povređenog.

Ključne reči:
povrede, multiple; toraks, povrede; jetra, povrede; humerus, prelomi; dijagnostičke tehnike i procedure; aspiracija, mehanička; disanje, mehaničko.

Introduction

Management of a polytraumatized patient is a problem that requires a multidisciplinary approach, in order to optimise patient's outcome. It involves a cohesive group of individuals working together with polytraumatized patient, with minimal waste of time, from the moment of admission to a health facility to the moment of release. The important milestone in the implementation of a systematic and structured care for traumas dates back to 1878, when the first Advanced Trauma Life Support (ATLS) course was conducted [1–3]. The role of trauma surgical procedures is not to provide a definitive anatomic reconstruction but to provide a normal physiological function of damaged tissues and organs. The most common surgical procedures include hemostasis, decontamination of injured body cavities and the rapid closure

Correspondence to: Slobodan Milisavljević, Clinical Centre Kragujevac, Clinic for General and Thoracic Surgery, 34 000 Kragujevac, Serbia. E-mail: s.milisavljevic65@gmail.com

of surgical wounds [4–6]. Resuscitation and following surgical treatment may not be life-saving procedures in polytraumatized patients – bodily functions may fail to return to normal oneswhich results in death, permanent disability, scarring, pain, as well as in the difficulties that affect all spheres of life (physical, psychological, social, and financial). Therefore, we should focus on prevention of trauma and pay more attention to the resources directed to work on the prevention. The purpose of this study was to show the approach in the treatment of a patient with a severe life-threatening polytrauma, including a personalized healthcare approach with the positive outcome, after the inadequate initial treatment.

Case report

The male patient, age 20, was admitted to Clinical Center Kragujevac with the injuries he sustained in a traffic accident. On admission the patient was intubated, without spontaneous breathing, unconscious, hemodynamically unstable, with blood pressure 100/65 mm Hg, heart rate of 110/min, blood oxygen saturation (SpO_2) 99%. Arterial blood gas analysis (also done on admission) revealed the following results: partial pressure of oxygen (PaO2) 7.4 kPa; partial pressure of carbon dioxide (PaCO2) 10.7 kPa, Bicarbonate (HCO_3) 26.6 mmol/L; Glasgow coma score (GCS) was 7, Injury Severity Score (ISS) was 50. There were the clinical signs of flail chest. He was previously treated in the Regional Health Centre. Diagnosis was hepatic rupture with conquasation and retroperitoneal hematoma and the patient underwent liver tamponade with 6 abdominal compressions; operative wound was sutured without placing an abdominal drains. During the initial treatment the patient received 6 units of blood. The volume and the seriousness of the injury required a multi disciplinary treatment approach. Computed tomography (CT) scan of endocranium showed normal findings. A chest CT scan showed a serial broken ribs on both sides with massive right sided traumatic hemothorax, left sided traumatic pneumothorax and massive lung contusion on both sides (Figure 1). CT of the abdomen showed: liver morphology wiped out in the posterior parts, unclearly contoured with conquasated tissue (the rest of the liver was heterogeneous structure but a clear morphology); compresses made sufficient tamponade (Figure 2); rupture of the right kidney with the formation of retroperitoneal hematoma; a larger amount of hemorrhagic contents intraperitoneally. Orthopaedic clinical and radiographic examination showed fracture of the right humerus (Figure 3). During 60 minutes after the admission to the hospital the CT findings showed that the immediate surgical treatment was necessary. The patient received 4 blood units more in our hospital and there was risk of massive transfusion. Drainage of the right pleural space was performed, and it evacuated about 3,000 mL hemorrhagic content; drainage of the left pleural space evacuated the air and about 200 mL of blood (Figure 4). Relaparotomy was immediately performed and an irregular laceration of the right lobe of the liver with elements of conquasation and active bleeding from the laceration was identified. The patient underwent repeated liver tamponade

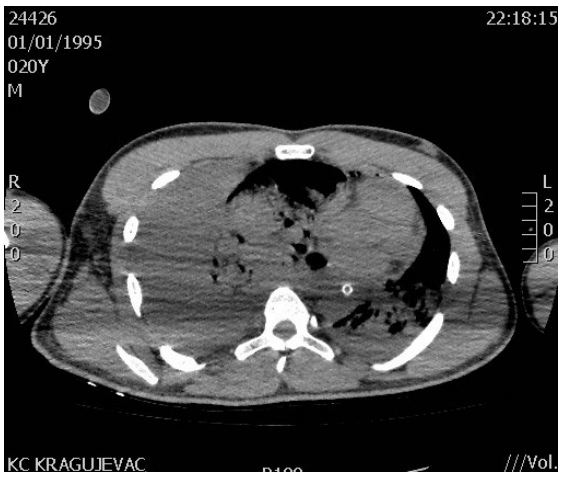

Fig. 1 – A chest computed tomography scan showed a serial broken ribs on both sides with massive right sided traumatic hemothorax, left sided traumatic pneumotorax and massive lung contusion on both sides.

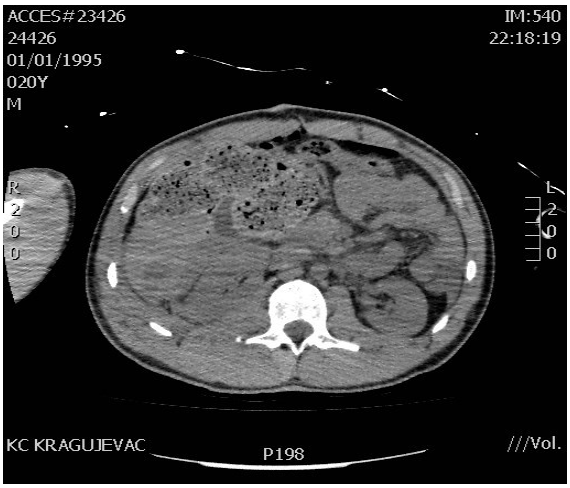

Fig. 2 – Computed tomography scan image of perihepatic packing after first operation.

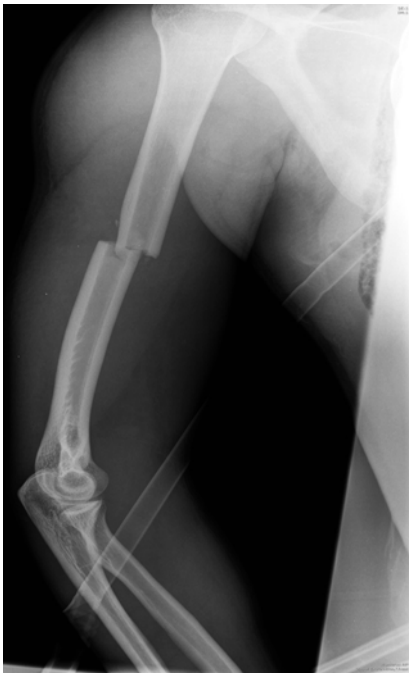

Fig. 3 – Fracture of the right humerus.

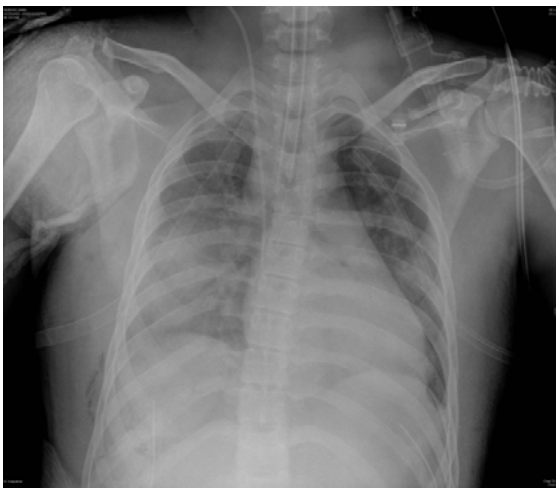

Fig. 4 – Drainage of both pleural cavities due to right sided hemothorax and left sided pneumothorax.

with sterile perforated bags with two abdominal compression, two subphrenically and two subhepatically placed, and it was managed to stop the bleeding from the liver. Due to the existence of retroperitoneal hematoma and CT findings of ruptured kidney, the urologist eliminated the need for surgical treatment of kidney infringement. The external fixation of the fracture of the right humerus was performed with the plaster splint by the orthopedists. After the liver tamponade hepatogram was aspartate aminotransferase (AST) 348 IUL/L (ref. range 0 – 40 IU/L), alanine aminotransferase (ALT) 408 IU/L (ref. range 0–40 IU/L), total bilirubin: 30.7 umol/L (ref. range 5,0–21 umol/L), direct bilirubin: 8.3 umol/L (ref. range 0,1–3,4 umol/L). The patient was connected to a breathing machine, a mechanical ventilation system, thus achieving an adequate gas exchange and an internal stabilization of the flail chest. Arterial blood gas analysis after the chest drainage revealed the following levels: PaO2 17. 2 kPa; PaCO2 5.7 kPa; HCO3 33.1 mmol/L. The patient received a greater number of blood units and blood derivatives: 20% albumin, platelet concentrate, cryoprecipitate. The adequate antibiotic therapy was determined according to the results of regularly taken swabs from surgical wounds, the thoracic and abdominal drains and biological samples for microbiological examination. On the seventh day of hospitalization, laparotomy was carried out in order to remove liver tamponade. There were no signs of active bleeding in the liver. Retroperitoneal hematoma was in regression. Hepatogram after the tamponade removal was AST 105 IU/L; ALT 104 IU/L; total bilirubin: 20.3 umol/L; direct bilirubin: 9.1 umol/L. On the 12th day of hospitalization thoracic drain on the left side of the chest was removed and control radiography registered the complete expansion of the left lung. The patient required long-term mechanical ventilation, and tracheostomy was performed on the 13th day of hospitalization. On the 21st day of hospitalization thoracic drain on the right side of the chest was removed. Regular radiographic examinations revealed lung reexpansion in the presence of contusion lesions (Figure 5). Conservative treatment of the fracture of the right humerus did not result in healing, and on the 25th day of hospitalization, dynamic compression plate (DCP) osteosynthesis of 8 holes was performed and cortical screws were placed with deliberation radial nerve (Figure 6).

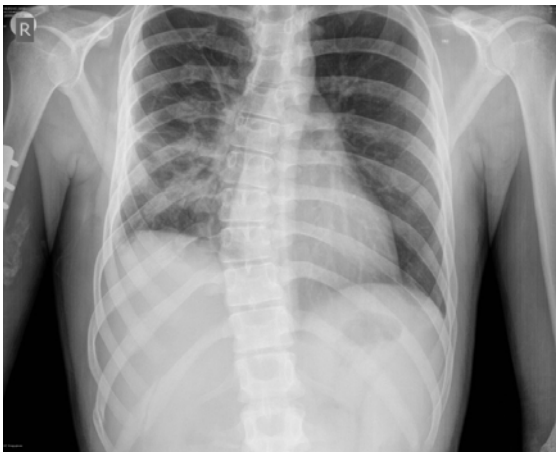

Fig. 5 – Complete reexpansion of the lung in the presence of contusion lesions.

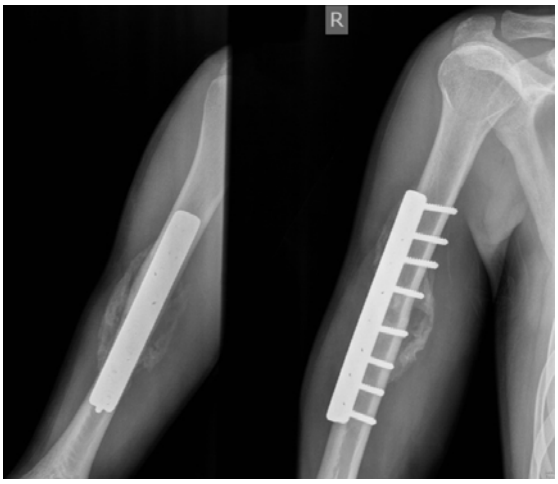

Fig. 6 – Humerus after plate osteosynthesis of 8 holes and cortical screws.

On the 26th day of hospitalization the treatment of the patient included the physical therapy and electrostimulation for radial nerve lesions verified before the osteosynthesis. On the 27th day of hospitalization, the patient was removed from mechanical ventilation. Tracheal cannula was removed, too. Tracheostoma was healing *per secundam*. The patient was taken to the Department of Thoracic Surgery for further observation, after a 30-day-stay in the Intensive Care Unit (ICU). Radiographic examinations of the chest registered rib fractures repairs and almost complete regression of lesions was in the lung parenchyma was found. Arterial blood gas analysis during spontaneous breathing: PaO2:12.3 kPa; PaCO2 5.8 kPa; HCO_3 – 23 mmol/L. Control CT scan performed on the 23rd day after the removing liver packs showed normal findings (Figure 7). We continued with physical therapy that led to regression of lesions of radial nerve. At discharge, neurological status of the patient's right hand – inability to dorsiflex the hand and inability to abduct the thumb. Thirty five days after the hospital treatment, the patient was discharged and recommended to continue to attend the rehabilitation programme.

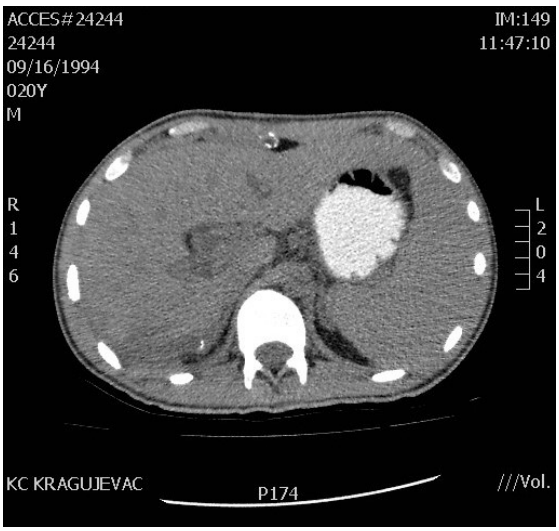

Fig. 7 – Computed tomography scan image of the liver on the 23rd day after removing liver packs with no signs of active bleeding.

Discussion

The multiple injuries with thoracic trauma jeopardize the patient's status significantly. Polytrauma associated with thoracic injuries is found with blunt trauma and is usually related to road traffic injury. In most cases thoracic trauma can be managed without thoracotomy. This fact should not be taken for granted. Each patient with thoracic trauma requires urgent and qualified assessment of severity of injuries as well as the certain actions and measures in order to manage the injuries and therefore to reduce the risk of fatal outcome [7].

CT diagnostics provides the identification and grading of injured organs and the quantification of fluid or blood in the cavities. This allows non-surgical treatment of stable patients, thus reducing the rate of nontherapeutic surgery [8–10]. Although thoracic trauma occupies approximately 10% to15% of all traumas, the mortality rate of thoracic trauma is very high, estimated at 25%. Rib fracture is the most common injury in thoracic trauma [11], but only 6% to 12% of trauma patients complain only of rib fracture – it is common for trauma patients to experience other organ injuries [12]. The increased number of fractured ribs increases the level of injury and its mortality rate [13]. Although the frequency of intraabdominal injury did not increase with the number of rib fractures, as shown in this study, the frequency of intraabdominal injuries requiring surgical treatment did [14]. The liver injury we identifed, according to Moore, was classified as grade III. Retamponade was performed due to hemodynamic instability, massive blood loss in the pleural space after chest tube placement and the risk of massive blood transfusions. After seven days we removed liver tamponade. The recent studies recommend relaparotomy after liver packing within 48 hours, [15] but in our case this could not be done earlier because of the risk of liver hemorrhage and the risk of massive transfusion. Caruso et al. [16] shows that removing liver tamponade up to 72 hours reduced the risk of rebleeding. Although the retamponade was removed 7 days after the surgery, there were not any complications of perihepatic tamponade in this period.

The current treatment of severe chest wall injuries such as flail chest causing instability of chest wall with paradoxical breathing with hypoventilation, disorders of arterial blood-gas (ABG) concentration levels and respiratory insufficiency, includes: nonsurgical management *via* intubation and intermittent positive pressure ventilation (internal pneumatic splint), analgesia, pulmonary toilet, and chest physiotherapy [17–19]. After initial treatment of chest trauma in our patients with both side drainage, although it was evacuated 3,000 mL of blood content from right side (hematocrit of this content was lower than that of the venous blood), the patient's respiratory status was stable and drained fluid was 50 mL and less during the first hour of post-drainage, so we gave up thoracotomy. During the first week of hospitalization, the primary goals of the treatment were the stabilization of hemodynamic parameters, improvement of respiratory function, improvement in hematological status, infection control and flail chest stabilization (flail resulted from mutual serial rib fractures). During the treatment there were no pleural complications. There are methods for flail chest surgical fixation and studies demonstrating the benefit of surgical treatment of severe chest wall injuries [20–24], but they are not widely accepted due to the lack of evidence. Flail chest treatment requires long-term mechanical ventilation. The type of mechanical ventilation that was used on our patient was intermittent positive pressure ventilation (IPPV) with positive end-expiratory pressure (PEEP). Early tracheostomy, compared to conducting tracheostomy after prolonged endotracheal intubation (longer than 14 days), reduced the incidence of pneumonia, duration of ventilatory dependence, ICU length of stay and tracheal complication rates [25–28].

Taking into account the available literature and current treatment guidelines, we treated this patient as described.

Conclusion

The management of each polytraumatized patient is based on the good clinical practice guide, though specific modifications of the approach are needed due to the severity of injuries and degree of organ systems damage, as it was shown in the case of the treatment of our patient and thoroughly explained in the report. Trauma is best managed by a team approach (there is no "I" in trauma). A thorough primary and secondary survey is key to identify life threatening injuries and to give adequate treatment. Once a life threatening injury is discovered, intervention should not be delayed.

Acknowledgement

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CASE REPORT

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Surgical treatment of secondary hip osteoarthritis using cementless total hip endoprosthesis with Fitmore® Hip Stem – a case report

Sekundarna osteoartroza kuka lečena ugradnjom totalne bescementne endoproteze sa Fitmore® stemom – prikaz bolesnika

Ivan Golubović*, Zoran Baščarević†, Predrag Stojiljković*, Zoran Radovanović‡, Ivana Golubović‡, Milan Radojković‡, Dušan Djordjević§, Aleksandar Mitić‡, Svetlana Milijić‡, Zoran Golubović*

Clinical Center Niš, *Clinic for Orthopaedic Surgery and Traumatology, Niš, Serbia; †Institute of Orthopaedic Surgery "Banjica", Belgrade, Serbia; University of Niš, ‡Faculty of Medicine, Niš, Serbia; §Military Hospital, Niš, Serbia

Abstract

Introduction. Hip dysplasia with subluxation represents insufficient coverage of the femur's head placed in the dysplastic acetabulum. This lack of coverage ranges from barely noticeable to condition where half of femur head is uncovered by acetabulum. The caput-collum-diaphyseal angle of the proximal femur and anteversion angle of collum are increased, Wiberg's angle is less than 15° and Ménard-Shenton line is interrupted. Hip joint degeneration occurs very early. When radiological signs of hip joint degenerative changes are discovered in elderly they are associated with pain, limited movements and leg shortening. **Case report**. We present a 53-year old female treated conservatively in childhood because of hip diyplasia with subluxation. After pregnancy, right hip pain emerged. Clinical and radiological examinations revealed hip subluxation with the signs of degenerative osteoarthritis. Initial treatment was conservative and included drugs and balneophysical procedures. Since pain and movement impairment progressed and became constant, a hip replacement using cementless total endoprosthesis with Fitmore® Hip Stem was done. In the pre-operative preparation, anteroposterior x-ray of the hip(with third of the proximal femur) was made. This X-ray enabled precise planning of implantation endoprosthesis component. The early postoperative course was uneventful with very good therapeutic effect. Following successful physical rehabilitation, the patient returned to work and full life activity. **Conclusion**. Implantation of the cementless endoprosthesis with Fitmore® Hip Stem in the treatment of secondary hip osteoarthritis is a good choice in the treatment of young patients with good bone quality. Future clinical and radiological follow-up and comparative studies are needed to show the advantages of this type of stem compared to the classical cementless long stem.

Keywords:
ostheoarthritis, hip; hip prosthesis; orthopedic procedures; treatment outcome.

Apstrakt

Uvod. Razvojni poremećaj kuka sa subluksacijom predstavlja nedovoljnu pokrivenost glave butne kosti koja se nalazi u displastičnom acetabulumu. Nepokrivenost glave butne kosti acetabulumom može biti jedva primetna do nepokrivensti jedne polovine glave. Postoji povećanje kolodijafizarnog ugla i ugla anteverzije, Wiberov ugao je manji od 15 stepeni, Shenton-Menardov luk je prekinut. Vrlo rano dolazi do degenerativnih promena u zglobu kuka što je praćeno bolovima, ograničenjem pokreta u kuku i skraćenjem noge. **Prikaz bolesnika**. U radu je prikazana bolesnica stara 53 godine, koja je kao dete lečena neoperativno zbog razvojnog poremećaja kuka. Nakon trudnoće i porođaja javili su se bolovi u desnom zglobu kuka. Urađen je klinički i radiološki pregled pri čemu je dijagnostikovana subluksacija desnog zgloba kuka sa znacima degenerativne osteoartroze. Lečenje je započeto neoperativno, medikamentoznom terapijom i balneo-fizikalnim procedurama. Nakon konzervativnog lečenja zbog stalnih bolova i ograničenja pokreta u kuku, odlučeno je da se lečenje nastavi ugradnjom endoproteze zgloba kuka. U preoperativnoj pripremi urađen je antero-posteriorni rendgenski snimak kuka sa gornjom trećinom butne kosti, koji omogućava precizno planiranje ugradnje komponenti endoproteze. Ugrađena je bescementna endoproteza zgloba kuka sa Fitmore® stemom. Rani postoperativni tok protekao je uredno. Dobijen je dobar klinički rezultat lečenja. Nakon sprovedene rahabilitacije bolesnica se vratila svojim radnim i životnim aktivnostima. **Zaključak**. Primena bescementne endoproteze zgloba kuka sa Fitmore stemom u lečenju sekundarne osteoartroze kuka, predstavlja dobar izbor u lečenju mlađih bolesnika sa dobrim kvalitetom koštanog tkiva. Buduća klinička i radiološka praćenja primene ove vrste stema uz komparativne studije, su neophodna da bi se pokazale njegove prednosti u odnosu na klasični bescementni dugi stem.

Ključne reči:
kuk, osteoartritis; kuk, proteza; ortopedske procedure; lečenje, ishod.

Correspondence to: Predrag Stojiljković, Clinical Center Niš, Clinic for Orthopaedic Surgery and Traumatology, Bulevar dr Zorana Djindjića 48, 18 000 Niš, Serbia. E-mail: predragsss@gmail.com

Introduction

Hip subluxation is characterized by insufficient coverage of femur's head placed in the dysplastic acetabulum. This lack of coverage ranges from barely noticeable to condition where half of femur's head is uncovered by acetabulum. In advanced cases, anteversion angle of collum and caput-collum-diaphyseal (CCD) angle are usually altered [1]. Hip subluxation may occur very early in life, even shortly after birth, when bone changes are minimal. However, it is much more common as a residue after conservative or early surgical treatment and often diagnosed after 15 years of life [2]. It is very commonly diagnosed between 15 and 30 year of life due to the occurrence of hip pain. When radiological signs of hip joint degenerative changes (osteoarthritis) are discovered in elderly they are associated with pain and limited movements [3].

We present a 53-year old female with advanced right femur's head subluxation and developed clinical and radiological signs of right hip osteoarthritis. Hip replacement using the cementless total endoprosthesis with Fitmore® Hip Stem was done. The postoperative follow-up period was 24 months.

Case report

A 53-year old female was admitted to the Orthopedics and Traumatology Clinic, Clinical Center Niš, for constant right hip pain and movement restrictions. Pain occurred after delivery in 1991. As a child, she was treated conservatively because of hip dysplasia (no medical records about the method of treatment). Clinical and radiological examination revealed right hip subluxation with the signs of osteoarthritis. The initial treatment included physical medicine procedures and antirheumatic medications which resulted in transient improvement of symptoms. On multiple occasions, she was hospitalized in the Institute "Niška banja" and treated with balneophysical procedures and antirheumatic agents. Nevertheless, in 2013 she contacted an orthopaedist for constant pain and limited movements in her right hip refractory to antirheumatic drugs. A plain right hip radiograph revealed subluxated femur's head with marked osteoarthritic changes that included narrowed and missing joint space, marginal osteophytes and bone cysts in femur head and acetabulum (Figure 1). Following conservative therapy that included drugs and balneophysical procedures, the patient was suggested surgical treatment due to constant and progressive hip pain and movement restriction, which she accepted.

It is important to take preoperative planning with particular precision before implantation of the cementless total endoprosthesis with Fitmore® Hip Stem in order to reduce the intraoperative and postoperative complications to a minimum. For preoperative planning, it is essential to have a good anteroposterior and lateral view of the hip which includes the proximal third of the femur. Preoperative planning provides the size and position of the acetabular component and femoral stem. Correct positioning of the acetabular and femoral components is declared to ensure optimal fixation of endoprosthesis components and restore hip biomechanics.

The cup templates were placed on the X-ray with the acetabular component in approximately 40 to 45 degrees of inclination. Several sizes were assessed to determine which acetabular component will provide the optimal fit with maximum coverage (Figure 2) [4].

In the stem template, the three stem families were displayed with different sizes. The correct family was chosen primarily based on the correct offset. To choose the correct stem family, position the overview template of the family that seemed most appropriate into the medullary canal was

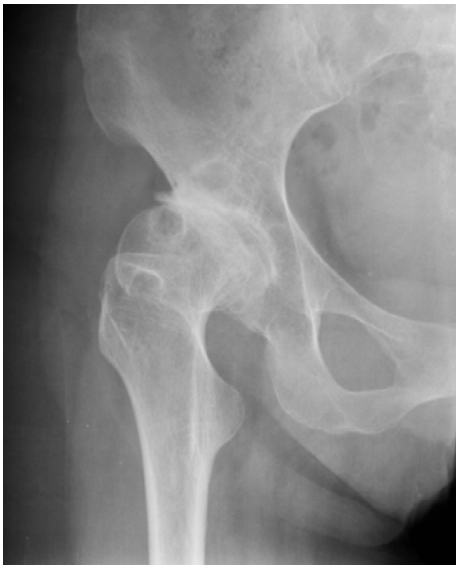

Fig. 1 – Right hip radiograph presenting subluxated femur head with marked osteoarthritic changes (narrowed and almost completely missing joint space, marginal osteophytes and bone cysts in femur head and acetabulum).

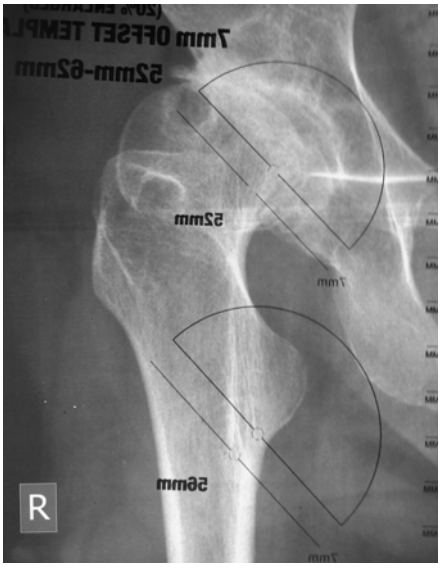

Fig. 2 – Preoperative planning of the acetabular component.

done so that the reference line of the femoral axis was parallel to the femur and that the medial contour of the prosthesis was aligned with the medial cortex (Figure 3) [4].

Following preoperative preparation, hip replacement using cementless total endoprosthesis with Fitmore® Hip Stem was done in spinal anaesthesia. The early postoperative course was uneventful. The patient was mobilized using underarm crutches with non weight bearing on operated leg. The postoperative radiograph showed placed right hip total cementless endoprosthesis with Fitmore® Hip Stem. Installed endoprosthesis components were in a good position (Figures 3 and 4).

After removing the stitches patient was referred to the Institute "Niška banja" for further rehabilitation. The physical therapy was continued. Full weight bearing on operated leg was allowed six weeks after the surgery. Full recovery was achieved and she returned to work and life activities four months after the operation.

Twenty-four months after the surgery she was moving without any aid with the confident, painless and stable walk. The excellent functional result (93 points) according to Harris hip score [5] was achieved. Follow-up radiograph showed the good integration of implanted total cementless right hip endoprosthesis with Fitmore® Hip Stem (Figure 5).

Migrating subluxation is most often a consequence of early conservative treatment or postoperative therapy. Here acetabulum is dysplastic, extended and with steep roof. Femur head is round, often oval and, due to the influence of strong biomechanical force, permanently tends to migrate laterally. One third to one half of femur head is uncovered, the hip joint is irregular and a medial acetabular wall is thickened (callous). CCD angle and anteversion angle are increased, Wiberg's (CE–Center-Edge) angle is less than 15° and Ménard-Shenton line is interrupted. Hip joint degenerative changes occur very early and are accompanied with pain and limited movements [3].

Disease progression leads to further decrease of movement range so that walking is gradually becoming more and more difficult and painful. In the advanced stage of the disease, movements in the hip joint are very restricted and leg shortening occurs so that a patient soon needs mobility aid and starts using crutches. While hip osteoarthritis treatment in the initial stage of the disease is conservative, the operative hip replacement is indicated in the advanced stage.

Total hip arthroplasty is a good solution for most patients with advanced, symptomatic osteoarthritis due to developmental dysplasia of the hip with subluxation. In many cases, hip arthroplasty is significantly more complex because of the associated anatomical abnormalities. Biomechanically, the primary surgical objective is the reconstruction of the femoral offset and anatomical centre of rotation [6].

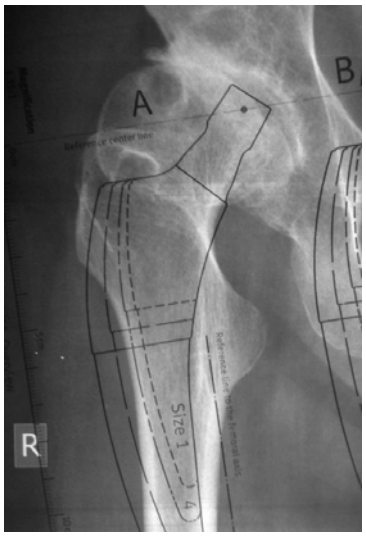

Fig. 3 – Preoperative planning of the femoral component.

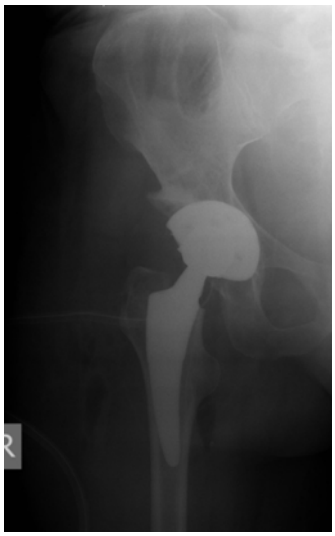

Fig. 4 – Right hip radiograph immediately after hip arthroplasty with total cementless endoprosthesis with Fitmore® Hip Stem.

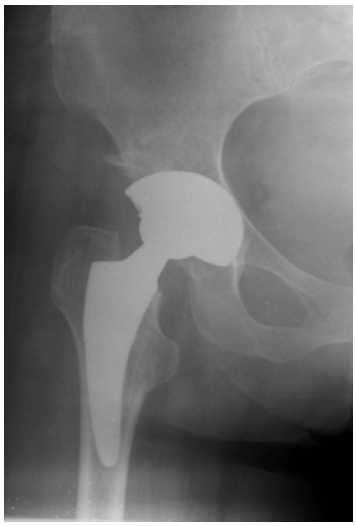

Fig. 5 – Right hip radiograph twenty-four months after the surgery.

Discussion

Based on clinical and radiological presentation, hip subluxations are divided on true (real) and migrating [2]. True subluxation is characterized by moderate acetabulum dysplasia without significant changes on the femur in regard to CCD and anteversion angle alterations. Most commonly this type of subluxation is discovered in older age when hip joint degenerative changes are developed and clinically presented as groin pain and leg movement restriction. True subluxation also may be frequently diagnosed in girls and younger women who suddenly experience hip pain and is associated with first delivery (1).

Cementless total hip arthroplasty shows excellent long term implant survival, with a mean of 94.7% after 16 years [7]. However, the main reasons for revision are based on aseptic loosening, caused by factors such as missing primary stability, stress shielding and wear-particle-induced osteolysis [8].

An implanted hip stem may change the bone structure in the proximal femur [9]. The aim of a modern cementless hip stem is to generate a metaphyseal fixation to reach a load transfer in

the subtrochanteric area closely comparable with physiological conditions. Proximal load transfer, therefore, reduces proximal stress shielding, which can lead to implant loosening [10].

Fitmore® Hip Stem saving the bone mass in the area of greater trochanter and diaphysis of the femur. It has different curves in order to restore the hip joint anatomy and achieve a good offset of the femoral neck [11].

In order to preserve greater trochanter bone tissue, Fitmore® Hip Stem has curved shape and trapezium-shaped cross-section allowing maximal rotational stability. Three-dimensional shape of the stem and Titan Vacuum Plasma Spray layer for press fit fixation enable good fixation and osteointegration, necessary for restoring hip joint biomechanics. The preoperative radiological templating is very important to determine the position of the prosthesis, its size, offset center of the rotation and leg length [4, 12].

The Fitmore® Hip Stem is a curved, uncemented, short-stem prosthesis which has been applied in clinical practice since 2007 [13]. Only a few studies have presented the clinical and radiographic outcomes for short-stem prostheses with a mid- to long-term follow-ups.

A 10-year follow-up study of Pipino et al. [14] reported an 82 % survival of short-stem prosthesis after 10 years.

The results of the first 162 Mayo short stems published by Morrey et al. [15] reported revision surgery in 6 % of total hip arthroplasties (THA) after a 6-year follow-up.

Gustke [11] published 500 Fitmore® Hip Stems report, with a mean follow-up of 1.3 years. He reported a survival rate of 99.4 %.

Gasbarra et al. [16] assessed correlation between osseointegration (with radiographic evaluation and bone densitometry) and functional results (Harris Hip Score) 12 months after surgery in 33 patients with a Fitmore® Hip Stem. They confirmed the good results and anticipated a long and stable fixation of this type of stem [16].

At our clinic, THAs were performed in 10 patients with primary and secondary osteoarthritis by using Fitmore® Hip Stem. After 2 years of follow-up, very good and excellent functional results according to Harris Hip Score and excellent radiological findings were achieved in all patients.

Conclusion

Total cementless hip endoprosthesis with Fitmore® Hip Stem in the surgical treatment of advanced degenerative osteoarthritis is a good choice for younger patients with good bone quality. It can be also used in complex cases of secondary hip osteoarthritis after hip dysplasia associated with anatomical abnormalities. Future clinical and radiological follow-up and comparative studies are needed to show the advantages of this type of short prosthesis stem compared to classical cementless long-stem one.

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Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

Detaljno uputstvo može se dobiti u redakciji ili na sajtu: www.vma.mod.gov.rs/vsp

VOJNOSANITETSKI PREGLED
VOJNOMEDICINSKA AKADEMIJA
Crnotravska 17, 11040 **Beograd**, **Srbija**
Tel/Fax: +381 11 2669689
vsp@vma.mod.gov.rs

Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

Godišnja pretplata za 2016. godinu iznosi: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za strane državljane i ustanove. Pretplate: Žiro račun br. 840-314849-70 MO – Sredstva objedinjene naplate – VMA (za Vojnosanitetski pregled), poziv na broj 12274231295521415. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om). Za zaposlene u MO i Vojsci Srbije moguća je i pretplata u 12 mesečnih rata putem trajnog naloga, tj. „odbijanjem od plate“. Popunjen obrazac poslati na adresu VSP-a.

PRIJAVA ZA PRETPLATU NA ČASOPIS „VOJNOSANITETSKI PREGLED“

Ime i prezime ili naziv ustanove	
Jedinstveni matični broj građana	
Poreski identifikacioni broj (PIB) za ustanove	
Mesto	
Ulica i broj	
Telefon / telefaks	

Pretplata na časopis „Vojnosanitetski pregled“ (zaokružiti):

1. Lično. Dokaz o pretplati dostavljam uz ovu prijavu.
2. Za pripadnike MO i Vojske Srbije: Dajem saglasnost da se prilikom isplate plata u Računovodstvenom centru MO iz mojih prinadležnosti obustavlja iznos mesečne rate (pretplate).
3. Virmanom po prijemu profakture.

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VOJNOMEDICINSKA AKADEMIJA
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Tel/Fax: +381 11 2669689
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