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The beginning of mass vaccination against the severe acute respiratory syndrome coronavirus (SARS-CoV-2) marked the end of the last year and the beginning of 2021. It is expected that this will put the COVID-19 pandemic under control and that normal life around the world will return.

Početak masovne vakcinacije protiv severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) obeležio je kraj prošle i početak nove 2021. godine. Očekuje se da će to dovesti do stavljanja pod kontrolu pandemije COVID-19 i povratka na normalan način života u celom svetu.

E D I T O R I A L

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The *Vojnosanitetski Pregled* in the year of COVID-19 pandemic

Vojnosanitetski pregled u godini pandemije COVID-19

Silva Dobrić

**University of Defence, Faculty of Medicine of the Military Medical Academy,
Center for Medical Scientific Information, Belgrade, Serbia**

The year 2020 will be remembered as one of the most difficult years for humanity, the year that united the entire planet against a common enemy, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of coronavirus disease 2019 (COVID-19), that took more than 1.5 million lives. For medical science, it was a year of great challenges, during which great efforts were made to find a vaccine against that virus, as well as the best therapeutic protocols for the treatment of COVID-19. The world's most famous journals made all the information about research of these topics published in them freely available and thus contributed to a faster and better understanding of the nature of the virus itself, the disease it causes and ways to prevent and treat it. Other medical topics seemed to be put in the background. However, a careful analysis of the published papers showed that other areas of medicine were very well represented in the professional medical literature. Although members of the medical profession, in general, were very engaged caring about COVID-19 patients, they still found the time to write papers from their narrow scientific fields. This is confirmed by the analysis of the papers submitted for publishing in the Vojnosanitetski Pregled (VSP) during 2020.

In the past year, a total of 280 manuscripts were received, which was almost identical to the number from 2019, when 276 manuscripts had been received [1]. Typically, the largest number was from the category 'Original articles' (177 or 63.21%) and 'Case reports' (79 or 28.21%) (Table 1). Of the submitted manuscripts, 72.86% were from domestic authors, primarily from the so-called civilian health and academic institutions (82.35%), while the authors of the remaining 17.65% of the manuscript were from the Military Medical Academy (MMA) in Belgrade, i.e. the Faculty of Medicine of the MMA of the University of Defence. Last year, the number of manuscripts from foreign authors increased significantly (27.14% of the total number of submitted manuscripts), which was slightly more than double compared to the last year's number of papers by foreign authors [1]. This indicates an increased interest in our journal in the international scientific community.

Table 1

Categories and the number of manuscripts submitted to the *Vojnosanitetski Pregled* in 2020

Category	Manuscripts n (%)
Original Articles	177 (63.21)
Case Reports	79 (28.21)
Current Topics	10 (3.57)
Practical Advice to Physicians	4 (1.43)
Preliminary Reports	1 (0.36)
History of Medicine	4 (1.43)
Letter to the Editor	5 (1.79)
Total	280 (100)

Of the 280 papers received in 2020, 103 manuscripts were rejected (36.8%), 94 (33.6%) were accepted for publication, and the remaining 83 (29.6%) are still in the process of peer review.

Regarding the published papers, there were 191 in 2020, which was about 4% more than in 2019. The structure of published papers is given in Table 2. As in previous years, most of the published papers were from the category 'Original Articles', which is understandable if we take into account that the number of received papers mostly belongs to that category. In addition, a total of 145 articles were published last year as Online first in electronic form with an assigned DOI number and they will be published in print form in future issues of the journal. As in previous years, the authors of published articles in 2020 were in more than 80% of cases domestic authors. Considering that the number of papers submitted by authors from abroad was significantly increased in the past year, it is to be expected that the number of published papers by these authors will increase in the next period, which will further affect the international recognition of the VSP.

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Table 2

Categories and the number of articles published in the *Vojnosanitetski Pregled* in 2020

Category	Articles n (%)
Editorial	3 (1.57)
Original Articles	128 (67.02)
Case Reports	40 (20.95)
General Review	2 (1.05)
Current Topics	3 (1.57)
Short Communication	7 (3.69)
Meta-analysis	1 (0.5)
History of Medicine	2 (1.05)
Letter to the Editor	5 (1.79)
Book Review	2 (1.5)
Special Article	1 (0.5)
Total	191 (100)

As it is known, the quality of a scientific journal largely depends on its reviewers. For years, the VSP has had the honor of cooperating with a large number of experts from various fields of medicine, pharmacy and dentistry. The names of those who were engaged in peer review of submitted manuscripts in 2020 are given in Table 3. On this occasion, I would like to thank them most warmly for their time and effort to improve the papers submitted for publication in our journal with their comments and suggestions. I would also like to thank our authors who showed interest in publishing their papers in the VSP, as well as all members of the Editorial Board and Editorial Office of the VSP, who with their great commitment contribute to our journal being among the most prestigious medical journals indexed in the Web of Science.

Table 3

Reviewers of the *Vojnosanitetski pregled* in 2020

Ač Nikolić Eržebet
Aleksić Zoran
Alempijević Đorđe
Amidžić Jelena
Antonijević Biljana
Antonijević Nebojša
Arsenijević Nebojša

Baletić Nenad
Balint Bela
Baščarević Vladimir
Baščarević Zoran
Begović Vesna
Bezmarević Mihajlo
Bogdanović Jovo
Bokonjić Dubravko
Bonkovsky L. Herbert
Borovčanin Milica
Bouhani Malek
Božić Milena
Brdareski Zorica
Brkić Zlata
Bulajić Nina

Carević Momir

Čolić Miodrag
Čutović Tatjana

Ćuk Vladimir

Daković Dragana
Damnjanović Tatjana
Davidović Lazar
Dedić Gordana
Dinčić Dragan
Dobrić Silva
Dominović-Kovačević Aleksandra
Dragojević Simić Viktorija
Dragović Tamara

Duka Miloš

Đenić Nemanja
Đorđević Boban
Đorđević Dragan
Đorđević Vladan
Đurić Dragan
Đurić Dragana
Đurić Mirna
Đurović Branka

Džopalić Tanja

Elez Marija

Filipović Branislav

Glišić Branislav
Glumac Sofija
Golocorbin Kon Svetlana

Hrvačević Rajko

Ilić Dragan
Ilić Tihomir
Ivanović Mirjana
Ivetić Dražen

Jakovljević Vladimir
Janićijević Petrović Mirjana
Janjić Zlata
Janković Borisav
Janković Janko
Janković Slobodan
Jevđić Jasna
Jokić Spasić Vesna
Jovanović Dragana
Jovanović Milan

Jovanović Vesna
Jović Jasna
Jović Nebojša
Jović Stošić Jasmina
Jović Zoran

Kačar Aleksandra
Kandolf Sekulović Lidija
Knežević Ušaj Slavica
Konstantinović Ljubica
Konstantinović Vitomir
Koračević Goran
Kostić Dejan
Kostić Smiljana
Kozarski Jefta
Kozomara Ružica
Krajnović Dušanka
Kukić-Marković Jelena
Kuzmanović Marija

Lajnert Vlatka
Lakić Aneta
Lalošević Dušan
Lazić Srđan
Lazović Biljana
Lepić Toplica
Lučić Miloš

Ljubić Aleksandar

Majkić Singh Nada
Manojlović Nebojša
Marčetić Mirjana
Marić Bojović Nađa
Marinković Nadica
Marinković Valentina
Marjanović Ivan
Marković Dejan
Martić Vesna

Matić Ivana
Matijević Stevo
Medić Stojanoska Milica
Micev Marjan
Mijušković Mirjana
Mijušković Željko
Mikić Dragan
Mikov Aleksandra
Milanović Slađan
Milenković Branislava
Milenković Saša
Mileusnić Dušan
Milev Boško
Milić Rade
Milisavljević Milan
Mirković Ljiljana
Mitrović Jovanović Ana
Mladenović Zorica
Mladnović Tihomir

Nedok Aleksandar
Nenadić Dane
Nićiforović Dijana
Nikolić Borislav
Nikolić Branka
Nikolić Ljubiša
Ninković Milica

Obradović Slobodan
Ostojić Gordana

Paunović Zoran
Pavlović Milorad
Pekić Sandra
Perić Aleksandar
Perić Aneta
Pljevljakušić Dejan
Polovina Snežana
Pucar Ana

Table 3 – *continued*

Rabrenović Violeta Rađen Slavica Radenović Lidija Radoičić Dragan Radović Milan Radunović Aleksandar Rančić Nemanja Rasulić Lukas Resan Mirko Ristanović Elizabeta Ristić Anđelka Ristić Dragana Ristić Gorica Ristić Ljubiša Ristić Medić Danijela Roganović Branka Savić Slobodan Savić Snežana	Sekulić Vuk Sotirović Jelena Spasojević Ivan Srzentić Snežana Stamenković Dragoslav Stamenković Jelena Stanković Ivan Stanković Nebojša Starčević Srđan Stijak Lazar Stojanović Zvezdana Šobić Šaranović Dragana Špirić Željka Šubarević Vladan Šupić Gordana Šurbatović Maja Šušnjar Snežana	Tadić Ivana Tadić Vanja Tarabar Dino Tatić Svetislav Tatomitović Željka Tepšić Ostojić Vesna Todorović Balint Milena Todorović Ljubomir Todorović Veljko Tomić Aleksandar Tomić Dragan Trifunović Zamlakar Danijela Trifunović Zoran Tušek Ivan Udovičić Ivo Vasilijić Saša Vasiljević Nađa	Vavić Neven Vezmar Kovačević Sandra Višnjić Milan Vojvodić Danilo Vojvodić Nikola Vučaj Ćirilović Viktorija Vučetić Dušan Vučević Dragana Vučinić Predrag Vukomanović Aleksandra Vukomanović Đurđević Biserka Vukosavljević Miroslav Vulović Maja Zeba Snježana Živković Slavoljub Životić-Vanović Mirjana

R E F E R E N C E S

1. *Dobrić S*. The year in review, 2019. Vojnosanit pregl 2020; 77(1): 5–7.

ORIGINAL ARTICLES

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Prevalence of C-shaped mandibular second molar canals in the population of central Serbia: a cone-beam computed tomography study

Učestalost kanala C-oblika kod drugih mandibularnih molara u populaciji centralne Srbije: studija sa kompjuterizovanom tomografijom konusnog snopa

Suzana Živanović, Miloš Papić, Mirjana Radović, Aleksandra Mišić, Miloš Živić, Milica Popović

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Abstract

Background/Aim. C-shaped canals are a complex morphological variation of the tooth root canal system that, if present, could greatly affect the outcome of endodontic therapy. The prevalence of these canal configurations varies between the populations of different ethnic and geographical origins. Therefore, the goal of this study was to analyze the prevalence and morphology of mandibular second molar C-shaped canals in the population of Central Serbia. **Methods.** The study included a total of 199 mandibular second molars receiving a cone-beam computed tomography (CBCT) examination and determining the presence of C-shaped canal systems, their configuration, minimal wall thickness and its relative position on axial cross-sections at the coronal, middle, and apical level. **Results.** The prevalence of C-shaped mandibular second molars was 5.53%. C1 canal configuration was the most frequent at the coronal cross-section, while C2 configuration was the most frequent at middle and apical cross-sections. Minimal wall thickness decreased going apically, with the mean value of 1.01 mm at the coronal, 0.87 mm at the middle, and 0.67 mm at the apical cross-sections. Minimal wall thickness was mostly directed lingually at all cross-sectional levels. **Conclusion.** C-shaped canals should be expected in mandibular second molars of the population of Central Serbia. CBCT was shown to be the most valuable technique to determine C-shaped canals and facilitate understanding of the C-shaped canal morphology; its implementation could improve the success of endodontic therapy, especially if the complex root canal configuration is present.

Key words:
endodontics; tooth root; tooth anomalies; molar; tomography; serbia.

Apstrakt

Uvod/Cilj. Kanali korenova C-oblika su kompleksne morfološke varijacije kanalnih sistema zuba, čije prisustvo može ozbiljno da utiče na ishod endodontske terapije. Učestalost ovih konfiguracija varira između populacija različitog etničkog i geografskog porekla. Stoga je cilj ove studije bio da analizira učestalost i morfologiju kanala C-oblika drugih mandibularnih molara u populaciji centralne Srbije. **Metode**. Studijom je bilo obuhvaćeno ukupno 199 drugih mandibularnih molara snimljenih kompjuterizovanom tomografijom konusnog snopa, na kojima je analizirano prisustvo kanalnog sistema C-oblika, njegova konfiguracija, najmanja debljina zida i njegova relativna pozicija na aksijalnim presecima na koronarnom, srednjem i apikalnom nivou. **Rezultati**. Učestalost drugih mandibularnih molara sa kanalnim sistemom C-oblika bila je 5,53%. Kanalna konfiguracija C1 bila je najčešća na koronarnom preseku, dok je konfiguracija C2 bila najčešća na srednjim i apikalnim presecima. Vrednosti najmanje debljine zida opadale su prema apeksu, sa srednjim vrednostima od 1,01 mm na koronarnom, 0,87 mm na srednjem i 0,67 mm na apikalnom preseku. Najmanje debljine zida najčešće su bile orijentisane lingvalno na svim presecima. **Zaključak**. Treba očekivati prisustvo kanala C-oblika na drugim mandibularnim molarima u populaciji centralne Srbije. Kompjuterizovana tomografija konusnog snopa pokazala se kao značajna tehnika za pomoć u razumevanju morfologije kanala C-oblika, a njena implementacija može poboljšati uspeh endodontske terapije, posebno ako je prisutna kompleksna konfiguracija kanala korena zuba.

Ključne reči:
endodoncija; zub, korenski kanal; zub, anomalije; molari; tomografija; srbija.

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Introduction

In order to ensure successful endodontic treatment and avoid procedural errors during chemo-mechanical preparation and definitive obturation of the tooth root canal, it is important to know and find out the exact root canal morphology [1, 2]. The internal morphology of the tooth root could be very complex, with differently shaped canals on cross-sections, including round, oval, or irregular. C-shaped canals were first described in 1979 as an anatomic variation of the root canal system in which individual mesial and distal canals are connected by a slit or a network of access canals forming the distinctive shape resembling the letter "C" on the axial cross-section [2–4]. Treatment of these canal systems may be impeded due to the varying canal lumen diameter and the dentine wall thickness, thus making it necessary to properly diagnose the C-shaped canal at the initiation of the treatment [5, 6].

The prevalence of the C-shaped canal is the highest in mandibular second molars, ranging from 2.7% to 44.5%. However, the literature showed that these types of canals could be present in maxillary second premolars and molars or even in the maxillary second incisors [2, 3]. It has been shown that the prevalence of C-shaped canals has a geographical, ethnic, and racial predilection. Asians have the highest prevalence of this canal system compared to the other racial groups. However, there is a difference in prevalence between the populations of East and West Asia [7]. Apart from the lower prevalence in Caucasians, the presence of the C-shaped canal system should not be overlooked [1].

Standard radiographic methods are insufficient to diagnose the C-shaped canals because of a superimposition of the structures on a two-dimensional image; thus, practitioners are encouraged to use cone beam computed tomography (CBCT) for treatment planning and therapy of these canals [8, 9]. Previous studies have shown that CBCT is a precise, non-invasive diagnostic tool that could be used for the visualization of complicated root canal morphology, even though it has not yet been introduced as a routine method in endodontics [2, 4].

The prevalence and configuration of C-shaped canal systems in mandibular second molars have never been examined in the Serbian population. Therefore, the goal of this retrospective study was to analyze the prevalence and morphology of the C-shaped canals in mandibular second molars in the population of Central Serbia. This study is part of major research of the tooth root morphology, conducted at the Faculty of Medical Sciences, University of Kragujevac, Serbia [10–12].

Methods

The study protocol was approved by the Ethics Committee of the Faculty of Medical Sciences, University of Kragujevac, Serbia (No: 01-15942), and it was conducted in accordance with the Helsinki Declaration and Guidelines for Good Clinical Practice.

Sample

This study included CBCT scans of 150 patients of both genders from a pre-existing database. All CBCT images were made in the Radiology Department at the Faculty of Medical Sciences, University of Kragujevac, in the period between October 2014 and October 2018. The scans were obtained using Orthophos XG 3D device (Sirona Dental Systems GmbH, Bensheim, Germany), with three-dimensional settings for recording, VOL1 or VOL1 HD, and a voxel size of 160 μm; the layer thickness was 0.16 mm with a large Field of view (FOV). The reasons for CBCT scanning were different (prosthetic, surgical, orthodontic, and endodontic).

The main image's inclusion criterion was the existence of at least one mandibular second molar. Other inclusion criteria were the following: the tooth is fully visible; has completed root growth; has no radiographically visible periapical lesion; has no radiographically visible external or internal root resorption; is not treated endodontically; has no prosthetic restoration.

C-shaped canal analysis

CBCT images were analyzed using GALAXIS v1.9.4 software (Sirona Dental Systems GmbH, Bensheim, Germany), on the axial cross-sections. Observations were conducted on the 23-inch Philips LED monitor, with a resolution of 1920 × 1080 pixels, in a room with dim lighting. Brightness and contrast were adjusted using the software.

All mandibular second molars' canal systems were analyzed for the presence of the following criteria for C-shaped canals defined by Fan et al. [13]: fused roots; presence of a longitudinal groove on the lingual or buccal surface of the root; at least 1 cross-section of the canal showed a C1, C2, or C3 configuration.

The orientation of the longitudinal groove of a C-shaped canal system was noted as lingual or buccal.

C-shaped canal configuration was analyzed from canal orifice to apical foramen at distinct three cross-sectional levels: C (coronal) – 2 mm from the root canal orifice; M (middle) – at the middle of the root canal length; A (apical) – 2 mm from the apical foramen (Figure 1).

At these cross-sections, C-shaped canal systems were classified in 5 configurations, according to Fan et al. [13] (Figure 2). Differentiation of C2 and C3 configurations was accomplished by measuring angles and shown in Figure 3 — C1: Continuous C-shaped canal; C2: Semicolon shaped because of a discontinuation in the ''C'' outline; however, either angle, α or β, should be no less than 60°; C3: 2 or 3 separate canals, and both angles, α and β, were less than 60°; C4: Single round or oval canal; C5: No canal lumen.

Measurement of the minimum thickness (*t*) between the inner wall of the canal to the outer root surface in the C-shaped canal system was performed at the same three cross-sections by drawing lines at six distinct points: from the most distal canal outline (Dt), from central canal outline (Ct), from

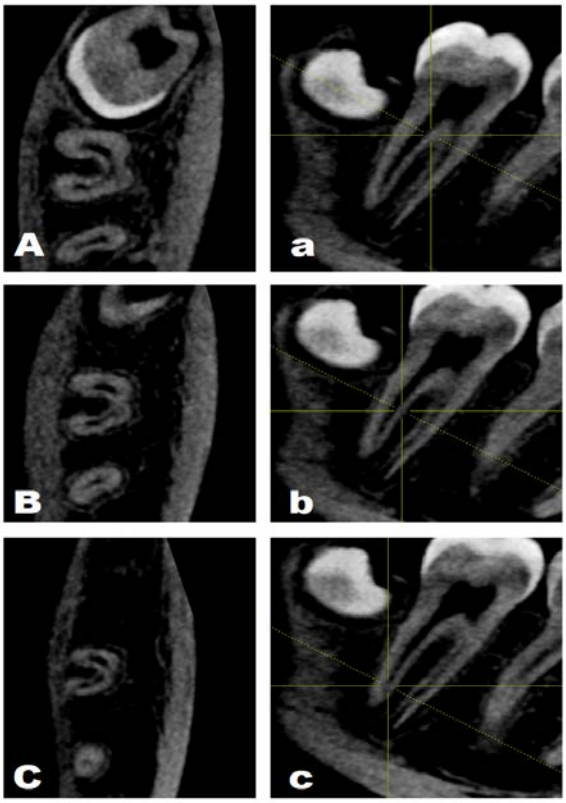

Fig. 1 – Cone-beam computed tomography (CBCT) axial cross-sections of the left mandibular second molar showing different types of C-shaped canal system (A, B, C) with corresponding levels of analysis on sagittal cross-sections (a, b, c).

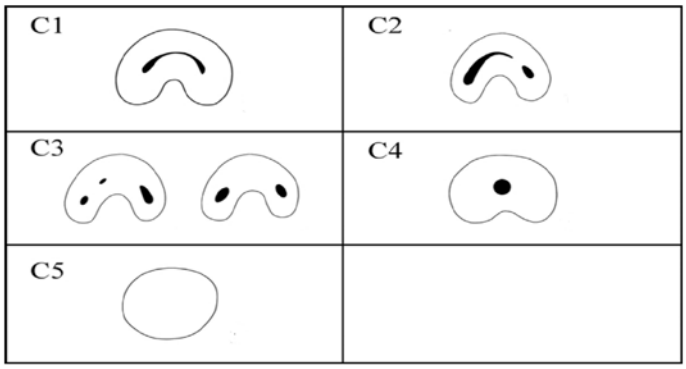

Fig. 2 – Classification of C-shaped canal system configurations.

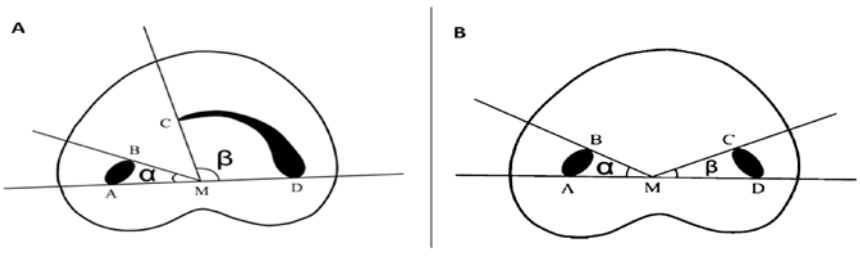

Fig. 3 – Schematic representation of measurement of the angles α and β to differentiate the C2 and C3 canal configurations: (A and B) – Ends of one canal cross-section; (C and D) – Ends of the other canal cross-section. M – middle point of line AD; α – angle between line AM and line BM; β – angle between line CM and line DM [2]. (A) C2 canal configuration, angle β > 60º; (B) C3 canal configuration, α < 60º, β < 60º.

the most mesial canal outline (Mt), from the middle between the most distal and central canal outline points (DCt), and from the middle between the most mesial and central canal outline points (MCt). The minimal thickness was noted, as well as its position according to the six directions of the tooth where it was measured (Figure 4).

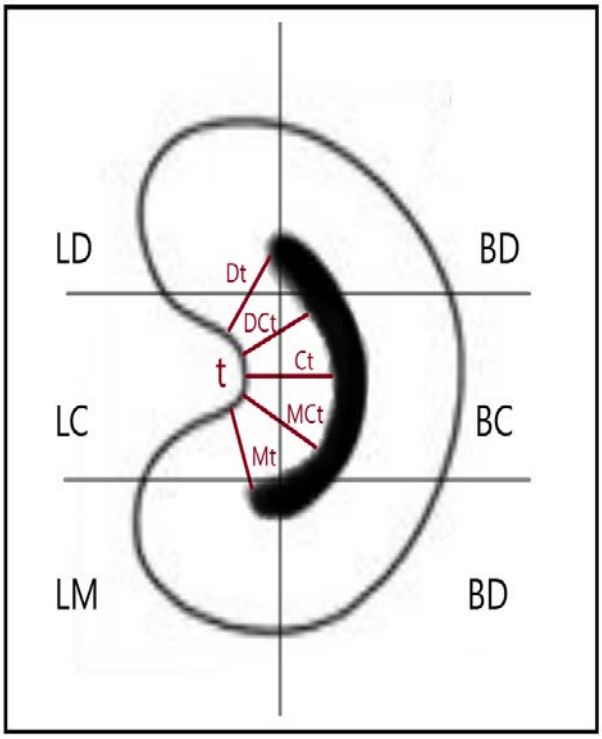

Fig. 4 – Representation of the measurement of the minimal thickness by drawing lines from the five points on the inner canal outline (Dt, DCt, Ct, MCt, Mt) to the closest outline of the root surface (red lines). At every cross-section, axial tooth view was divided into the following sixths: LD – linguo-distal, LC – linguo-central, LM – linguo-mesial, BD – bucco-distal, BC – bucco-central, and BD – bucco-distal. Depending on the sixth where most of the line was located, a minimal thickness value was added to one of the six directions.

In order to analyze the frequencies of different configurations of C-shaped canal systems and the values of minimal thickness and its position, all the collected data were entered into the commercial software for statistical analysis SPSS v20.0 (SPSS Inc., Chicago, IL, USA).

Results

The study sample included CBCT scans from 150 patients, 73 (49%) female, and 77 (51%) male, with a total of 233 mandibular second molars. The average age of the patients was 39 years old (the minimum age being 15, and the maximum 72 years old). Out of the total number of teeth, 199 had reached inclusion criteria, of which 11 teeth (5.53%) had presented C-shaped canal systems. Only one tooth had a buccally oriented longitudinal groove, while the rest had a lingually oriented groove.

Table 1 shows the prevalence of C-shaped canal configurations in mandibular second molars. Most teeth presented a C1 configuration of the C-shaped canal system at the coronal cross-section and C2 configuration at the middle and apical cross-sections.

Table 1

Number of teeth with different configurations of C-shaped canals at the coronal, middle, and apical cross-sections

Type	Number of teeth (%)		
	coronal	middle	apical
C1	8 (72.7)	- (0)	3 (27.3)
C2	1 (9.1)	6 (54.5)	5 (45.4)
C3	2 (18.2)	4 (36.4)	2 (18.2)
C4	- (0)	- (0)	- (0)
C5	- (0)	1 (9.1)	1 (9.1%)
Total		11 (100)	

Minimal *t* values ranged from 0.89 mm to 1.05 mm (mean – 1.01 mm) at the coronal, from 0.54 mm to 1.05 mm (mean – 0.87 mm) at the middle, and from 0.51 mm to 0.83 mm (mean – 0.67 mm) at the apical cross-section (Figure 4). The minimal *t* value was differently oriented at the coronal, middle, and apical levels, but all teeth mostly presented one of the lingual directions at the coronal and middle cross-sections, while in the apical regions, minimal *t* value was presented equally in lingual and buccal directions (Table 2).

Table 2

Number of teeth with different directions of the minimal thickness (t) values at the coronal, middle, and apical cross-sections

Direction	Number (%) of teeth		
	coronal	middle	apical
LM	3 (27.3)	5 (50)	2 (20)
LC	2 (18.2)	- (0)	3 (30)
LD	3 (27.3)	2 (20)	- (0)
Total lingual	8 (72.7)	7 (70)	5 (70)
BM	2 (18.2)	2 (20)	- (0)
BC	1 (9.1)	- (0)	4 (40)
BD	- (0)	1 (10)	1 (10)
Total buccal	3 (27.3)	3 (30)	5 (30)
Total	11 (100)	10* (100)	10* (100)

***One case had presented C5 configuration at the middle and apical cross-section. Thus, the t value and its direction could not be analyzed.**
LD – linguo-distal, LC – linguo-central, LM – linguo-mesial, BD – bucco-distal, BC – bucco-central, and BD – bucco-distal.

Diagrammatic representation of the maximum, mean, and minimum values of the minimal wall thickness for C-shaped canals are presented in Figure 5.

Two cases with complex C-shaped canal systems at coronal, middle and apical cross-section are shown in Figure 6.

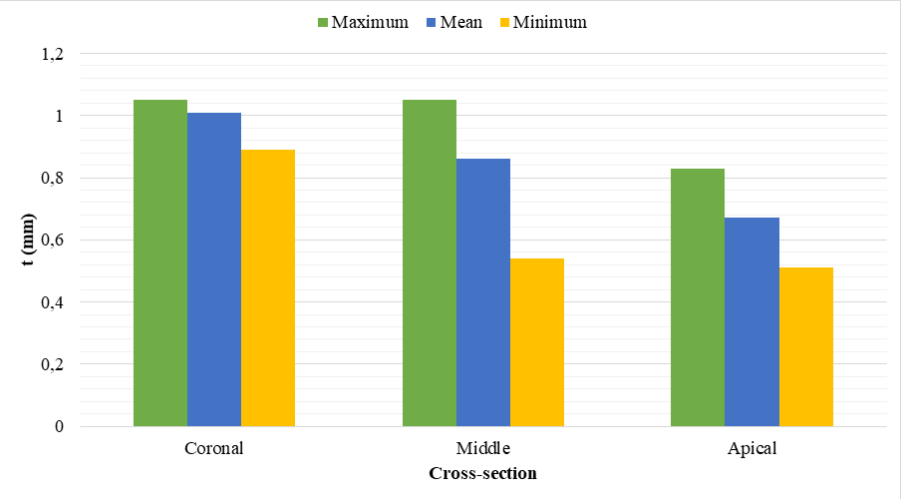

Fig. 5 – Diagrammatic representation of maximum, mean, and minimum values of the minimal wall thickness (t) for C-shaped canals at the coronal, middle, and apical cross-section.

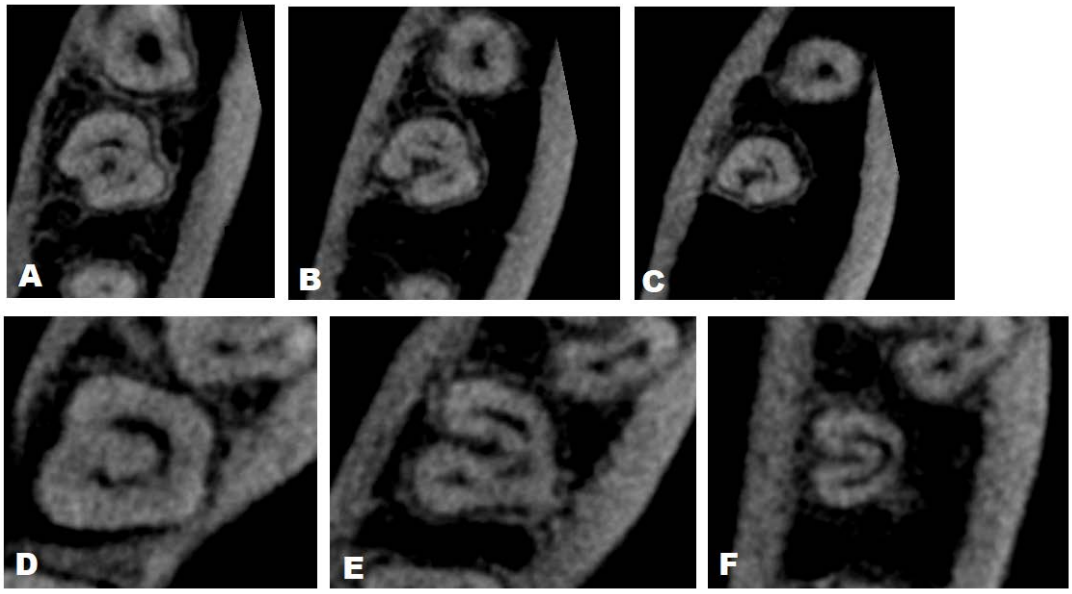

Fig. 6 – Two cases with complex C-shaped canal systems at the coronal, middle, and apical cross-sections. The first case presented C3 configuration at the coronal (A), C2 at the middle (B), and C1 at the apical cross-section (C). The second case is showing a more complex configuration starting with C1 at the coronal (D), dividing to C3 with three canals at the middle (E), and finishing with C1 configuration at the apical cross-section (F).

Discussion

Mandibular second molar has two roots and three canals in most cases, although many variations in the number of roots and the internal canal morphology have been reported [14]. Since anatomical and morphological characteristics of the root canals greatly affect the outcome of the endodontic therapy, practitioners should be familiar with the possible anatomical complexities and variations in the canal system [15]. Different techniques were used in the preoperative analysis of canal morphology, with varying outcomes. The ideal technique should be non-invasive, non-destructive, feasible, and precise in the *in vivo* conditions [5]. Digital dental radiography is an important diagnostic method in endodontics, but it is not sufficient in assessing the teeth with complicated morphology [2]. The European Society of Endodontology has proposed that CBCT should be considered if complicated root canal morphology was suspected [9].

In order to consider a possible presence of a C-shaped canal system, a longitudinal groove should exist on the root surface, buccally or lingually. The most widely accepted theory for the formation of C-shaped canals is the failure of Hertwig's epithelial root sheath to fuse either at the buccal or at the lingual root surface, thus forming the groove on the side contrary to the fuse failure [1, 16]. The orientation of this

groove determines the direction of instruments during chemo-mechanical root canal preparation in order to avoid the possibility of canal perforation and other procedural errors [6, 17]. Most teeth have a lingually oriented longitudinal groove, as shown in a study in China, where no buccally oriented groove was found [7]. Nevertheless, a buccally oriented groove could be present in a smaller percentage, as shown in two cases in a study in the Turkish population [2] and one case in our study. A higher occurrence was found in the Portuguese population in 22% of the examined teeth [18]. These variations could be attributed to the population's race and ethnicity, or the study sample size and methodology of root canal analysis [2, 7, 19].

The prevalence of C-shaped mandibular second molars' canals is quite diverse between the populations from different geographical origins. Regardless of the canal morphology analysis method, the highest prevalence of C-shaped canals was reported in East Asia, up to 44.5% in China and Korea [7, 20]. Going west, the prevalence reduces, as shown in Sri Lanka and India, 6% and 7.5% [21, 22]. A relatively high prevalence of C-shaped canals was also reported in the Middle East region, ranging from 7.2% to 10.6% in Iran, Israel, and Saudi Arabia [14, 16, 23]. In Brazil, the prevalence of C-shaped canals was shown to range from 3.5% to 15% depending on the used methods [5]. The presence of C-shaped canals was also reported in the European population, with differences between countries; for example, in Portugal, the prevalence of C-shaped canals was found to be 8.5%, similarly to Turkey, where the prevalence was 8.9% [2, 18, 19]. A lower prevalence of C-shaped canals, similar to our results, was found in a Greek study, 4.6% [24]. These results, as well as ours, coincide within the range (2.7%–8.1%) shown in studies in the Caucasian race [3, 22–24], which suggests that these differences could be racial or ethnic.

Because of their complexity, C-shaped canal systems could complicate the endodontic treatment, which may be even more complicated when they present configurations with a variable number of canals at different axial cross-sections [6, 17], such as a C-shaped canal presented in Figure 6; for example, that canal presented the C1 configuration at the coronal cross-section, then the C3 in the medial cross-section with three separate canals, merging again to the C1 configuration at the apical cross-section [25–27] (Figure 6, E, F, G). Frequencies of different configurations on cross-sectional levels have also been shown to differ by population. Our results showed that at the coronal and apical cross-sections, most canals have the C1 configuration, which was similar to the Israeli population [16], but contrary to our results, they found the highest prevalence of the C3 configuration at the middle cross-section. The highest frequency of the C2 configuration at all cross-sectional levels was found in the studies of Yang et al. [28], Jayasinghe and Li [29], and Seo and Park [30], while a study in Portugal showed that the most common was the C3 canal configuration [18]. Fernandes et al. [1] claimed that these differences could be racially or ethnically determined, but they could also be caused by differences in study sample sizes or used methodology.

Seo et al. [4] stated that the thickness of the canal wall and the related position of the thinnest wall should be thoroughly analyzed in order to avoid procedural errors during canal instrumentation. Therefore, we have examined the minimal thickness of the inner wall at the coronal, middle, and apical cross-sections. Our results indicate that the minimal wall thickness decreases going apically, similarly to the results of Seo et al. [4]. In addition, we analyzed the direction of the thinnest wall on axial cross-sections. Even though most canals showed lingual directions, we found a high frequency of the thinnest wall in buccal directions at all cross-sectional levels, contrary to the previous findings [4]. These results could help practitioners in endodontic treatment of C-shaped canals, where they could reduce the incidence of root wall perforations during a chemo-mechanical preparation by directing instrumentation opposite to the less thin wall [1, 2, 6, 17].

Conclusion

Our results showed that C-shaped canals should be expected in mandibular second molars of the population of Central Serbia with a prevalence of 5.53%. Configuration of C-shaped canals varied in morphology and the number of canals on different axial cross-sections. Knowing and recognizing the root canal morphology facilitates preoperative and operative canal identification, prevents the unnecessary removal of a healthy tooth structure, reduces the incidence of procedural errors, and thus increases the overall success of endodontic therapy.

REFERENCES

1. *Fernandes M, de Ataide I, Wagle R.* C-shaped root canal configuration: A review of literature. J Conserv Dent 2014; 17(4): 312–9.
2. *Sinanoglu A, Helvacioglu-Yigit D.* Analysis of C-shaped canals by panoramic radiography and cone-beam computed tomography: root-type specificity by longitudinal distribution. J Endod. 2014; 40(7): 917–21.
3. *Cooke HG, Cox FL.* C-shaped canal configurations in mandibular molars. J Am Dent Assoc 1979; 99(5): 836–9.
4. *Seo DG, Gu Y, Yi YA, Lee SJ, Jeong JS, Lee Y*, et al. A biometric study of C-shaped root canal systems in mandibular second molars using cone-beam computed tomography. Int Endod J 2012; 45(9): 807–14.
5. *Ladeira DB, Cruz AD, Freitas DQ, Almeida SM.* Prevalence of C-shaped root canal in a Brazilian subpopulation: a cone-beam computed tomography analysis. Braz Oral Res 2014; 28(1): 39–45.
6. *Kirilova J, Topalova-Pirinska S.* C-Shaped Configuration of the Root Canal System – Problems and Solutions. J IMAB 2014; 20(1): 504–9.
7. *Zheng Q, Zhang L, Zhou X, Wang Q, Wang Y, Tang L*, et al. C-shaped root canal system in mandibular second molars in a

Chinese population evaluated by cone-beam computed tomography. Int Endod J 2011; 44(9): 857–62.
8. *Lopes DS, Câmara AC, Aguiar CM, do Nascimento MD, Farias de Araújo L.* A C-Shaped Canal in a Maxillary Second Molar: Prexion 3D Cone-Beam Computed Tomography Analysis. Acta Stomatol Croat 2016; 50(4): 354–8.
9. European Society of Endodontology. *Patel S, Durack C, Abella F, Roig M, Shemesh H, Lambrechts P,* et al. European Society of Endodontology position statement: the use of CBCT in endodontics. Int Endod J 2014; 47(6): 502–4.
10. *Popovic M, Papic M, Zivanovic S, Acovic A, Loncarevic S, Ristic V.* Cone-Beam Computed Tomography Study of the Root Canal Morphology of Mandibular Anterior Teeth in Serbian Population. Ser J Exp Clin Res 2018; 19(1): 27–34.
11. *Popovic M, Papic M, Acovic A, Zivanovic S, Kanjevac T.* Cone-beam computed tomography study of root number and root canal configuration of premolars in Serbian population. Med Pregl 2018; 71(3–4): 100–7.
12. *Popovic M, Zivanovic S, Vucicevic T, Grujovic M, Papic M, Kanjevac T.* Cone-beam computed tomography study of tooth root and canal morphology of permanent molars in a Serbian population. Vojnosanit Pregl 2018; doi: 10.2298/VSP180322100P (In Press).
13. *Fan B, Cheung GSP, Fan M, Gutmann JL, Bian Z.* C-shaped canal system in mandibular second molars: Part I--Anatomical features. J Endod 2004; 30(12): 899–903.
14. *Rahimi S, Shahi S, Lotfi M, Zand V, Abdolrahimi M, Es'haghi R.* Root canal configuration and the prevalence of C-shaped canals in mandibular second molars in an Iranian population. J Oral Sci 2008; 50(1): 9–13.
15. *Vertucci FJ.* Root canal morphology and its relationship to endodontic procedures. Endod Top 2005; 10(1): 3–29.
16. *Shemesh A, Levin A, Katzenell V, Itzhak J Ben, Levinson O, Avraham Z,* et al. C-shaped canals – prevalence and root canal configuration by cone beam computed tomography evaluation in first and second mandibular molars—a cross-sectional study. Clin Oral Investig 2017; 21(6): 2039–44.
17. *Raisingani D, Gupta S, Mital P, Khullar P.* Anatomic and diagnostic challenges of C-shaped root canal system. Int J Clin Pediatr Dent 2014; 7(1): 35–9.
18. *Martins JN, Mata A, Marques D, Caramês J.* Prevalence of C-shaped mandibular molars in the Portuguese population evaluated by cone-beam computed tomography. Eur J Dent 2016; 10(4): 529–35.
19. *Helvacioglu-Yigit D, Sinanoglu A.* Use of cone-beam computed tomography to evaluate C-shaped root canal systems in mandibular second molars in a Turkish subpopulation: a retrospective study. Int Endod J 2013; 46(11): 1032–8.
20. *Jin GC, Lee SJ, Roh BD.* Anatomical study of C-shaped canals in mandibular second molars by analysis of computed tomography. J Endod 2006; 32(1): 10–3.
21. *Peiris R, Takahashi M, Sasaki K, Kanazawa E.* Root and canal morphology of permanent mandibular molars in a Sri Lankan population. Odontology 2007; 95(1): 16–23.
22. *Neelakantan P, Subbarao C, Subbarao CV, Ravindranath M.* Root and canal morphology of mandibular second molars in an Indian population. J Endod 2010; 36(8): 1319–22.
23. *Al-Fouzan KS.* C-shaped root canals in mandibular second molars in a Saudi Arabian population. Int Endod J 2002; 35(6): 499–504.
24. *Lambrianidis T, Lyroudia K, Pandelidou O, Nicolaou A.* Evaluation of periapical radiographs in the recognition of C-shaped mandibular second molars. Int Endod J 2001; 34(6): 458–62.
25. *Weine FS.* The C-shaped mandibular second molar: incidence and other considerations. Members of the Arizona Endodontic Association. J Endod 1998; 24(5): 372–5.
26. *Cimilli H, Cimilli T, Mumcu G, Kartal N, Wesselink P.* Spiral computed tomographic demonstration of C-shaped canals in mandibular second molars. Dentomaxillofac Radiol 2005; 34(3): 164–7.
27. *Martins JN, Marques D, Mata A, Caramês J.* Root and root canal morphology of the permanent dentition in a Caucasian population: a cone-beam computed tomography study. Int Endod J 2017; 50(11): 1013–26.
28. *Yang ZP, Yang SF, Lin YC, Shay JC, Chi CY.* C-shaped root canals in mandibular second molars in a Chinese population. Dent Traumatol 1988; 4(4): 160–3.
29. *Jayasinghe RD, Li TKL.* C-shaped canals in mandibular second molars in the Hong Kong population: a computed tomographic study. Hong Kong Dent J 2008; 5(1): 27–30.
30. *Seo MS, Park DS.* C-shaped root canals of mandibular second molars in a Korean population: clinical observation and in vitro analysis. Int Endod J 2004; 37(2): 139–44.

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Efficacy of surgical treatment in patients with cervical spondylotic myelopathy

Efikasnost hirurškog lečenja bolesnika sa cervikalnom spondilotičnom mijelopatijom

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Abstract

Background/Aim. Treatment options for cervical spondylotic myelopathy (CSM) are the topic for discussion due to the lack of controlled randomized prospective studies. Also, the natural history of CSM is unpredictable and the efficacy of surgical decompression is still controversial. The aim of this prospective study was to describe the results of surgical treatment of patients with CSM in a single institution. **Methods.** Fifty-nine patients with symptomatic CSM were enrolled in this single-center prospective study. At the end of the follow-up period of 12 months, 50 patients were analyzed. All patients were operated on; surgical decompression was performed by anterior or posterior surgical approach. Outcome evaluations were obtained preoperatively and 12 months postoperatively by using the following outcome measures: the modified Japanese Orthopedic Association (mJOA) scale, the Nurick score and the Neck Disability Index (NDI). The functional recovery ratio was calculated postoperatively by using Hirabayashi's formula. **Results.** According to our results, significant improvements were detected in all outcome variables (mJOA score, Nurick score and NDI). Also, a statistically significant improvement was observed in all three categories of patients according to the preoperative mJOA score (mild, moderate, severe). Twenty-three patients (46%) had a satisfactory functional recovery, while twenty-seven (54%) had an unsatisfactory functional recovery rate. **Conclusion.** Surgical treatment of CSM is a very effective treating method and it resulted in a significant improvement in all outcome measures for a 1-year follow-up period. New studies could be recommended to evaluate the course of the disease, define the optimal surgical strategy, and better determine surgical outcome predictors.

Key words:
spinal cord diseases; neck; surgical procedures, surgery; decompression, surgical; recovery of function.

Apstrakt

Uvod/Cilj. Izbor tretmana u lečenju cervikalne spondilotične mijelopatije (CSM) predstavlja temu za diskusiju zbog nedostatka kontrolisanih randomizovanih prospektivnih studija. Prirodan tok CSM nije moguće predvideti, a sa druge strane efikasnost hirurške dekompresije je i dalje diskutabilna. Cilj ove prospektivne studije bio je prikazivanje rezultata hirurškog lečenja bolesnika sa CSM u jednoj hirurškoj ustanovi. **Metode.** U studiju je uključeno 59 bolesnika sa simptomatskom CSM u jednom centru, na kraju perioda praćenja od 12 meseci, analizirano je 50 bolesnika. Svi bolesnici u studiji su operisani, a dekompresija je urađena prednjim ili zadnjim hirurškim pristupom. Ishod je praćen preoperativno i postoperativno nakon 12 meseci pomoću skala za merenje ishoda: modifikovana skala Japanskog Udruženja Ortopeda (mJOA), Nurick-ov skor i Neck Disability Index (NDI – indeks ograničenja sposobnosti zbog bola u vratu). Funkcionalni oporavak računat je nakon operacije, korišćenjem Hirabayashijeve formule. **Rezultati.** Prema našim rezultatima, značajno poboljšanje je registrovano prema svim skalama za merenje ishoda (mJOA, Nurick-ov skor, NDI). Pored toga, zabeleženo je statistički značajno poboljšanje u sve tri grupe bolesnika u odnosu na preoperativni mJOA (blaga, umerena i teška mijelopatija). Funkcionalni oporavak bio je zadovoljavajući kod 23 bolesnika (46%), dok je 27 bolesnika (54%) imalo nezadovoljavajući oporavak. **Zaključak.** Hirurško lečenje CSM veoma je efikasna metoda lečenja, ono dovodi do značajnog poboljšanja prema svim skalama za merenje ishoda u periodu praćenja od jedne godine. Takođe, mogu se predložiti nova istarživanja radi boljeg praćenja toka bolesti, definisanja optimalne hirurške strategije i boljeg definisanja faktora koji utiču na ishod operativnog lečenja CSM.

Ključne reči:
kičmena moždina, bolesti; vrat; hirurgija, operativne procedure; dekompresija, hirurška; funkcija, povratak.

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Introduction

Cervical spondylotic myelopathy (CSM) is a progressive disease in its nature; it is also a degenerative disease and a common cause of neurologic impairment in the elderly [1–3]. Spondylosis or the degenerative disease of the cervical spine is the most common cause of the cervical myelopathy. The disease is age-related due to the direct mechanical compression of the spinal cord and the onset of symptoms commonly occurs in a slow stepwise pattern with fine motor dysfunction, decreased hand dexterity and gait and balance worsening [1, 2]. Degenerative changes in the cervical spine can be identified in the majority of individuals beyond the fifth life decade, but most of them are asymptomatic. Symptoms in the population with cervical degeneration are estimated and may be present in as much as 5% of the general population and exist along with a wide spectrum of symptoms, from axial neck pain to radiculopathy and spondylotic myelopathy [4]. The natural history of CSM is mixed, but generally progressive and approximately 20% to 60% of patients with symptomatic CSM will deteriorate over time without surgical intervention in a stepwise fashion [5]. According to the results from a recent study, surgery improves neurological outcomes, functional status and the quality of life in patients with CSM [6]. The effects of surgery on the full spectrum of CSM cases is still in question, and accurate prediction of the results from surgical interventions continues to be a challenge for surgeons.

The aim of this prospective study was to describe the results of the surgical treatment of patients with CSM in a single institution.

Methods

This prospective study was conducted in the Department of Neurosurgery at the Military Medical Academy in Belgrade, with the approval of the Hospital Ethics Committee. Fifty-nine symptomatic patients with symptomatic CSM were enrolled in this single-center study. Our study included the patients who were 18 years old or above, had a clinical diagnosis of CSM, radiographically detected cervical cord compression, with or without hyperintensity on T2W sequences on magnetic resonance images (MRI) and had no prior cervical spine surgery. All patients in our study were operated on by anterior or posterior surgical approach at the discretion of the attending surgeon and under the approval of the Collegium of Neurosurgeons at the Department. Nine patients were excluded from the study (one patient died from another disease in the follow-up period, two had a second operation in the adjacent cervical segment, and 6 were lost in the follow-up); 50 patients were analyzed in total (follow-up ratio: 85%). Patients with asymptomatic CSM, active infections, neoplastic diseases, rheumatoid arthritis, trauma, psychiatric diseases, previous surgeries in the cervical spine and patients with concomitant lumbar spinal stenosis were excluded.

Outcome assessments

Outcome evaluations included three outcome measures: the modified Japanese Orthopedic Association (mJOA) scale, the Nurick score and the Neck Disability Index (NDI). The values of measurements were obtained preoperatively and 12 months postoperatively, and functional recovery ratio was evaluated by using Hirabayashi's formula [7–10]. According to the preoperative mJOA scores, the patients were classified as having mild (mJOA > 15), moderate (mJOA 12–14) and severe myelopathy (mJOA < 12).

Statistical analysis

The collected data were presented by descriptive statistics, using the means and standard deviations for continuous variables and percentages for categorical variables. The *t*-test for paired data or the Wilcoxon test was used for continuous variables for comparing preoperative and postoperative measurements. The one-way ANOVA with repeated measures test was used to evaluate the changes in the outcome measurements recorded between the pre and postoperative measurements and sorted into three categories according to the preoperative mJOA score (mild, moderate, severe).

All statistical analyses were performed using software RStudio (0.098.976) and SPSS 17.0 (Chicago, IL).

Results

There were 59 subjects in the study, one patient died during the follow-up period of unrelated causes, two patients were excluded due to the second operation in the adjacent cervical segment, and six were lost during the follow-up period. After that, 50 subjects were analyzed. There were 37 men and 13 women and the average age was 59.06 (SD 10.98). Twenty-eight patients were operated on by an anterior approach and twenty-two underwent a posterior approach. The patients in the anterior surgical group were operated on by anterior cervical discectomy and fusion (ACDF) or ACDF and anterior cervical plate (ACP) fixation, and those in the posterior surgical group underwent laminectomy or laminectomy with lateral mass screw fixation. Table 1 summarizes demographic, clinical and outcome characteristics of the subjects in the study. In this study, there were 10 patients with one stenotic level, 18 with two, 16 with three, and 6 patients with four stenotic levels. The most involved segment was C5/C6 (35.6%), the second was C4/C5 (32.2%), on the third place was C6/C7 (19.5%), and at the end was C3/C4 (12.7%). Among the subjects in our group, 34 had high signal intensity on T2-weighted magnetic resonance images, while 16 subjects had normal T2 signal intensity. According to the mJOA scores of subjects in the study, 9 patients had mild myelopathy, 19 moderate and 22 had severe myelopathy preoperatively, whereas postoperatively, 26 patients had mild myelopathy, 14 moderate and 10 had severe cervical spondylotic myelopathy. Table 2 presents statistics for outcome

Table 1

Descriptive subjects' characteristics

Parameters	Values
Sex, n (%)	
male	37 (74)
female	13 (26)
Age (years), mean ± SD	59.06 ± 10.98
Number of stenotic levels, n (%)	
1	10 (20)
2	18 (36)
3	16 (32)
4	6 (12)
Anatomical level of stenosis, n (%)	
C3-C4	15 (12.7)
C4-C5	38 (32.2)
C5-C6	42 (35.6)
C6-C7	23 (19.5)
T2W signal, n (%)	
hyper	34 (68)
normal	16 (32)
Nurick score, mean ± SD	
preoperative	2.70 ± 1.18
postoperative	1.78 ± 1.39
mJOA score, mean ± SD	
preoperative	11.90 ± 2 .48
postoperative	14 .46 ± 2 .89
mJOA score (preoperative), n (%)	
mild	9 (18)
moderate	19 (38)
severe	22 (44)
mJOA score (postoperative), n (%)	
mild	26 (52)
moderate	14 (28)
severe	10 (20)
Functional recovery rate, n (%)	
satisfactory	23 (46)
unsatisfactory	27 (54)
NDI score, mean ± SD	
preoperative	25.88 ± 8.40
postoperative	15.54 ± 10.75
Surgical approach, n (%)	
anterior	28 (56)
posterior	22 (44)

mJOA – modified Japanese Orthopedic Association; NDI – Neck Disability Index; n (%) – number (percentage) of the patients; SD – standard deviation.

measures: Nurick score, mJOA and NDI preoperatively and postoperatively, and also the analysis of changes in outcome parameters between baseline and 12-month follow-up period for all patients. The extent of improvement in all outcome assessment measures shows that all outcome variables improved significantly from their baseline values after the operation ($p < 0.001$). Table 3 shows the analysis of changes in the outcome parameter mJOA pre and postoperatively. The patients were divided according to the preoperative mJOA score into three groups, mild (mJOA > 15), moderate (mJOA 12–14) and severe myelopathy (mJOA < 12). The extent of improvement was statistically significant in all three categories of patients according to the mJOA score ($p < 0.001$). The extent of functional recovery (recovery ratio, RR) was calculated by using the formula of Hirabayashi et al. [10] [postoperative mJOA score - preoperative mJOA score/full score – preoperative mJOA score) x 100]. A recovery rate in the mJOA that was less than 50% was considered as an unsatisfactory outcome. The mean RR was 47.99% (SD 35.05). In our study, 23 patients (46%) had a satisfactory functional recovery, while 27 (54%) of all patients had an unsatisfactory functional recovery by Hirabayashi's method, after operative treatment. No matter how it looks, Hirabayashi's formula and the recovery rate according to it, are very demanding, and this recovery ratio could be accepted as a very important fact for patients with CSM.

Table 3

Improvement in the mJOA score according to the category of myelopathy

Category of myelopathy	Preoperative mJOA score (mean ± SD)	Postoperative mJOA score (mean ± SD)	*p*
Severe	9.59 ± 1.62	12.41 ± 2.84	< 0 .001
Moderate	12.95 ± 0.85	15.47 ± 1.95	< 0 .001
Mild	15 ± 0.01	17.22 ± 0.97	< 0 .001

For abbreviations see under Table 1.

Discussion

Cervical spondylotic myelopathy is the most common cause of spinal cord dysfunction in those over 50 years of age worldwide. Despite that, there remains a lack of a guiding strategy for the surgical management of patients with CSM [1–3]. CSM is caused by spinal cord ischemia, axonal stretch-associated injuries, and the combination of static factors and dynamic repeated compression of the spinal cord that lead to CSM in some patients [11]. The management of

Table 2

Outcome variables (pre- and postoperative values)

Score	Preoperative (mean ± SD)	Postoperative (mean ± SD)	Difference (mean ± SD)	*p*
Nurick	2.70 ± 1.18	1.78 ± 1.39	0.92 ± 0.88	< 0.001
mJOA	11.84 ± 2.44	14.44 ± 2.95	2.56 ± 1.96	< 0.001
NDI	25.88 ± 8.40	15.54 ± 10.75	10.34 ± 8.96	< 0.001

For abbreviations see under Table 1.

CSM is controversial. The fact that the surgical treatment of CSM is better than no treatment seems to be well established. However, even this assumption continues to be questioned. The results of some studies indicate that the outcome of surgically treated CSM has not improved in comparison to the natural history or nonoperative therapy and notes that there are no clear guidelines for selecting patients who will benefit from the surgery, while other studies favored surgical management [6, 12, 13]. Reasons for that are: the presence of confounding variables, variable course of the disease, and the absence of randomized, controlled studies to allow evidence-based treatment choice [6, 13]. Decompressive surgery for CSM has been the treatment of choice for most patients with neurological deficits. CSM is usually treated with either anterior or posterior decompression with or without fusion, and each has its advantages and disadvantages. The choice between the two is often determined by multiple and obscure rules [14, 15]. However, the choice of strategy for the treatment of CSM is unclear and even today confusion about those topics remains. Numerous publications favor the nonoperative treatment in patients with a stable disease. Kadaňka et al. [16] performed a prospective randomized study comparing conservative and operative treatment of patients with mild and moderate CSM (mJOA > 12) with no or very slow progression and long duration of symptoms. They analyzed clinical outcomes by modified JOA scores, recovery rates, results from a timed 10 m walk test, daily activity scores recorded by video, and subjective assessment of patients. The results of this study suggest that there was no significant difference in clinical outcome measures between groups in the 2-year follow-up period, which means that the surgery did not show better results than the conservative treatment during the follow-up period. Also, Kadanka et al. [17, 18] continued to follow their prospective, randomized study group in a 3 and 10-year follow-up period, and again they concluded there was no significant difference in clinical outcomes in that follow-up period. On the contrary, there were other studies that favor surgical treatment in patients with CSM, and it could not be concluded that surgery for CSM is not useful [6, 11, 19, 20]. Therefore, Fehlings et al. [6] , in their prospective study of 278 patients, found a statistically significant improvement in mJOA after surgery, regardless of the surgical approach. In our prospective study, we also observed the significant improvement in all outcome measurements (mJOA scor, Nurick score and NDI). We found a 2.56 ± 1.96 mean improvement in mJOA score after surgery; in the Nurick score, it was 0.92 ± 0.88, and there was also a significant improvement in the NDI (10.34 ± 8.96). We analyzed the results of surgical treatment of CSM according to the preoperative mJOA (mild, moderate and severe), and we found a significant improvement in all three groups after the surgical treatment.

Conclusion

CSM is a very complex, multifactorial and heterogeneous disease with unpredictable natural history and the most common cause of spinal cord impairment in the elderly. Surgical treatment of CSM resulted in significant improvements in all outcome measures for a 1-year follow-up period, also, surgery prevents further disease progression. The anterior and posterior surgical approaches could be effective in treating CSM and preventing the devastating consequences of this disease. However, new studies are recommended for evaluating the course of the disease to define the optimal surgical strategy and better determine the surgical outcome predictors.

REFERENCES

1. *Kalsi-Ryan S, Karadimas SK, Fehlings MG.* Cervical spondylotic myelopathy: the clinical phenomenon and the current pathobiology of an incrisingly prevalent and devastating disorder. Neuroscientist 2013; 19(4): 409–21.
2. *Nouri A, Tetreault L, Singh A, Karadimas SK, Fehlings MG.* Degenerative Cervical Myelopathy: epidemiology, genetics, and pathogenesis. Spine 2015; 40(12): E675–93.
3. *Tretault L, Tan G, Kopjar B, Côté P, Arnold P, Nugaeva N*, et al. Clinical and surgical predictors of complications following surgery for the treatment of cervical spondylotic myelopathy: results from the multicenter, prospective AOSpine international study of 479 patients. Neurosurgery 2016; 79(1): 33–44.
4. *Côté P, Cassidy JD, Carroll LJ, Kristman V.* The annual incidence and course of neck pain in the general population: a population-based cohort study. Pain 2004; 112(3): 267–73.
5. *Karadimas SK, Erwin WM, Ely CG, Dettori JR, Fehlings MG.* The pathophysiology and natural history of cervical spondylotic myelopathy. Spine (Phila Pa) 2013; 38(22 Suppl 1): S21–36.
6. *Fehlings MG, Wilson JR, Kopjar B, Yoon ST, Arnold PM, Massicotte EM,* et al. Efficacy and safety of surgical decompression in patients with cervical spondylotic myelopathy: results of the AOSpine North America multi-centre study. J Bone Joint Surg Am 2013; 95(18): 1651–8.
7. *Benzel EC, Lancon J, Kesterson L, Hadden T.* Cervical laminectomy and dentate ligamnent section for cervical spondylotic myelopathy. J Spinal Disord 1991; 4(3): 286–95.
8. *Nurick S.* The natural history and the results of surgical treatment of the spinal cord disorder associated with cervical spondylosis. Brain 1972; 95(1): 101–8.
9. *Vernon H, Mior S.* The Neck Disability Index: a study of reliability and validity. J Manipulative Physiol Ther 1991; 14(7): 409–15.
10. *Hirabayashi K, Miyakawa J, Satomi K, Maruyama T, Wakano K.* Operative results and postoperative progression of ossification among patients with ossification of cervical posterior longitudinal ligaments. Spine (Phila Pa 1976) 1981; 6(4): 354–64.
11. *Henderson FC, Geddes JF, Vaccaro AR, Woodard E, Berry KJ, Benzel EC.* Strech-associated injury in cervical spondylotic myelopathy: new concept and review. Neurosurgery 2005; 56(5): 1101–13; discussion 1101–13.
12. *Rowland LP.* Surgical treatment of cervical spondylotic myelopathy: time for a controlled trial. Neurology 1992; 42(1): 5–13.

13. *Tetreault L, Willson JR, Kotter MLN, Côté P, Nouri A, Kopjar B,* et al. Is preoperative duration of symptoms a significant predictors of functional outcomes in patients undergoing surgery for the treatment of degenerative cervical myelopathy? Neurosurgery 2018; https://doi.org/10.1093/neuros/nyy474 (In Press)
14. *Fehlings MG, Tetreault L, Kurpad S, Brodke DS, Wilson JR, Smith JS,* et al. Change in functional impairment, disability, and quality of life following operative treatment for degenerative cervical myelopathy: a systematic review and meta-analysis. Global Spine J 2017; 7(3 Suppl): 53S–69S.
15. *Fehlings MG, Ahuja CS, Mroz T, Hsu W, Harrop J.* Future advances in spine surgery: The AOSpine North America perspective. Neurosurgery 2017; 80(3 Suppl): S1–S8.
16. *Kadanka Z, Bednarík J, Vohánka S, Vlach O, Stejskal L, Chaloupka R*, et al. Conservative treatment versus surgery in spondylotic cervical myelopathy: a prospective randomised study. Eur Spine J 2000; 9(6): 538–44.
17. *Kadanka Z, Mares M, Bednaník J, Smrcka V, Krbec M, Stejskal L*, et al. Approaches to sponylotic cervical myelopathy: conservative versus surgical results in a 3-year follow-up study. Spine (Phila Pa 1976) 2002; 27(20): 2205–10; discussion 2210–1.
18. *Kadaňka Z, Bednařík J, Novotný O, Urbánek I, Dušek L.* Cervical spondylotic myelopathy: conservative versus surgical treatment after 10 years. Eur Spine J 2011; 20(9): 1533–8.
19. *Ghogawala Z, Benzel EC, Riew KD, Bisson EF, Heary RF.* Surgery vs conservative care for cervical spondylotic myelopathy: surgery is appropriate for progressive myelopathy. Neurosurgery 2015; 62(Suppl 1): 56–61.
20. *Badhiwala JH, Witiw CD, Nassiri F, Akbar MA, Mansouri A, Wilson JR*, et al. Efficacy and safety of surgery for mild degenerative cervical myelopathy: results of the AOSpine North America and International prospective multicenter study. Neurosurgery 2019; 84(4): 890–7.

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Gender differences in ischemic heart disease among the Middle-Eastern population

Razlike među polovima kod ishemijske bolesti srca u populaciji Bliskog istoka

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Abstract

Background/Aim. Despite substantial improvements in the outcomes of ischemic heart disease (IHD) in women, it continues to be the leading cause of morbidity and mortality. This paper aimed to study the gender-based differences among the Middle-Eastern population presented with IHD. **Methods.** This was a prospectively designed study where IHD patients who had an indicated coronary angiography (CA) performed at the tertiary cardiac center between 1st September 2014 to 1st September 2015 were analyzed. IHD patients were classified into two groups: stable IHD (SIHD) and acute coronary syndrome (ACS). **Results.** A total of 400 IHD patients had completed coronary angiographic data. About 70% of the patients were diagnosed with ACS and 30% with SIHD. Females were older (64 ± 12 years *vs* 59 ± 13 years, $p < 0.004$) and had higher body mass index (34 ± 7 kg/m2 *vs* 29 ± 5 kg/m2, $p < 0.001$) compared to males. Females were more diagnosed with hypertension (87% *vs* 62%, $p < 0.001$) and diabetes mellitus (76% *vs* 58%, $p < 0.001$) compared to males. Among patients with ACS, males tended to have more ST-elevation myocardial infarction (STEMI) (37% *vs* 12%, $p < 0.001$) whereas females presented more with non-STEMI (45% *vs* 17%, $p < 0.001$. **Conclusion.** Middle-Eastern females tended to have more adverse risk factors, presented more with non-STEMI, and had fewer rates of in-hospital complications.

Key words:
cardiac catheterization; coronary angiography; percutaneous coronary intervention; acute coronary syndrome; middle east; gender.

Apstrakt

Uvod/Cilj. Uprkos znatnom poboljšanju ishoda liječenja ishemijske bolesti srca (IBS) kod žena, ona i dalje predstavlja vodeći uzrok mortaliteta i morbiditeta. Cilj je bio ispitati razlike vezane za ishemijsku bolest srca zasnovane na razlikama kod polova u populaciji Bliskog istoka. **Metode.** Prospektivnom studijom analizirani su bolesnici sa IBS kojima je urađena elektivna koronarna angiografija u kardiološkom centru tercijernog nivoa u periodu od 1.9. 2014. do 1.9.2015. godine. Bolesnici sa IBS su bili podeljeni na dve grupe: grupu sa stabilnom ishemijskom bolešću srca (SIBS) i grupu sa akutnim koronarnim sindromom (AKS). **Rezultati.** Ukupan broj bolesnika kod kojih je urađena koronarna angiografija je 400. Oko 70% bolesnika je bilo dijagnostifikovano kao AKS, a 30% kao SIBS. Žene su bile starije (64 ± 12 godina *vs* 59 ± 13 godina, $p < 0,004$) i imale su veći indeks telesne mase (34 ± 7 kg/m² *vs* 29 ± 5 kg/m², $p < 0,001$) u poređenju sa muškarcima. Žene su češće imale povišen krvni pritisak (87% *vs* 62%, $p < 0,001$) i šećernu bolest (76% *vs* 58%, $p < 0,001$). Među bolesnicima sa AKS, muškarci su češće imali infarkt miokarda sa elevacijom ST segmenta (STEMI) (37% *vs* 12%, $p < 0.001$), dok su se žene češće prezentovale sa non-STEMI (45% *vs* 17%, $p < 0,001$). **Zaključak.** Žene sa Bliskog istoka su češće imale faktore rizika, prezentovale se češće sa non-STEMI i imale su manju stopu intrahospitalnih komplikacija.

Ključne reči:
kateterizacija srca; angiografija koronarnih arterija; perkutana koronarna intervencija; akutni koronarni sindrom; bliski istok; pol.

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Introduction

Ischemic heart disease (IHD) is the most common cause of morbidity and mortality worldwide [1]. Chest pain is a common presentation of the acute coronary syndrome (ACS). However, women tend to have more atypical symptoms [2, 3]. Studies have shown that men presented more with ST-elevation myocardial infarction (STEMI), whereas women presented more with non-STEMI (NSTEMI) [4]. Symptomatic women undergoing coronary angiography (CA) tend to have less extensive and severe coronary artery disease (CAD), but more adverse prognosis compared to men [4].

The gender differences in mortality after reperfusion are predominantly explained by baseline differences, including advanced age and greater comorbidity in women. Procedural success in percutaneous coronary intervention (PCI) is similar in men and women, although women tend to experience more bleeding complications [5]. Women also face higher mortality from IHD due to their relatively higher prevalence of "female-pattern" IHD [6]. Application of guidelines therapy is improving outcomes in women. However, mechanisms and interventions directed at gender differences in IHD are still a matter for debate [7].

In this prospectively designed study, the CA data registry was used to study the gender-based differences among the Middle-Eastern population presented with IHD in the tertiary interventional cardiac center.

Methods

The study was designed as a prospective observational cohort study. The study subjects were enrolled with ACS, referred to the Chest Diseases Hospital (CDH) from the public sector (Ministry of Health Hospitals) as well as the private hospitals, for an indicated cardiac CA with possible PCI if needed.

Study subjects and data collection

Study subjects were prospectively enrolled from 1st September 2014 to 1st September 2015. According to the inclusion criteria patients with the age of 18 years and above with IHD diagnosis were included in the study.

IHD diagnosis including both stable and ACS variables was based on the American College of Cardiology clinical data standards. Briefly, stable IHD (SIHD) patients were those who had unacceptable ischemic symptoms despite medical therapy and who were amenable to, and candidates for coronary revascularization, or whose clinical characteristics and results of noninvasive testing (exclusive of stress testing) indicated a high likelihood of severe IHD, and who were amenable to, and candidates for coronary revascularization, as well as those who could not undergo diagnostic stress testing, or had indeterminate or non-diagnostic stress tests when there was a high likelihood that the findings would result in important changes to therapy [7].

ACS was defined as a clinical presentation consistent with unstable angina (UA)/NSTEMI or STEMI within 8 days of admission, associated with any one of the following: ECG changes, elevated biomarkers of myocardial necrosis (any one of CPK-MB/troponin-T or troponin-I). STEMI was diagnosed if ECG showed evidence of ST segment elevation in ≥ 2 contiguous leads or a new left bundle branch block in addition to chest pain or elevated cardiac markers. The rest of the cases were labeled as NSTEMI/UA based on the presence of elevated biomarkers of myocardial necrosis with or without chest pain.

The study protocol was approved by the institutional review board, and all patients provided written informed consent, which included consent for the CA. Patients younger than 18 years of age and patients refusing to give consent were excluded from the study.

Statistical methods

Demographic and baseline characteristics, treatment patterns, angiographic status, and in-hospital outcomes were compared between men and women overall and according to ACS status: UA/NSTEMI and STEMI. Since patients often had multiple lesions intervened upon during a single PCI laboratory visit, lesion characteristics were assigned as follows: for each characteristic, the highest risk value of any lesion intervened upon during the index PCI was recorded. Continuous variables were described as medians (with inter-quartile ranges) and categorical variables were described as frequencies. Continuous and ordinal categorical variables were compared using stratum adjusted Wilcoxon rank sum test, whereas nominal categorical variables were compared using stratum adjusted χ^2 test where stratification is done by hospital. User-defined missing values were treated as missing. In examining the relationship between gender and outcomes, as well as gender and medical treatments, comparison adjusting for ACS status alone was initially performed. A *p*-value of 0.05 was established as the level of statistical significance for all tests. All analyses were performed using SAS software (versions 8.2, SAS Institute, Cary, NC).

Results

A total of 400 IHD patients had completed coronary angiographic data. Their mean age was 61 ± 12 years, and 64% were males. About 70% of the patients were diagnosed with ACS, and 30% were diagnosed with SIHD. Females were much older (64 ± 12 years *vs* 59 ± 13 years, $p < 0.004$) and had higher body mass index (34 kg/m^2 ± 7 *vs* 29 ± 5 kg/m^2, $p < 0.001$) compared to males. Females tended to have more adverse risk factors. Hypertension was diagnosed in 87% females and 62% males ($p < 0.001$), and diabetes mellitus was diagnosed in 76% females and 58% males ($p < 0.001$) (Tables 1 and 2).

Table 1

Demographics and gender differences among patients with IHD

Patients	All patients (n = 400)	ACS (group A) (n = 279)	SIHB (group B) (n = 121)	*p*
Sex, n (%)				
male	256 (64)	177 (63.4)	79 (65.3)	0.724
female	144 (36)	102 (36.6)	42 (34.7)	
Age (years), mean ± SD				
total population	60.8 ± 12.5	60.5 ± 12.5	61.7 ± 12.0	0.004
male	58.9 ± 12.6	58.8 ± 12.8	59.1 ± 12.1	male *vs* female (group A)
female	64.2 ± 11.7	63.3 ± 12.1	66.4 ± 10.3	
Marital status, n (%)				
single	6 (1.5)	2 (0.7)	4 (3.3)	
married	330 (82.5)	230 (82.4)	100 (82.6)	0.268
widowed	53 (13.3)	39 (14)	14 (11.6)	
divorced	11 (2.8)	8 (2.9)	3 (2.5)	
Height (cm), mean ± SD				
total population	164.6 ± 8.9	164.6 ± 8.5	164.5 ± 9.6	0.917
male	168.9 ± 6.8	168.9 ± 6.2	168.8 ± 7.9	0.926
female	157 ± 6.7	157.2 ± 6.6	156.4 ± 7	0.544
Weight (kg), mean ± SD				
total population	83.3 ± 16.7	83.3 ± 16.2	83.4 ± 17.8	0.978
male	83.2 ± 15.7	82.7 ± 14.9	84.3 ± 17.4	0.446
female	83.5 ± 18.3	84.3 ± 18.2	81.5 ± 18.7	0.402
BMI (kg/m^2), mean ± SD				
total population	30.8 ± 6.3	30.9 ± 6.4	30.8 ± 6.2	0.936
male	29.1 ± 5	29 ± 4.8	29.5 ± 5.3	0.000
female	33.9 ± 7.2	34.6 ± 7.3	33.2 ± 6.9	male *vs* female (group A)
BMI classification, n (%)				
underweight	6 (1.5)	5 (1.8)	1 (0.8)	
normal	63 (15.8)	41 (14.7)	22 (18.2)	0.890
overweight	137 (34.3)	98 (35.1)	39 (32.2)	
obese	194 (48.5)	135 (48.4)	59 (48.8)	
Waist circumference (cm), mean ± SD				
total population	104.3 ± 16.3	104.8 ± 15.5	103.1 ± 18.1	0.355
male	101.8 ± 14.2	101.7 ± 13	102.1 ± 16.6	0.000
female	108.6 ± 18.8	110.1 ± 17.9	105 ± 20.5	male *vs* female (group A)

IHD – ischemic heart disease; ACS – acute coronary syndrome; SIHD – stable IHD; BMI – body mass index; SD – standard deviation.

Table 2

Risk factors and gender differences among patients with IHD

Category	All patients (n = 400)	Total population male (n = 256)	Total population female (n = 144)	*p*	ACS (group A) (n = 279)	SIHD (group B) (n = 121)	*p*
Hypertension	284 (71)	159 (62.1)	125 (86.8)	0.000	196 (70.3)	88 (72.7)	0.617
Dyslipidemia	209 (52.3)	133 (52)	76 (52.8)	0.874	147 (57.2)	62 (51.2)	0.791
Diabetes mellitus	257 (64.3)	148 (57.8)	109 (75.7)	0.000	184 (65.9)	73 (60.3)	0.283
Smoking history							
non-smoker	208 (52)	75 (29.3)	133 (92.4)		150 (53.8)	58 (47.9)	
previous smoker (stopped > 1 year ago)	48 (12)	46 (18)	2 (1.4)	0.000	28 (10)	20 (16.5)	
recent smoker (stopped 1 year, 1 month ago)	8 (2)	4 (1.6)	4 (2.8)		6 (2.2)	2 (1.7)	0.738
current smoker	136 (34)	131 (51.2)	5 (3.5)		95 (34.1)	41 (33.9)	
Prior MI	128 (32)	86 (33.6)	42 (29.2)	0.364	82 (29.4)	46 (38)	0.090
Prior PCI	82 (20.5)	59 (23)	23 (16)	0.093	49 (17.6)	33 (27.3)	0.027
Prior CABG	25 (6.3)	18 (7)	7 (4.9)	0.391	15 (5.4)	10 (8.3)	0.274
Prior HF	38 (9.5)	16 (10.2)	12 (8.3)	0.552	28 (10)	10 (8.3)	0.580
Prior stroke	38 (9.5)	24 (9.4)	14 (9.7)	0.910	32 (11.5)	6 (5)	0.041
Peptic ulcer	25 (6.3)	19 (7.4)	6 (4.2)	0.198	19 (6.8)	6 (5)	0.484
Chronic renal failure	28 (7)	19 (7.4)	9 (6.3)	0.660	20 (7.2)	8 (6.6)	0.842
on dialysis	6 (1.5)	5 (2)	1 (0.7)	0.379	3 (1.1)	3 (2.5)	0.204
Chronic lung disease	39 (9.8)	18 (7)	21 (14.6)	0.014	23 (8.2)	16 (13.2)	0.124

IHD – ischemic heart disease; ACS – acute coronary syndrome; SIHD – stable ischaemic heart disease; MI – myocardial infarction; PCI – percutaneous coronary intervention; CABG – coronary artery by-pass grafting; HF – heart failure.

Gender differences among patients with ACS

Of the 279 ACS patients, 60 patients were diagnosed with UA (32 males and 28 females), 122 were diagnosed with NSTEMI (34 males and 89 females), and 97 patients were diagnosed with STEMI (74 males and 23 females). Females presented more with NSTEMI (45% females *vs* 17% males, $p < 0.001$), whereas males presented more with STEMI (37% males *vs* 12% females, $p < 0.001$). There were no statistically significant differences between males or females concerning UA (16% males *vs* 14% females, $p = 0.57$) (Table 3).

Coronary angiography and gender difference

Of the 400 IHD patients, CA was diagnosed as normal in 30%. Of these, females had less normal coronaries (36 females *vs* 85 males). About 24% had single-vessel disease (54 males *vs* 40 females), 95 had double-vessel disease (60 males *vs* 35 females), and 57 had triple-vessel disease (36 males *vs* 21 females). Left main disease was diagnosed in 3 patients (1 male *vs* 2 females) (Table 4).

Table 3

Gender differences among patients with ACS

Characteristics	All patients n (%)	Male n (%)	Female n (%)	*p*
Unstable angina at presentation	60 (15)	32 (16)	28 (14)	0.570
NSTEMI at presentation	122 (31)	34 (17)	89 (45)	< 0.001
STEMI at presentation	97 (24)	74 (37)	23 (12)	< 0.001
Positive EET	96 (24)	50 (25)	46 (23)	0.640
Abnormal nuclear	140 (35)	62 (31)	78 (39)	0.090
Prior PCI	46 (12)	22 (11)	24 (22)	0.752
Prior CABG	38 (98)	24 (12)	14 (7)	0.081
Pre-procedural aspirin	390 (98)	196 (98)	194 (97)	0.521
Pre-procedural clopidogrel	372 (93)	191 (96)	181 (91)	0.051

NSTEMI – non-ST-elevation myocardial infarction; STEM – myocardial infarction with ST-elevation; ACS – acute coronary syndrome; PCI – percutaneous coronary intervention; CABG – coronary artery by-pass grafting; EET – treadmill exercise test.

Table 4

Coronary angiography findings among patients with IHD

Category	All patients (n = 400)	Male (n = 256)	Female (n = 144)	*p*
Vessel disease status, n (%)				
Normal	121 (30.3)	85 (33.3)	36 (25)	
SVD	94 (23.5)	54 (21.1)	40 (27.8)	
DVD	95 (23.8)	60 (23.4)	35 (24.7)	
TVD	57 (14.2)	36 (14.1)	21 (14.6)	0.471
LM	3 (0.3)	1 (0.4)	2 (1.4)	
LM + DVD	18 (4.5)	14 (5.5)	4 (2.8)	
LM + TVD	12 (3)	6 (2.3)	6 (4.2)	
Vessel classifications, n (%)				
LM stenosis	21 (5.3)	10 (3.9)	11 (7.6)	0.109
LAD stenosis	210 (52.5)	128 (50)	82 (56.9)	0.183
LCX stenosis	152 (38)	99 (38.7)	53 (36.8)	0.713
RCA stenosis	154 (38.5)	99 (38.7)	55 (38.2)	0.925
Treatment options, n (%)				
Medical	153 (38.3)	106 (41.4)	47 (32.6)	
PCI	196 (49)	120 (46.9)	76 (52.8)	0.095
CABG	51 (12.8)	30 (11.7)	21 (14.6)	
PCI, n (%)				
BMS used	20 (10.2)	9 (7.5)	11 (14.5)	0.117
DES used	176 (89.8)	111 (92.5)	65 (85.5)	0.117
PCI to ISR	2 (0.5)	2 (0.8)	0	0.270
PCI to bifurcation	4 (1)	3 (1.2)	1 (1.3)	0.570
Average waiting period for PCI/CABG, n (%)				
1 day	389 (97.3)	249 (97.3)	140 (97.2)	0.980
2 days	11 (2.8)	7 (2.7)	4 (2.8)	

IHD – ischemic heart disease: SVD – single-vessel disease; DVD – double-vessel disease; TVD – triple-vessel disease; LM – left main coronary artery disease; LAD – left anterior descending coronary artery; Lcx – left circumflex artery; RCA – right coronary artery; PCI – percutaneous coronary intervention; CABG – coronary artery bypass grafting; BMS – bare metal stents;
DES – drug-eluting stents; ISR – in-stent restenosis.

In-hospital outcomes

Stent thrombosis tended to be more frequent in males than females (3% *vs* 0%), but females had more heart failure than males (4% vs 1%). The most significant complication differences were in acute kidney injury. Males tended to have more frequent kidney injuries than females (5% *vs* 1%), while women had more fever compared to males (8% *vs* 3%) (Table 5).

Table 5

In-hospital outcomes (PCI complications) among IHD population

Characteristics	All patients n (%)	Male n (%)	Female n (%)	*p*
Failure of PCI	2 (1)	1 (1)	1 (1)	0.990
Bifurcation Stenting	10 (3)	4 (2)	6 (3)	0.052
PCI of ISR	6 (2)	3 (2)	3 (2)	0.990
Stent thrombosis	3 (1)	3 (2)	0 (0)	0.080
Heart Failure	10 (3)	2 (1)	8 (4)	0.050
CIN	10 (3)	9 (5)	1 (1)	0.010
VT/VF	6 (2)	3 (2)	3 (2)	0.990
Infection (fever)	20 (5)	5 (3)	15 (8)	0.020
Pseudo aneurysm	6 (2)	3 (2)	3 (2)	0.990
Groin hematoma	10 (3)	6 (3)	4 (2)	0.520
Retroperitoneal bleeding	2 (1)	1 (1)	1 (1)	0.990
MI as a complication	9 (2)	3 (2)	6 (3)	0.50
CVA	3 (1)	0 (0)	3 (2)	0.240
Death	1 (1)	0 (0)	1 (1)	0.990

PCI – percutaneous coronary intervention; IHD – ischemic heart disease; ISR – in-stent restenosis; CIN – contrast-induced nephropathy; VT/VF – ventricular tachycardia/ventricular fibrillation; MI – myocardial infarction; CVA – cerebrovascular accidents.

Discussion

Techniques of treatment should be custom designed for each gender, as our registry revealed lesser high-risk angiographic features but more in-hospital complication rates in females than in males. This should not only be instrumental in reducing post-intervention complications but shall also aid to improve the appropriate antiplatelet therapy adherence and efficacy.

In our study, patients were admitted to general hospitals for non-invasive cardiology services. This indicates low referral rates for invasive strategies. The same is in harmony with the results of the recent ACS registry published in Kuwait, which shows the rates of in-hospital coronary angiography cases as significantly lower (21% for NSTEMI, 17% for STEMI, and 15% for UA [8]) as compared to the Global Registry of Acute Coronary Events (GRACE) rate of 53% for NSTEMI, 55% for STEMI, and 42% for UA [9]. In Kuwait, there was an acute lack of onsite cardiac cath-labs in general hospitals which was stretching the capacity of the sole invasive cardiac centre, which was our site. This might have been a major cause for the lesser number of in-hospital coronary angiograms, the theory which was also proposed worldwide by Fox et al. [10]. In addition, there may be many intrinsic biological mechanisms that require more studies, specifically at the basic level.

The rate of women presenting with UA/NSTEMI and having atypical symptoms was significantly higher compared to men, which was similar to the findings in Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO) IIb study, which attributed this to the differences in anatomy, the pathophysiology of CAD, and clinical characteristics in the two genders [11]. However, this might also be due to lesser utilization of the acute antiplatelet therapy on admission in females and due to unnoticed reasons, even though it was proven in other trials [12, 13], as well as our own, that women with ACS are older and have more co-morbidities. Nevertheless, as opposed to the findings of Blomkalns et al. [14] that women with ACS present more often with both prior and current signs of congestive heart failure, we found similar or better left ventricular functions in terms of ejection fraction (EF) in women.

Chest pain was not a common finding in elderly males or females, and if found, it was milder or more often absent in females than in their male counterparts , possibly owing to the higher comorbidities like diabetes mellitus. However, even though chest pain incidences were predominant in many cases of those below 55 years of age, regardless of the ACS type, women in the same group seemed to have higher asymptomatic presentation rates [3]. Although there were high similarities between our patient cohort and those of the Euro Heart Survey II [15], our survey into the treatment and short-term prognosis revealed some noticeable differences.

Even though women mostly presented with atypical cases and Killip I (as against Killip III for males), and were mostly diagnosed as NSTEMI, most males could be managed by PCI while women had higher CABG rates.

Given the information that the results of PCI may be inferior to CABG, most subjects did not defer from choosing PCI over surgery. Higher mortality rates of post-PCI in females have been decreasing [16–19], and as for the National

Heart, Lung and Blood Institute, registries have slumped from 2.6% in 1985 to 1.5% in 1994 (*p*-value not significant) [20].

Variations in procedural outcomes owing to differences from presentation, diagnosis, management, and treatment in the two genders were identified. However, more work needs to be done to identify and explain these based on inherent biological differences between males and females [21].

Conclusion

In this single-centre cohort study, it was found that among the Middle-Eastern population, females tended to have more adverse risk factors, presented more with non-STEMI, and had fewer rates of in-hospital complications than males.

R E F E R E N C E S

1. *Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ.* Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet 2006; 367(9524): 1747–57.
2. *Akhter N, Milford-Beland S, Roe MT, Piana RN, Kao J, Shroff A.* Gender differences among patients with acute coronary syndromes undergoing percutaneous coronary intervention in the American College of Cardiology-National Cardiovascular Data Registry (ACC-NCDR). Am Heart J 2009; 157(1): 141–8.
3. *Khan NA, Daskalopoulou SS, Karp I, Eisenberg MJ, Pelletier R, Tsadok MA*, et al. Sex differences in acute coronary syndrome symptom presentation in young patients. JAMA Intern Med 2013; 173(20): 1863–71.
4. *Canto JG, Rogers WJ, Goldberg RJ, Peterson ED, Wenger NK, Vaccarino V*, et al. Association of age and sex with myocardial infarction symptom presentation and in-hospital mortality. JAMA 2012; 307(8): 813–22.
5. *Maas AH, Appelman YE.* Gender differences in coronary heart disease. Neth Heart J 2010; 18(12): 598–602.
6. *Khan NA, Daskalopoulou SS, Karp I, Eisenberg MJ, Pelletier R, Tsadok MA*, et al. Sex differences in prodromal symptoms in acute coronary syndrome in patients aged 55 years or younger. Heart 2017; 103(11): 863–9.
7. *Fihn SD, Blankenship JC, Alexander KP, Bittl JA, Byrne JG, Fletcher BJ*, et al. 2014 ACC/AHA/AATS/PCNA/SCAI/STS focused update of the guideline for the diagnosis and management of patients with stable ischemic heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines, and the American Association for Thoracic Surgery, Preventive Cardiovascular Nurses Association, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. J Thorac Cardiovasc Surg 2015; 149(3): e5–23.
8. *Zubaid M, Rashed WA, Saad H, Attiya A, Al-Banat BA, Ridha M*, et al. Kuwait acute coronary syndromes registry: baseline characteristics, management practices and in-hospital outcomes of patients hospitalized with acute coronary syndromes in Kuwait. Med Princ Pract 2007; 16(6): 407–12.
9. *Steg PG, Goldberg RJ, Gore JM, Fox KA, Eagle KA, Flather MD,* et al. Baseline characteristics, management practices, and in-hospital outcomes of patients hospitalized with acute coronary syndromes in the Global Registry of Acute Coronary Events (GRACE). Am J Cardiol 2002; 90(4): 358–63.
10. *Fox KA, Goodman SG, Klein W, Brieger D, Steg PG, Dabbous O,* et al. Management of acute coronary syndromes. Variations in practice and outcome; findings from the Global Registry of Acute Coronary Events (GRACE). Eur Heart J 2002; 23(15): 1177–89.
11. *Hochman JS, Tamis JE, Thompson TD, Weaver WD, White HD, Van de Werf F*, et al. Sex, clinical presentation, and outcome in patients with acute coronary syndromes. Global Use of Strategies to Open Occluded Coronary Arteries in Acute Coronary Syndromes IIb Investigators. N Engl J Med 1999; 341(4): 226–32.
12. *Anand SS, Xie CC, Mehta S, Franzosi MG, Joyner C, Chrolavicius S,* et al. Differences in the management and prognosis of women and men who suffer from acute coronary syndromes. J Am Coll Cardiol 2005; 46(10): 1845–51.
13. *Vaccarino V, Rathore SS, Wenger NK, Frederick PD, Abramson JL, Barron HV*, et al. Sex and racial differences in the management of acute myocardial infarction, 1994 through 2002. N Engl J Med 2005; 353(7): 671–82.
14. *Blomkalns AL, Chen AY, Hochman JS, Peterson ED, Trynosky K, Diercks DB,* et al. Gender disparities in the diagnosis and treatment of non-ST-segment elevation acute coronary syndromes: large-scale observations from the CRUSADE (Can Rapid Risk Stratification of Unstable Angina Patients Suppress Adverse Outcomes With Early Implementation of the American College of Cardiology/American Heart Association Guidelines) National Quality Improvement Initiative. J Am Coll Cardiol 2005; 45(6): 832–7.
15. *Mandelzweig L, Battler A, Boyko V, Bueno H, Danchin N, Filippatos G*, et al. The second Euro Heart Survey on acute coronary syndromes: Characteristics, treatment, and outcome of patients with ACS in Europe and the Mediterranean Basin in 2004. Eur Heart J 2006; 27(19): 2285–93.
16. *Fang J, Alderman MH.* Gender differences of revascularization in patients with acute myocardial infarction. Am J Cardiol 2006; 97(12): 1722–6.
17. *Radovanovic D, Erne P, Urban P, Bertel O, Rickli H, Gaspoz JM.* AMIS Plus Investigators. Gender differences in management and outcomes in patients with acute coronary syndromes: results on 20,290 patients from the AMIS Plus Registry. Heart 2007; 93(11): 1369–75.
18. *Abramson JL, Veledar E, Weintraub WS, Vaccarino V.* Association between gender and in-hospital mortality after percutaneous coronary intervention according to age. Am J Cardiol 2003; 91(8): 968–71, A4.
19. *Mikhail GW.* Coronary revascularisation in women. Heart 2006; 92(Suppl 3): iii19–23.
20. *Jacobs AK, Kelsey SF, Yeh W, Holmes DR Jr, Block PC, Cowley MJ*, et al. Documentation of decline in morbidity in women undergoing coronary angioplasty (a report from the 1993-94 NHLBI Percutaneous Transluminal Coronary Angioplasty Registry). National Heart, Lung, and Blood Institute. Am J Cardiol 1997; 80(8): 979–84.
21. *Jacobs AK.* Women, ischemic heart disease, revascularization, and the gender gap: what are we missing? J Am Coll Cardiol 2006; 47(3 Suppl): S63–5.

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Morphometric analysis of glomeruli, clinical features and outcome in obese and non-obese patients with focal segmental glomerulosclerosis patients

Morfometrijska analiza glomerula, klinički tok i ishod bolesti kod gojaznih i negojaznih bolesnika sa fokalno segmentnom glomerulosklerozom

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Abstract

Background/Aim. In the past three decades, focal segmental glomerulosclerosis (FSGS) was commonly regarded as a part of obesity-related glomerulopathy (ORG), a distinct entity featuring proteinuria, glomerulomegaly, progressive glomerulosclerosis, and a decline of renal function. The present study aimed to evaluate the glomerular morphometry, clinical features, and a two-year outcome in the obese and non-obese FSGS patients. **Methods.** The study included 35 FSGS patients (23 males, aged 46.5 ± 15.2 years) divided into two groups: obese [body mass index (BMI) ≥ 27 kg/m^2 (18 patients, aged 47.2 ± 15.5 years)] and non-obese [BMI < 27 kg/m^2 (17 patients, aged 45.7 ± 15.2 years)]. The serum concentrations of proteins, albumin, cholesterol, triglyceride, and creatinine were determined at the time of the biopsy, and 6, 12, and 24 months after the biopsy. Cockcroft-Gault (BMI < 27 kg/m^2) and Cockcroft-Gault$_{LBW}$ (BMI ≥ 27 kg/m^2) formulas were calculated. Glomerular radius (GR), glomerular volume (GV), and glomerular density (GD) were compared morphometrically between the two groups. **Results.** At the time of the kidney biopsy and 6 months later, the obese had significantly lower glomerular filtration rate (GFR) compared to the non-obese. After 24 months of follow-up, there were not any differences between the groups. The obese had a significantly higher GR (109.44 ± 6.03 μm vs. 98.53 ± 14.38 μm) and GV (3.13 ± 0.49 × 10^6 μm^3 vs. 2.26 ± 0.83 × 10^6 μm^3), and only slightly lower GD (1.91 ± 0.39/mm^2 vs. 1.95 ± 0.61/mm^2) compared to the non-obese. A significant positive association between GV and BMI (r = 0.439) was found. After 12 months of follow-up, a significantly higher percentage of the non-obese patients reached complete remission compared to the obese (71.4% vs. 37.5%, respectively; $p = 0.041$), but after 24 months there were no significant differences. **Conclusion.** Obese patients, at the time of the kidney biopsy and 6 months later, had already a significantly lower kidney function compared to the non-obese ones. However, 12 and 24 months after, this difference was not statistically significant. Also, 24 months after, there was no significant difference between the two groups in the percentage of patients with complete remission of the nephrotic syndrome.

Key words:
biopsy; glomerular filtration rate; glomerulosclerosis, focal segmental; kidney glomerulus; obesity; risk assessment; treatment outcome.

Apstrakt

Uvod/Cilj. U poslednje tri decenije fokalno segmentna glomeruloskleroza (FSGS) je predstavljena kao oblik glomerulopatije uslovljene gojaznošću (GUG), poseban entitet karakterisan proteinurijom, glomerulomegalijom, progresivnom glomerulosklerozom i smanjenjem bubrežne funkcije. Cilj ove studije bio je odrediti morfometriju glomerula, klinički tok i ishod nakon dve godine praćenja gojaznih i negojaznih FSGS bolesnika.

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Metode. Studija je obuhvatila 35 FSGS bolesnika (23 muškaraca, starosti od 46,5 ± 15,2 godina), podeljenih u 47,2 ± 15,5 godina] i negojazni [BMI < 27 kg/m² (17 bolesnika, starosti od 45,7 ± 15,2 godina)]. Merena je serumska koncentracija proteina, albumina, holesterola, triglicerida i kreatinina u momentu biopsije, kao i 6, 12 i 24 meseca nakon biopsije. Jačina glomerulske filtracije (JGF) procenjena je pomoću formula Cockcroft-Gault (BMI < 27 kg/m²) i Cockcroft-Gault$_{LBW}$ (BMI ≥ 27 kg/m²). Između dve grupe morfometrijski su poređeni poluprečnik glomerula (PG), volumen glomerula (VG) i gustina glomerula (GG) **Rezultati.** U vreme biopsije i nakon 6 meseci, gojazni su imali značajno nižu JGF u poređenju sa negojaznim. Nakon 24 meseca praćenja, nije bilo razlike između grupa. Gojazni su imali statistički značajno viši PG (109,44 ± 6,03 μm vs. 98,53 ± 14,38 μm) i VG (3,13 ± 0,49 x 10^6 μm³ vs. 2,26 ± 0,83 x 10^6 μm³), ali nižu GG bez značajne razlike u poređenju sa negojaznim (1,91 ± 0,39/mm² vs. 1,95 ± 0,61/mm²). dve grupe: gojazni [body mass index (BMI) – indeks telesne mase ≥ 27 kg/m² – 18 bolesnika, starosti od Pronađena je značajna, pozitivna korelacija između VG i BMI (r = 0,439). Nakon 12 meseci praćenja, značajno viši procenat negojaznih bolesnika ušlo je u kompletnu remisiju u poređenju sa gojaznim (71,4% vs. 37,5%; p = 0,041), ali, nakon 24 meseca nije bilo značajne razlike između grupa. **Zaključak.** Gojazni bolesnici su u vreme biopsije bubrega i nakon 6 meseci praćenja imali značajno nižu JGF u poređenju sa negojaznim bolesnicima. Međutim, nakon 12 i 24 meseca, ova statistički značajna razlika se izgubila. Takođe, posle 24 meseca praćenja nije bilo značajne razlike između dve grupe u procentu bolesnika sa kompletnom remisijom nefrotskog sindroma.

Ključne reči:
biopsija; glomerulska filtracija, brzina; glomeruloskleroza, fokalna, segmentna; bubreg, glomerul; gojaznost; rizik, procena; lečenje, ishod.

Introduction

Focal segmental glomerulosclerosis (FSGS), with the increasing prevalence worldwide, describes both a common lesion in progressive kidney disease and a disease characterized by marked proteinuria and podocyte injury [1]. Thus, FSGS defines several clinical and pathological syndromes that may be primary (idiopathic) or secondary, mediated by adaptive structural-functional responses. These adaptive forms include not only patients with congenital anomalies, but also patients with an acquired reduction of the functional nephron mass. Other secondary forms are associated with hemodynamic stress placed on an initially normal nephron population (hypertension, atheroembolism, sickle cell anemia, increased lean body mass, and obesity) [2]. In the past three decades, kidney biopsy findings of focal and segmental glomerulosclerosis were commonly regarded as a part of the obesity-related glomerulopathy (ORG), a distinct entity featuring proteinuria, glomerulomegaly, progressive glomerulosclerosis, and progressive renal functional decline. This pathohistological entity is described as a secondary form of glomerular disease in obese patients with morphological characteristics of FSGS and enlargement of the glomeruli or only by enlargement of the glomeruli. Fortunately, not all obese persons develop ORG [3, 4]. A typical clinical feature of ORG is medium to massive proteinuria without reducing serum albumin levels or without developing nephrotic syndrome. This clinical feature is important in the differential diagnosis of ORG from primary FSGS in which massive proteinuria is followed by the development of full-blown nephrotic syndrome [5]. Moreover, the progression of ORG to end-stage renal disease (ESRD) is slower than in primary FSGS (5 years renal survival, 75% vs. 50%), even though 10% to 30% of ORG patients start the dialysis treatment [4, 6–8].

During the last 15 years, there has been an equivalent dramatic rise in the prevalence of obesity and ESRD, increasing the interest in the role of obesity-related kidney disease. Not only does obesity increase the risk of preexisting renal disease progression but is also in itself an independent risk factor of renal injury [9].

Usually, in everyday clinical practice, it is not easy to distinguish primary from secondary forms of FSGS, especially in obese patients. On the one hand, the main histopathological features in ORG patients are FSGS with subtle differences from primary FSGS (perihilar FSGS variant, glomerulomegaly, foot process effacement usually in less than 50% of glomerular surface area) [4]. On the other hand, obesity can accelerate the progression of an already existing renal injury.

Methods

Patients

The study included 35 adult FSGS patients (23 males) with a mean age of 46.5 ± 15.2 years (range 21–72 years). Indications for kidney biopsy were: nephrotic syndrome, pathological proteinuria without the nephrotic syndrome, or abnormal urinary sediment. Renal biopsies from patients with secondary FSGS other than ORG and with diabetic nephropathy were cautiously excluded.

Obesity was defined as BMI ≥ 27 kg/m² and patients were divided into two groups: obese with BMI ≥ 27 kg/m² (18 patients, 14 males, mean age 47.2 ± 15.5, mean BMI 32.41 ± 3.47 kg/ m²) and non-obese with BMI < 27 kg/m² (17 patients, 9 males, mean age 45.7 ± 15.2, mean BMI 23.99 ± 2.11 kg/m²).

The study protocol was conformed with ethical guidelines, approved by the Faculty of Medicine, Belgrade University Ethics Committee (number 29/III-9), and informed consent was obtained from each participant.

After the histopathological diagnosis, the participants were treated according to the established protocols for FSGS. Some of them received oral corticosteroid therapy 1 mg/kg with symptomatic therapy, and some of them were only

symptomatically treated. The symptomatic therapy included angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor type 1 blockers (ARBs) [10]. The special nutrition diet for obese patients was not strictly recommended. All the patients were carefully followed up 6, 12, and 24 months after the kidney biopsy. Complete remission of the nephrotic syndrome was defined with daily proteinuria less than 1 g/day with normalization of protein, albumin, and lipids serum concentration and partial remission with daily proteinuria between 1–3 g/day.

Laboratory methods

Hematological, as well as biochemical analyses were done at the time of kidney biopsy, as well as 6, 12, and 24 months after the biopsy. A hematological analyzer (The Beckman Coulter HmX) was used to provide a complete hematological profile. The serum concentration of protein, albumin, cholesterol, triglyceride, and creatinine was determined on the biochemical analyzer DXC 800, Beckman Coulter. The serum creatinine level was measured according to the Jaffe method. The proteinuria was determined by spectrophotometry with the pyrogall red protein assay. Only samples with a sterile urine culture were processed. Urine sediments with more than 3 red blood cells (RBC)/hpf or 5 white blood cells (WBC)/hpf were defined as clinically significant erythrocyturia or leukocyturia.

The estimated glomerular filtration rate (eGFR) was calculated according to the following formulas:

A.Cockcroft-Gault – for participants with BMI < 27 kg/m^2 [11]:

eGFR = [(140–age) × body weight/(72 × serum creatinine)] × 0.85 (correction factor for female);

B.Cockcroft-Gault$_{LBW}$ – for participants with BMI ≥ 27 kg/m^2:

Cockcroft Gault$_{LBW}$ = (140–age) × lean body weight (LBW)/serum creatinine × correction factor (correction factor for male = 1.23; correction factor for female = 1.04)

Lean body weight (LBW) = 9720 × body weight/6680 + 216 × BMI for male;

LBW = 9720 × body weight / 8780 + 244 × BMI for female [12].

The morphometric analysis of glomeruli

A percutaneous biopsy of the inferior pole of the left kidney was done under ultrasound control. The samples were relatively equal in the number of glomeruli and approximately of the same size. All tissue samples were routinely processed, cut into 5 μm thick sections, and stained using the Periodic Acid-Schiff method (PAS). Whole tissue sections were analyzed (Olympus BX51Tokyo, Japan) and captured (Olympus DP70 camera) at magnification x 12.5. The number of glomeruli in each section is determined. All present glomeruli were also captured at magnification × 400. Microphotographs were analyzed using a computer-assisted image analysis system, ImageJ [13].

The volumes of all glomeruli contained entirely within the serially sectioned material were measured in each case (n = 20 ± 10 glomeruli). Glomerular volume was calculated by the maximal profile area (MPA) method (V_{GMA}) identifying the profile of each glomerulus with the largest area. An ideal radius r_o was derived from the area of the largest profile (APmax) based on the assumption that the profile was a circle:

$$r_o = \sqrt{APmax/\pi}.$$

The volume corresponding to the MPA (V_{GMA}) was then calculated based on the assumption that the glomerulus was a sphere:

$$V_{GMA} = 4/3\ \pi\ r_o^3.$$

Glomerular density was expressed as the average area of tissue in the biopsy sample per one glomerulus in a group of obese and non-obese patients [14].

Statistics

Data are presented as mean values and standard deviation (SD). The Kolmogorov-Smirnov test was used to check the normal distribution of the variables. Data were analyzed using Student's *t*-test (or Mann-Whitney due to distribution) and Pearson's χ^2 test (for nominal data). Relationships between variables were estimated using Pearson's parametric correlation method. Statistical analysis is performed using SPSS software 17.0. Statistical significance is defined as the conventional *p*-value, with the effects being considered significant at $p < 0.05$.

Results

The study included 35 FSGS patients. The patients were divided into two groups: obese with BMI ≥ 27 kg/m^2 (18 patients) and non-obese with BMI < 27 kg/m^2 (17 patients). There was no significant difference between the groups in age and gender. In both groups, the nephrotic syndrome was the major indication for kidney biopsy (72.2% obese vs. 70.6% non-obese); all of the patients had some levels of pathological proteinuria (Table 1).

Table 1

Age, gender, and indications for kidney biopsy in two patient groups

Groups	Age (years) mean ± SD	Gender (m/f), n	Proteinuria n (%)	Eritrocyturia and proteinuria n (%)	*Syndroma nephroticum* n (%)
Obese	47.2 ± 15.5	14/ 4	4 (22.2)	1 (5.6)	13 (72.2)
Non-obese	45.7 ± 15.2	9/ 8	3 (17.6)	2 (11.8)	12 (70.6)
Total	45.5 ± 15.2	23/ 12	7 (20.0)	3 (8.6)	25 (71.4)
p	0.773	0.212		0.783	

m – male; f – female; SD – standard deviation.

Table 2 shows clinical and laboratory data for the two patient groups at the time of kidney biopsy, and 6, 12, and 24 months after the biopsy. At the time of kidney biopsy, the obese patients had only significantly higher serum creatinine concentration and significantly lower eGFR compared to the non-obese patients. In other measured parameters, there were no significant differences. Six months later, eGFR was still lower in the obese than non-obese patients, daily proteinuria was lower in the non-obese patients but not significantly, and there were no other differences between the groups. Twelve months after the kidney biopsy, the non-obese patients had significantly lower daily proteinuria, as well as cholesterol serum concentration, and higher serum protein and albumin concentrations compared to the obese patients. After 24 months of follow-up, no statistically significant difference in the examined variables between the groups could be found (Table 2).

Table 2

Clinical and laboratory data in two patient groups

Variable before kidney biopsy	Obese (mean ± SD)	Non obese (mean ± SD)	*p*
Hemoglobin (g/L)	136.33 ± 25.20	135.35 ± 21.56	0.903
Serum protein (g/L)	54.14 ± 11.29	56.72 ± 9.95	0.556
Serum albumin (g/L)	27.16 ± 10.40	29.11 ± 9.02	0.559
Cholesterol (mmol/L)	8.22 ± 2.68	7.47 ± 2.27	0.381
Triglyceride (mmol/L)	2.96 ± 1.48	2.54 ± 1.36	0.398
Serum creatinine (μmol/L)	144.83 ± 84.98	95.52 ± 43.22	0.040
Cockcroft- Gault (mL/min)#	62.22 ± 31.30	95.01 ± 49.23	0.032
Proteinuria (g/day)	8.29 ± 6.73	7.17 ± 7.83	0.654
6 months after kidney biopsy			
Hemoglobin (g/L**)**	138.05 ± 20.81	140.26 ± 15.49	0.725
Serum protein (g/L)	54.69 ± 10.09	57.76 ± 8.22	0.332
Serum albumin (g/L)	31.89 ± 7.20	34.91 ± 6.35	0.198
Cholesterol (mmol/L)	7.57 ± 2.09	7.70 ± 1.68	0.843
Triglyceride (mmol/L)	2.56 ± 0.95	3.03 ± 1.31	0.237
Serum creatinine (μmol/L)	105.33 ± 69.54	82.21 ± 31.49	0.218
Cockcroft-Gault (mL/min)#	67.9 ± 29.07	114.4 ± 40.03	0.009
Proteinuria (g/day)	5.04 ± 4.69	3.41 ± 3.60	0.259
12 months after kidney biopsy			
Hemoglobin (g/L)	138.00 ± 13.36	143.23 ± 8.99	0.190
Serum protein (g/L)	62.97 ± 9.28	62.48 ± 15.44	0.911
Serum albumin (g/L)	35.68 ± 5.22	35.58 ± 9.06	0.971
Cholesterol (mmol/L)	6.40 ± 1.46	6.06 ± 1.17	0.461
Triglyceride (mmol/L)	2.76 ± 1.02	2.70 ± 1.13	0.870
Serum creatinine (μmol/L)	115.20 ± 80.92	109.76 ± 44.29	0.809
Cockcroft- Gault (mL/min)#	61.87 ± 32.73	74.0 ± 35.19	0.711
Proteinuria (g/day)	4.08 ± 5.65	2.23 ± 1.92	0.210

 # for obese patients – Cockcroft-Gault$_{LBW}$ (mL/min).

Figure 1 presents mean glomerular volume and density in both obese and non-obese patients. Not only did the obese patients have significantly higher glomerular radius (109.44 ± 6.03 μm vs. 98.53 ± 14.38 μm) compered to the non-obese ones (t = 2.729; p = 0.011) but they also had higher glomerular volume (Figure 1 A) (3.13 ± 0.49 x 10^6 μm^3 vs. 2.26 ± 0.83 x 10^6 μm^3) in comparison with the non-obese patients (t = 3.545; p = 0.001). Obese patients had lower glomerular density (1.91 ± 0.39/mm^2 vs. 1.95 ± 0.61/mm^2) but without significant difference (Figure 1 B).

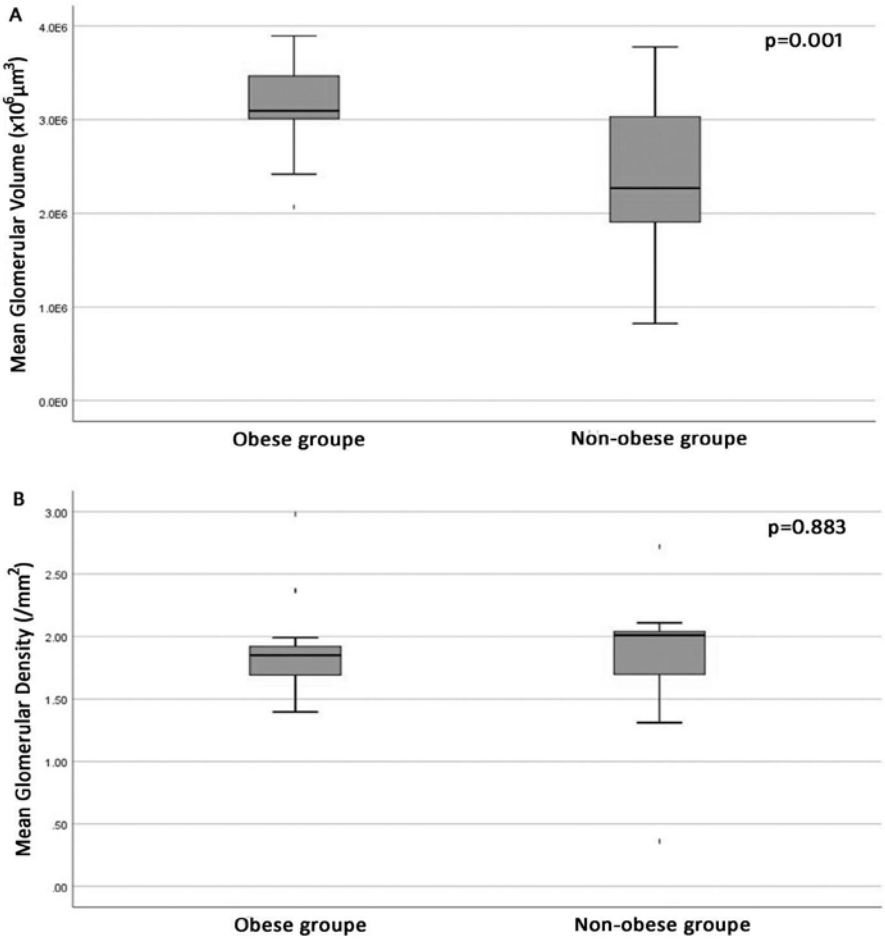

Fig. 1 – Mean glomerular volume (A) and mean glomerular density (B) in obese and non-obese focal segmental glomerulosclerosis (FSGS) patients.

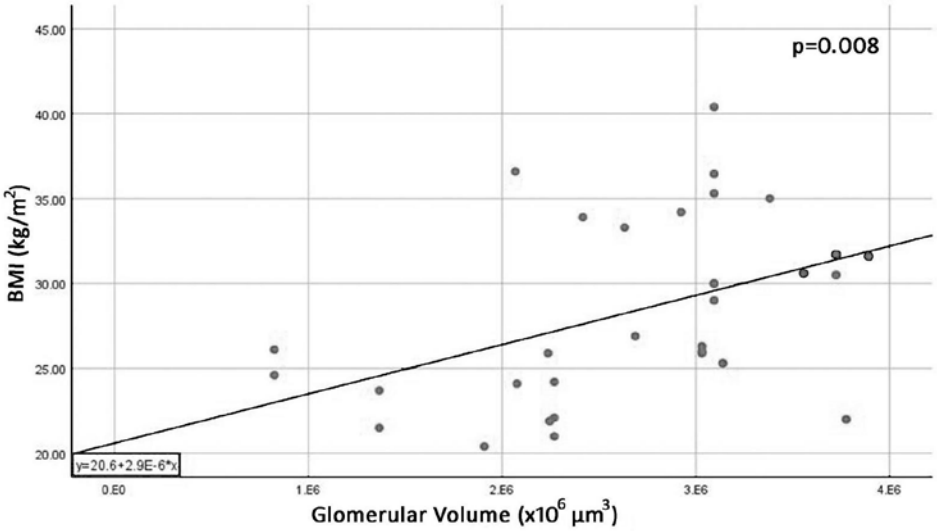

Fig. 2 – Correlation between glomerular volume and body mass index (BMI).

Significantly positive association between mean glomerular volume and BMI was found (r = 0.439; p = 0.008) (Figure 2). There were no significant correlations between glomerular volume and daily proteinuria but also between age, gender, and eGFR.

After 6 months of follow-up, there was no significant difference in the outcome between the obese and non-obese patients. Complete remission reached 23.1% of obese and 36% of non-obese patients, while partial remission was reached in 15.4% of obese and 9.1% of non-obese patients.

Without remission were 61.5% of obese and 54.5% of non-obese patients. After 12 months of follow-up, a significantly higher percentage of non-obese patients reached complete remission compared to obese patients (71.4% vs. 37.5%; p = 0.041). After 24 months of follow-up, there was no significant difference in the outcome between the obese and non-obese patients. Complete remission reached the same percentage of the obese and non-obese patients (33.3%), partial remission was accomplished in 11.1% of obese and 16.7% of non-obese patients. Almost half of the examined patients in both groups were without remission after two years of follow-up (55.6% vs. 50%) (Figure 3).

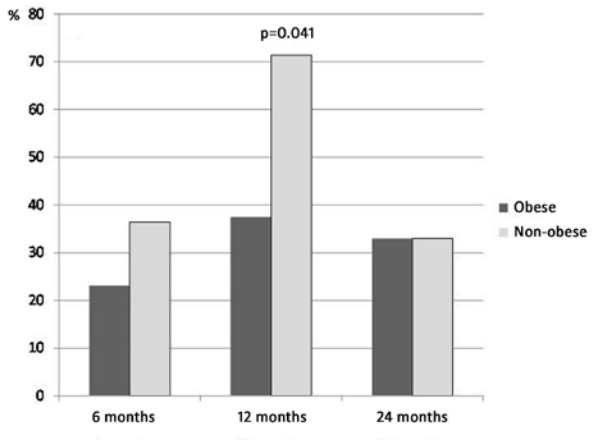

Fig. 3 – Percentage of obese and non-obese patients with complete remission of the nephrotic syndrome after 6, 12, and 24 months of follow-up.

Discussion

The study included 35 patients with FSGS, 18 obese patients with BMI ≥ 27 kg/m^2, and 17 non-obese patients with BMI < 27 kg/m^2. In both study groups, the nephrotic syndrome was the major indication for kidney biopsy (72.2% obese vs. 70.6% non-obese). In Danilewicz and Wagrowska-Danielwicz [15] study, almost the same huge percent of ORG and primary FSGS patients had nephrotic syndrome as a major indication for kidney biopsy.

In the present study, the morphometric glomerular analysis in FSGS patients showed that obese patients had significantly higher radius and significantly higher glomerular volume as well as lower glomerular density, although with no significant difference in comparison with non-obese patients. The morphometric study on glomerular parameters confirmed the earlier findings of Praga et al. [7] that glomerulomegaly and lower glomerular density in obese FSGS patients was significantly increased compared to non-obese patients. Additionally, Kambham et al. [6] and Danilewicz and Wagrowska-Danielwicz [15] showed the same results. Although the pathogenesis of ORG was not clearly defined, it has been shown that the enlarged glomeruli found in animal models of rats may have a close relationship with intraglomerular hyperfiltration and hypertension. It has been suggested that relative reductions in the number of nephrons, as a result in body size increases, can play a role in the pathogenesis of ORG. Decreased nephron mass in experimental animal models is clinically analogous to congenital renal agenesis or nephrectomy [3]. Praga et al. [16] showed that obese patients could develop significant proteinuria after unilateral nephrectomy. Fukuda et al. [17] demonstrated that hypertrophy of the glomerular podocytes could be a compensatory mechanism for renal injury associated with obesity – ORG. It could be suggested that the appearance of FSGS in obese patients depends not only on obesity-related increases in glomerular volume, but also on podocyte hypertrophic responses. Moreover, the relative reduction in the coating area of glomerular podocytes on the glomerular surface could be found in ORG patients.

In the present study, the level of proteinuria was the same in both groups at the time of kidney biopsy. Six months later, daily proteinuria was lower in the non-obese patients but without significance, and 12 months after biopsy, proteinuria was significantly lower, and protein and albumin serum concentrations were higher than in the obese patients. Therefore, a significantly higher percentage of non-obese patients had complete remission compared to obese patients. However, 24 months after the kidney biopsy, there were no statistical differences in percentages of patients with complete remission.

Forty years ago, the association between proteinuria and obesity was first reported [18]. In the 1980s, there were several case reports and autopsy series studies of ORG [19, 20]. In 2001, Kambham et al. [6] published the first large study on this entity. In obese patients, the degree of proteinuria can be variable, but it can reach the nephrotic range (≥ 3.5 g/24 h) in a significant number of cases. Interestingly, obese patients with ORG hardly ever develop hypoproteinemia, hypoalbuminemia, oedema, or other typical findings of nephrotic syndrome even in the presence of massive proteinuria [5, 7]. This occurrence could be very useful in the differential diagnosis with other proteinuric renal diseases (idiopathic FSGS, membranous nephropathy, minimal change disease) that can also affect obese patients [7]. The reason why ORG patients do not develop oedema and have a lower incidence of nephrotic syndrome when compared to idiopathic FSGS patients is unclear. One of the explanations could be the slow progression of proteinuria in ORG patients that may allow the development of hepatic compensation for protein synthesis, and the other one may relate to lower grade of podocyte injury, the selectivity of proteinuria, and the ability of the tubules to reabsorb and catabolize the filtered protein in a different manner [7]. Several studies have shown that weight loss either induced by low-calorie diets, physical exercise, or bariatric surgery [21] and pharmacotherapy (ACE inhibitors or ARBs) are associated with important antiproteinuric effect [22]. In the present study, at the time of kidney biopsy, the same percentage of obese and non-obese patients had nephrotic range proteinuria with full-blown nephrotic syndrome.

In the current study, clinical and laboratory analyses showed that obese patients, at the time of kidney biopsy and 6 months later, had significantly lower kidney function than non-obese patients; but after 12 and 24 months, with the

progression of chronic kidney disease in non-obese patients, there were no significant differences between the groups. Additionally, only 12 months after biopsy, a significantly higher number of patients in the non-obese patient group had complete remission compared to the obese patients, but after 24 months, there were no differences in the clinical outcome. Some studies pointed out that obesity can accelerate the progression of chronic kidney disease. Bonnet et al. [23] reported that a BMI > 25 kg/m^2 or higher is a significant risk factor for the progression of chronic renal failure in IgA nephropathy patients, and Morales et al. [24] found that weight loss is effective for attenuating the progressive loss of kidney function in obese patients with diabetic and non-diabetic kidney diseases. Bertoux et al. [25] have demonstrated in a cohort of 331 IgA nephropathy patients that normal or elevated BMI status at the time of biopsy was associated with a worse presentation at diagnosis in the overweight/obese IgA nephropathy patients (more patients with hypertension; more patients with proteinuria ≥ 1g/day). Moreover, the absolute renal risk (ARR) score for dialysis/death was also significantly worse in obese patients compared to the non-obese ones. As expected, the final outcome was globally worse in obese IgA nephropathy patients. Praga et al. [7] followed patients 5 and 10 years after the renal biopsy, and the conclusion was that the estimated probability of renal survival in obese FSGS patients was significantly higher compared to non-obese FSGS patients. On the other hand, some studies revealed slower chronic kidney progression in obese patients with FSGS compared to non-obese FSGS patients [5, 7].

Conclusion

Morphometric analysis of glomeruli, clinical features, and treatment outcome in obese and non-obese FSGS patients showed that obese patients had significantly higher glomerular volume and insignificantly lower glomerular density. Obese patients at the time of kidney biopsy and after 6 months of follow-up had significantly lower kidney function compared to non-obese patients. However, 12 and 24 months after, with the progression of chronic kidney disease in non-obese patients, this difference was without statistical significance. It can be speculated that the progression of FSGS in obese patients is slower than in non-obese patients. The lack of the present study is the short time of the follow-up period and is in extension.

R E F E R E N C E S

1. *Fogo AB.* Causes and pathogenesis of Focal segmental glomerulosclerosis. Nat Rev Nephrol 2015; 11(2): 76–87.
2. *D'Agati VD, Kaskel FJ, Falk RJ.* Focal segmental glomerulosclerosis. N Engl J Med 2011; 365(25): 2398–411.
3. *Tsuboi N, Utsunomiya Y, Hosoya T.* Obesity-related glomerulopathy and the nephron complement. Nephrol Dial Transplant 2013; 28(Suppl 4): iv108–13.
4. *D´Aggati VD, Chagnac A, De Vries AP, Levi M, Porrini E, Herman-Edelstein M,* et al. Obesity related glomerulopathy: clinical and pathological characteristics and pathogenesis. Nat Rev Nephrol 2016; 12(8): 453–71.
5. *Praga M, Morales E, Herrero JC, Pérez Campos A, Dominquez-Gil B, Alegre R,* et al. Absence of hypoalbuminemia despite massive proteinuria in focal segmental glomerulosclerosis secondary to hyperfiltration. Am J Kidney Dis 1999; 33(1): 52–8.
6. *Kambham N, Markowitz GS, Valeri AM, Lin J, D´Agati VD.* Obesity-related glomerulopathy:an emerging epidemic. Kidney Int 2001; 59(4): 1498–509.
7. *Praga M, Hernández E, Morales E, Campos AP, Valero MA, Martínez MA,* et al. Clinical features and long-term outcome of obesity-associated focal segmental glomerulosclerosis. Nephrol Dial Transplant 2001; 16(9): 1790–8.
8. *Tsuboi N, Koike K, Hirano K, Utsunomiya Y, Kawamura T, Hosoya T.* Clinical features and long-term renal outcomes of Japanese patients with obesity-related glomerulopathy. Clin Exp Nephrol 2013; 17(3): 379–85.
9. *Chandra A, Biersmith M, Tolouian R.* Obesity and kidney protection. J Nephropathol 2014; 3(3): 91–7.
10. Chapter 6: Idiopathic focal segmental glomerulosclerosis in adults. Kidney Int Suppl (2011). 2012; 2(2): 181–5.
11. *Poggio ED, Wang X, Greene T, Van Lente F, Hall PM.* Performance of the modification of diet in renal disease and Cockcroft-Gault equations in the estimation of GFR in health and in chronic kidney disease. J Am Soc Nephrol 2005; 16(2): 459–66.
12. *Janmahasatian S, Duffull SB, Ash S, Ward LC, Byrne NM, Green B.* Quantification of lean bodyweight. Clin Pharmacokinetics 2005; 44(10): 1051–65.
13. *Collins TJ.* ImageJ for microscopy. Biotechniques 2007; 43(1 Suppl): 25–30.
14. *Pagtalunan ME, Drachman JA, Meyer TW.* Methods for estimating the volume of individual glomeruli. Kidney Int 2000; 57(6): 2644–9.
15. *Danilewicz M, Wagrowska-Danielwicz M.* Morphometric and immunohistochemical insight into focal segmental glomerulosclerosis in obese and non- obese patients. Nefrologia 2009; 29(1): 35–41.
16. *Praga M, Hernandez E, Herrero JC, Morales E, Revilla Y, Diaz-Gonzales R,* et al. Influence of obesity on the appearance of proteinuria and renal insufficiency after unilateral nephrectomy. Kidney Int 2000; 58(5): 2111–8.
17. *Fukuda A, Chowdhury MA, Venkatareddy MP, Wang SQ, Nishizono R, Suzuki T,* et al. Growth-dependent podocyte failure causes glomerulosclerosis. J Am Soc Nephrol 2012; 23(8): 1351–63.
18. *Weisinger JR, Kempson RL, Eldridge FL, Swenson RS.* The nephrotic syndrome: A complication of massive obesity. Ann Intern Med 1974; 81(4): 440–7.
19. *Kasiske BL, Napier J.* Glomerular sclerosis in patients with massive obesity. Am J Nephrol 1985; 5(1): 45–50.
20. *Jennette JC, Charles L. Gruhb W.* Glomerulomegaly and focal segmental glomerulosclerosis associated with obesity and sleep apnea syndrome. Am J Kidney Dis 1987; 10(6): 470–2.
21. *Soto FC, Higa-Sansone G, Copley JB, Berho M, Kennedy C, LoMenzo E,* et al. Renal failure, glomerulonephritis and morbid obesity improvement after rapid weught loss following laparoscopic gastric by pass. Obese Surg 2015; 15(1): 137–40.
22. *Engeli S, Böhnke J, Gorzelniak K, Janke J, Schiling P, Bader M,* et al. Weight loss and the renin- angiotenzin – aldosterone system. Hypertension 2005; 45(3): 356–62.

23. *Bonnet F, Deprele C, Sassolas A, Moulin P, Alamartine E, Berthezène F*, et al. Excessive body weight as a new independent risk factor for clinical and pathological progression in primary IgA nephritis. Am J Kidney Dis 2001; 37(4): 720–7.
24. *Morales E, Valero MA, León M, Hernández E, Praga M.* Beneficial effects of weight loss in overweight patients with chronic proteinuric nephropathies. Am J Kidney Dis 2003; 41(2): 319–27.
25. *Berthoux F, Mariat C, Maillard N.* Overweight/ obesity revisited as a predictive risc factor in primary Ig A nephropathy. Nephrol Dial Transplant 2013; 28(Suppl.4): iv160–6.

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ORIGINAL ARTICLE

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Potential effect of decreased levels of folic acid and vitamin B12 on herpes simplex virus keratitis reactivation

Mogući uticaj sniženih nivoa folne kiseline i vitamina B12 na reaktivaciju herpes simpleks virusnog keratitisa

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Abstract

Background/Aim. Most cases of herpetic keratitis present a recurrent disease as a result of herpes simplex virus type 1 reactivation from latency in the nearest sensory ganglia. Therefore, understanding the mechanisms of latency and reactivation of the latent virus is an important link in comprehending the onset of the recurrent eye disease itself. Epigenetic regulation of virus reactivation, as a result of the presence of transcriptionally active Latency-Associated Transcript (LAT) region in the latent viral genome, has already been demonstrated in several studies. The activity of the LAT region is directed to the chromatin arrangement. Epigenetic modulation of DNA methylation is associated with folate and vitamin B12 intake or their serum concentrations. To our knowledge, there is no report on the potential role of vitamin B12 and folic acid in herpes simplex virus keratitis reactivation. The aim of this study was to analyze the potential role of folic acid and vitamin B12 in the control of ocular herpes simplex keratitis reactivation. **Methods.** The study included 50 patients older than 18 years of age with recurrent herpes simplex virus eye disease. Levels of vitamin B12 and folic acid were measured in the acute phase of the disease. All patients were followed up for at least one year and episodes of recurrent herpetic eye diseases were recorded. **Results.** The recurrence rate of herpetic keratitis was statisticall significantly lower in patients with a higher blood level of vitamin B12. In addition, the recurrence rate of herpetic keratitis was lower in patients with a higher blood level of folic acid. However, statistical significance was lower in comparison with that for vitamin B12. **Conclusion.** The decreased levels of vitamin B12 and folic acid might have a vital role in herpes simplex keratitis reactivation.

Key words:
herpesvirus 1, human; folic acid; keratitis; recurrence; risk factors; vitamin b 12.

Apstrakt

Uvod/Cilj. Herpetični keratitis u većini slučajeva je rekurentna bolest, a nastaje kao rezultat reaktivacije herpes simpleks virusa tipa 1 iz latentnosti u najbližoj senzornoj gangliji. Zbog toga je razumevanje mehanizma latentnosti i reaktivacije latentnog virusa važna karika u razumevanju nastanka recidivne bolesti oka. Sve više studija potvrđuje epigenetsku regulaciju reaktivacije virusa kao posledicu prisustva transkripciono aktivnog transkripta vezanog za latenciju (LAT) regiona u latentnom virusnom genomu. Aktivnost LAT regiona usmerena je na hromatinsko uređenje. Epigenetska modulacija metilacije DNA je povezana sa unosom folata i vitamina B12 ili njihovim koncentracijama u serumu. Prema našim saznanjima, ne postoji izveštaj o potencijalnoj ulozi vitamina B12 i folne kiseline u reaktivaciji HSV keratitisa. Cilj rada bio je analiza moguće uloge folne kiseline i vitamina B12 u kontroli reaktivacije okularnog herpetičnog keratitisa. **Metode.** Studijom je bilo obuhvaćeno 50 bolesnika starijih od 18 godina sa različitim formama herpetičnog keratitisa, kao posledicom reaktivacije herpes simpleks virusa tipa 1. Nivoi vitamina B12 i folne kiseline mereni su u akutnoj fazi bolesti. Svi bolesnici su praćeni najmanje godinu dana, a beležen je broj recidiva virusne infekcije. **Rezultati.** Stopa recidiva herpetičnog keratitisa bila je statistički značajno niža kod bolesika sa višim nivoom vitamina B12 u krvi. Pored toga, stopa recidiva herpetičnog keratitisa je bila niža i kod bolesnika sa višim nivoom folne kiseline u krvi, ali sa nižom statističkom značajnošću u poređenju sa onom za vitamin B12.

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Zaključak. Sniženi nivoi vitamina B12 i folne kiseline mogu imati važnu ulogu u reaktivaciji herpetičnog keratitisa.

Ključne reči:
herpesvirus 1, humani; folna kiselina; keratitis; recidiv; faktori rizika; vitamin b12.

Introduction

The results of recent studies have shown that 50%–90% of adult humans have serum antibodies to herpes simplex virus (HSV) type 1 (HSV-1) [1, 2].

The annual incidence of all types of new ocular HSV infections has recently been estimated at 11.8 to 31.5 per 100,000 persons a year [3, 4]. The epithelial dendritic lesion is the most frequent type of recurrent keratitis, with prevalence as high as 56.3%, followed by stromal keratitis, 29.5% [4]. The clinical manifestations of primary HSV ocular infection are rare [5]. Reactivation of the latent virus in the ophthalmic branch of trigeminal ganglion can result in its shedding with subsequent infection of the overlying corneal epithelium [6, 7]. Herpetic keratitis occurs in various forms, and this largely depends on the depth of virus penetration into a corneal tissue. The direct effect of the virus and potent immune response to the viral proteins trigger corneal inflammation and neovascularisation leading to corneal thinning and scarring [8].

Most cases of herpetic keratitis represent a recurrent disease that occurs as a result of HSV-1 reactivation from latency. Due to its recurrent nature, after cataract, herpes virus keratitis is the second leading cause of corneal blindness in the developed world. Therefore, understanding the mechanism and causes of HSV-1 reactivation from the latent state has long been the holy grail of herpes virologists. In animal models and later humans, the latency of the virus may have an epigenetic regulation, primarily because the latent viral genome has a transcriptionally active Latency-Associated Transcript (LAT) region. The activity of the LAT region is directed to chromatin arrangement without the encoding of known proteins [9]. Although viral mutants lacking LATs are still able to establish and maintain reactivation from latency, recent findings indicate that the LAT-region increases the reactivation efficiency and, in some way, controls the latency of the virus itself [10, 11].

The aim of this study was to analyze the potential role of folic acid and B12 vitamin in the control of ocular HSV-1 reactivation. To our knowledge, there is no report on the potential role of vitamin B12 and folic acid in keratitis reactivation.

Methods

This study was conducted in compliance with the institutional review board regulations, the informed consent regulation, and it adhered to the tenets of the Declaration of Helsinki. It included 50 patients older than 18 years of age, regardless of gender, with recurrent ocular HSV-1 disease. Recurrent herpetic keratitis was confirmed by slit lamp examination based on clinical findings. The recurrences were classified as epithelial keratitis, stromal keratitis, endothelitis, iridocyclitis, or as combinations of these conditions. Exclusion criteria were as follows: existing history of associated ophthalmic comorbidities, previous ocular surgery, some form of anemia, or systemic and neurological diseases.

All patients received at least a one-year follow-up between January 2017 and January 2018 at the Clinic for Eye Diseases, Clinical Center of Serbia in Belgrade. In all patients, levels of vitamin B12 and folic acid were measured during the acute phase of the recurrent ocular HSV disease.

All patients were fasting for 8 hours before having their blood samples taken. In addition, none of the included patients took any form of vitamin B complex supplementation for at least 12 months prior to the blood sample harvesting. Two milliliters of venous blood was collected in a standard biochemical tube. Vitamin B12 level was measured on the "Roche Cobas 6000" analyzer, (ECLIA Method) and folate level on the "Roche Cobas E411" analyzer, (ECLIA Method). Blood samples were analyzed in the same laboratory, certified by the Total Quality Management (TQM) quality system. Reference values for serum levels of vitamin B12 and folic acid were 6.9–44.4 ng/mL and 4.6–18.7 ng mL, respectively. Statistical analysis was performed using SPSS Statistics 17.

Results

The analysis of our results showed that in all patients, blood levels of vitamin B12 and folic acid were in the lower reference range. According to the scatter diagrams (Figures 1 and 2), there was a drop in the number of relapses (as dependent variable) depending on both the blood level of vitamin B12 and folic acid (independent variable).

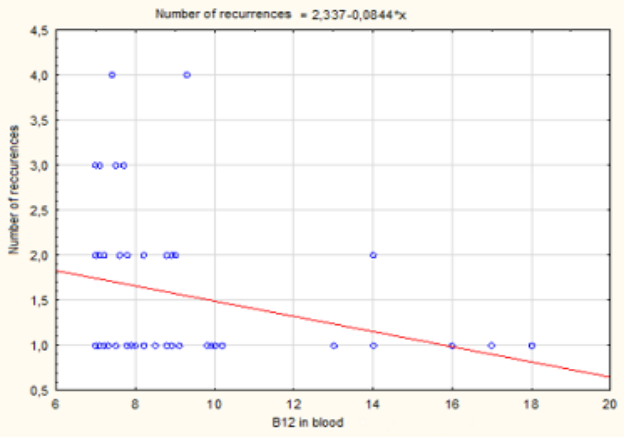

Fig. 1 – Scatter plot for vitamin B12 levels vs. number of herpetic keratitis recurrences.

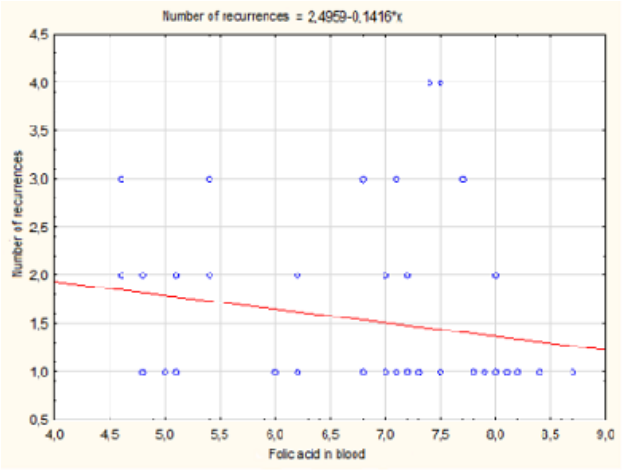

Fig. 2 – Scatter plot for folic acid levels vs. number of herpetic keratitis recurrences.

Analysis of Variance (ANOVA) and F-test (F = 5.031) (Table 1) showed that there were highly statistically significant difference between dependent (number of recurrences) and independent variables (B12 level in the blood); thus, the model had a statistical significance.

Pearson's correlation coefficient (Table 2) showed a statistically significant correlation between the level of vitamin B12 in the acute phase of the disease recurrence and the number of HSV keratitis recurrences. A higher level of vitamin B12 was associated with a reduced rate of disease recurrences. A higher folate level also had an impact on the decrease of the number of HSV keratitis recurrences; however, it was not as significant as vitamin B12 impact.

Discussion

Both vitamin B12 and folic acid are involved in the methylation process of DNA molecules. Methylation of DNA molecules is associated with folate intake and serum folate concentrations in the body [12]. A better insight into the epigenetic nature of the virus itself might help control HSV reactivation by using additional supplements in patients at higher risk of the disease recurrence. Interestingly, a case study from 1956 did not consider the epigenetic nature of virus reactivation. However, additional vitamin B12 supplementation significantly improved the clinical course of herpetic keratitis. Those patients experienced a milder clinical picture of recurrent herpetic eye disease [13, 14].

Reactivation of the virus from a latent phase of the disease into active HSV-1 keratitis may depend on the minimal deficiency of vitamin B12 or folic acids.

In our study, all patients had lower reference values of these vitamins in the acute phase of the disease. Therefore, this may be a potential trigger for virus reactivation and more severe clinical manifestations of herpetic keratitis.

Several studies have also found similar results with other viruses. Interestingly, Piyathilake et al. [15] evaluated the influence of plasma folate and vitamin B12 concentrations on cervical cancer risk. Folate and vitamin B12 may play a critical role in lowering the human papillomavirus (HPV) 16

Table 1

Differences between number of herpetic keratitis recurrences and vitamin B12 level in blood

Model		Sum of squares	df	Mean square	F	Sig.
1	Regression	3.265	1	3.265	5.031	0.030
	Residual	31.155	48	0.649		
	Total	34.420	49			

Dependent variable: number of recurrences; Predictors: (constant), vitamin B12 level in blood

Table 2

Correlation between the levels of vitamin B12 and folic acid and the number of herpetic keratitis recurrences

Variable	B12 in blood	Folic acid in blood	Number of recurrences
B12 in blood			
correlation coefficient	1	0.587	-0.308
sig. (2-tailed)		0.000	0.030
Folic acid in blood			
correlation coefficient		1	-0.200
sig. (2-tailed)			0.164
Number of recurrences			
correlation coefficient			1
sig. (2-tailed)			

sig. – significance.

methylation-associated risk of developing higher grades of cervical intraepithelial neoplasia. Likewise, Lopes et al. [16] revealed that vitamin B12 intake was inversely associated with nononcogenic HPV persistence. Recently, it has been observed that recurrent aphthous stomatitis, including herpetic etiology, was also related to iron and vitamin B12 deficiency [17].

HSV establishes a latent infection in sensory neurons. The fact that the latent viral genome has a transcriptionally active LAT region that encodes the protein and transcriptionally inactive lytic gene regions suggests epigenetic regulation. The LAT region itself records various forms of histone arrangement [9, 18, 19].

Immunological control of virus reactivation should also be considered. The environmental and physiologic factors that induce HSV-1 reactivation from latency include exposure to UV light, stress, and immune suppression, suggesting a possible role for T cells in preventing viral reactivation [21–23]. Studies in rabbits and mice also demonstrated that T cells infiltrate sensory neurons of the eye region around 8–10 days after corneal infection and remain there [24, 25].

The virus does not produce proteins in the latency period, and in that way, it 'hides' from the immune system. Therefore, what maintained the attraction of CD8+ T cells for latently infected neurons was unclear. At this juncture, a definition of the terms latency and reactivation is important. The virus is able to hide from the host immune system during latency since the immune system can only respond to viral protein synthesis.

As recently postulated, asymptomatic virus latency may also be related to the epigenetic nature of the virus. In our study, higher blood levels of both folic acid and vitamin B12 were associated with a reduced rate of recurrent herpetic keratitis. Future clinical and molecular epigenetic studies are necessary to clarify this further.

Conclusion

Our study showed that all patients in the acute phase of the disease had lower reference values of vitamin B12 and folic acid. Moreover, the recurrence rate of herpetic keratitis was lower in patients with higher blood levels of vitamin B12 and folic acid during the follow-up period.

Here, it is assumed that the reactivation of the HSV virus may be related to the minimal deficiency of vitamin B12 and folic acid during the latent phase of the disease.

Therefore, additional supplementation with vitamin B12 and folic acid may be helpful in preventing the reactivation of herpetic keratitis, potentially due to the epigenetic nature of virus reactivation. Further molecular epigenetic research and clinical studies may contribute to understanding and applying epigenetic therapy in herpetic eye disease.

REFERENCES

1. *Rabenau HF, Buxbaum S, Preiser W, Weber B, Doerr HW.* Seroprevalence of herpes simplex virus types 1 and type 2 in the Frankfurt am Main area, Germany. Med Microbiol Immunol 2002; 190(4): 153–60.
2. *Xu F, Sternberg MR, Kottiri BJ, McQuillan GM, Lee FK, Nahmias AJ,* et al. Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. JAMA 2006; 296(8): 964–73.
3. *Young RC, Hodge DO, Liesegang TJ, Baratz KH.* Incidence, recurrence, and outcomes of herpes simplex virus eye disease in Olmsted County, Minnesota, 1976–2007: the effect of oral antiviral prophylaxis. Arch Ophthalmol 2010; 128(9): 1178–83.
4. *Labetoulle M, Auquier P, Conrad H, Crochard A, Daniloski M, Bouee S,* et al. Incidence of herpes simplex virus keratitis in France. Ophthalmology 2005; 112(5): 888–95.
5. *Darougar S, Wishart MS, Viswalingam ND.* Epidemiological and clinical features of primary herpes simplex virus ocular infection. Br J Ophthalmol 1985; 69(1): 2–6.
6. *Shimeld C, Hill TJ, Blyth WA, Easty DL.* Passive immunization protects the mouse eye from damage after herpes simplex virus infection by limiting spread of virus in the nervous system. J Gen Virol 1990; 71(Pt 3): 681–7.
7. *Shimeld C, Hill TJ, Blyth WA, Easty DL.* Reactivation of latent infection and induction of recurrent herpetic eye disease in mice. J Gen Virol 1990; 71(Pt 2): 397–404.
8. *Jones BR.* The clinical features of viral keratitis and a concept of their pathogenesis. Proc R Soc Med 1958; 51(11): 917–24.
9. *Umbach JL, Kramer MF, Jurak I, Karnowski HW, Coen DM, Cullen BR.* MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. Nature 2008; 454(7502): 780– 3.
10. *Bertke AS, Swanson SM, Chen J, Imai Y, Kinchington PR, Margolis TP.* A5-positive primary sensory neurons are nonpermissive for productive infection with herpes simplex virus 1 in vitro. J Virol 2011; 85(13): 6669–77.
11. *Yang L, Voytek CC, Margolis TP.* Immunohistochemical analysis of primary sensory neurons latently infected with herpes simplex virus type 1. J Virol 2000; 74(1): 209–17.
12. *Anderson OS, Sant KE, Dolinoy DC.* Nutrition and epigenetic: An interplay of dietary methyl donors, one-carbon metabolism, and DNA methylation. J Nutr Biochem 2012; 23(8): 853–9.
13. *Maclatchy RS.* Herpes ophthalmicus. Br J Ophthalmol 1956; 40(12): 762–4.
14. *Rossler H.* Zur Behandlung des Herpes zoster ophthalmicus. Klin Mbl Augenheilk 1956; 128: 727. (German)
15. *Piyathilake CJ, Macaluso M, Chambers MM, Badiga S, Siddiqui NR, Bell WC,* et al. Folate and vitamin B12 may play a critical role in lowering the HPV 16 methylation-associated risk of developing higher grades of CIN. Cancer Prev Res (Phila) 2014; 7(11): 1128–37.
16. *Lopes RDVC, Teixeira JA, Marchioni D, Villa LL, Giuliano AR, Luiza Baggio M, Fisberg RM.* Dietary intake of selected nutrients and persistence of HPV infection in men. Int J Cancer 2017; 141(4): 757–65.
17. *Ślebioda Z, Krawiecka E, Szponar E, Dorocka-Bobkowska B.* Haematinic deficiencies and patient clinical profiles in Polish patients with recurrent aphthous stomatitis (RAS). J Oral Pathol Med 2018; 47(5): 531–7.
18. *Bloom DC, Giordani NV, Kwiatkowski DL.* Epigenetic regulation of latent HSV-1 gene expression. Biochim Biophys Acta 2010; 1799(3–4): 246–56.
19. *Cliffe AR, Garber DA, Knipe DM.* Transcription of the herpes simplex virus latency-associated transcript promotes the for-

mation of facultative heterochromatin on lytic promoters. J Virol 2009; 83(16): 8182–90.

20. *Blyth WA, Hill TJ, Field HJ, Harbour DA.* Reactivation of herpes simplex virus infection by ultraviolet light and possible involvement of prostaglandins. J Gen Virol 1976; 33(3): 547–50.
21. *Bonneau RH, Sheridan JF, Feng N, Glaser R.* Stress-induced modulation of the primary cellular immune response to herpes simplex virus infection is mediated by both adrenal-dependent and independent mechanisms. J Neuroimmunol 1993; 42(2): 167–76.
22. *Sainz B, Loutsch JM, Marquart ME, Hill JM.* Stress-associated immunomodulation and herpes simplex virus infections. Med Hypotheses 2001; 56(3): 348–56.
23. *Vicetti Miguel RD, Sheridan BS, Harvey SA, Schreiner RS, Hendricks RL, Cherpes TL.* 17-beta estradiol promotion of herpes simplex virus type 1 reactivation is estrogen receptor dependent. J Virol 2010; 84(1): 565–72.
24. *Gebhardt BM, Hill JM.* T lymphocytes in the trigeminal ganglia of rabbits during corneal HSV infection. Invest Ophthalmol Vis Sci 1988; 29(11): 1683–691.
25. *Liu T, Tang Q, Hendricks RL.* Inflammatory infiltration of the trigeminal ganglion after herpes simplex virus type 1 corneal infection. J Virol 1996; 70(1): 264–71.

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Performance on the Rey-Osterrieth complex figure test and the correlation with the magnetic resonance imaging brain lesion volume in multi-infarct versus small vessel disease dementia

Postignuća na Rey-Osterrieth testu složene figure i njihova povezanost sa volumenom ishemijskih lezija mozga vizualizovanih tehnikom magnetne rezonancije kod multi-infarktne demencije u odnosu na demenciju u okviru ishemijske bolesti malih krvnih sudova

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Abstract

Background/Aim. Regarding several cognitive domains, including visuospatial and visuoconstructional abilities, little is known about the differences between vascular dementia (VaD) subtypes, even in the most common subtypes, such as multi-infarct dementia (MID) and subcortical ischemic small vessel disease dementia (SSVD). This paper aimed to identify the differences between the performances on the Rey-Osterrieth Complex Figure (ROCF) test in MID and SSVD and correlate the ROCF scores in both groups with magnetic resonance imaging (MRI) ischemic lesion load. **Methods.** Sixty VaD patients with matching severity of dementia, age, and education were included in this study: 32 with SSVD and 28 with MID according to the NINDS-AIREN (*National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherché et l'Enseignement en Neurosciences*) neuroradiological criteria. A quantitative scoring system was performed. ROCF was given to all subjects in three test conditions: copy, immediate recall after 3 minutes, and delayed recall after 45 min. Magnetic resonance imaging (MRI) of the ischemic brain volumes of anterior and posterior lesions, left and right hemispheric lesions, left and right-sided basal ganglia lesions, and total lesion load (TLL) were calculated in both groups. **Results.** The MID group was more impaired than SSVD on ROCF copy ($p = 0.008$), immediate recall ($p= 0.005$) and delayed recall ($p = 0.001$). There were significant correlations between ROCF copy score and the TLL ($p < 0.05$) and posterior brain lesion volume ($p< 0.05$) in the MID group. **Conclusion.** The importance of visuospatial, visuoconstructional deficit and impairment of visual memory is disregarded in VaD subtypes. These impairments are more severe in MID than SSVD and the deficit of ROCF copying in MID patients correlates with posterior and total MRI lesion volume.

Key words:
dementia, vascular; cerebrovascular disorders; neurologic manifestations; neurologic examination; magnetic resonance imaging; memory disorders.

Apstrakt

Uvod/Cilj. U odnosu na kognitivne domene, uključujući i vizuospacijalne i vizuokonstrukcione sposobnosti, do sada su malo opisivane razlike između podtipova vaskularne demencije (VaD), pa čak i kod najčešćih kao što su multi-infarktna demencija (MID) i demencija u okviru supkortikalne ishemijske bolesti malih krvnih sudova (SSVD). Cilj rada bio je da se utvrdi da li postoji razlika između obolelih od MID i SSVD u odnosu na postignuća na

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Rey-Osterrieth testu složene figure (ROCF), kao i da li postoji povezanost između postignuća na ROCF i volumena ishemijskih lezija na magnetnoj rezonanci (MR) mozga. **Metode.** U studiju je bilo uključeno 60 obolelih od VaD ujednačenih u odnosu na težinu demencije, starosnu strukturu i stepen obrazovanja, i u odnosu na NINDS-AIREN (*National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherché et l'Enseignement en Neurosciences*) neuroradiološke kriterijume. Grupa ispitanika je bila podeljena na grupu MID sa 28 bolesnika i grupu SSVD sa 32 bolesnika. Kod svih ispitanika izražen je kvantitativni skor kopiranja ROCF, neposredno prisećanje ROCF nakon 3 minuta i odloženo prisećanje ROCF nakon 45 minuta. Izračuniati su: volumen ishemijskih lezija mozga kod obe ispitivane grupe, volumeni anteriornih i posteriornih lezija, lezija leve i desne hemisfere, lezija bazalnih ganglija sa leve i desne strane i ukupni volumen lezija mozga. **Rezultati.** U grupi MID bilo je teže oštećenje nego u grupi SSVD na testu kopiranja ROCF (p = 0,008), neposrednog prisećanja (p = 0,005) i odloženog prisećanja ROCF (p = 0,001). U MID grupi pronađene su značajne povezanosti između kopiranja ROCF i volumena ukupnih ($p < 0.05$) i posteriornih lezija mozga ($p < 0.05$) na MR. **Zaključak.** Kod podtipova VaD zanemaren je značaj vizuospacijalnog i vizuokonstrukcionog deficita, kao i oštećenja vizuelnog pamćenja. Ova oštećenja su teža kod MID nego kod SSVD. Postoji povezanost deficita kopiranja ROCF sa volumenom ukupnih i posteriornih lezija na MR kod obolelih od MID.

Ključne reči:
demencija, vaskularna; cerebrovaskularni poremećaji; neurološke manifestacije; neurološko ispitivanje; magnetna rezonanca, snimanje; pamćenje, poremećaji.

Introduction

The association between vascular brain lesions and cognitive deficits has been described over the past decades through the concept of vascular dementia (VaD) and vascular cognitive impairment [1–4].

The heterogeneity of VaD influenced the problem of classification and terminology within the category, in which numerous VaD subtypes are recognized [4–7]. Different etiologies, pathogenesis, and pathomorphological substrates in the VaD subtypes have affected the specificity of their cognitive profiles. The two most common VaD subtypes are large vessel disease dementia, or, as many authors call it, multi-infarct dementia (MID), and subcortical ischemic small vessel disease dementia (SSVD).

MID occurs most commonly as a result of multiple major cortical infarcts, and the impairments of cognitive functions in MID depend on the localization of infarction and include focal neuropsychological symptoms such as alexia, agraphia, acalculia, agnosia, apraxia, visuospatial and visuoconstructive disorders, and impairment of verbal and nonverbal memory.

The most common pathological substrate of SSVD includes subcortical lacunar infarcts and extensive white matter ischemic disease, which manifests as the lacunar state or the Binswanger's disease or their overlap. SSVD's cognitive profile is characterized by impairment of executive functions, decreased information processing speed, impaired attention and working memory.

Visuospatial skills involve the person's skill to identify the object visually, as well as to determine its localization, spatial coordinates, and relationships with other objects. Tests for assessing visuospatial abilities measure the subject's ability for visual discrimination, i.e. identification of the shape, the wholeness, details, understanding the similarities and differences in visual material, the ability to synthesize visual information, and the ability to imagine the object. Constructive praxia implies the ability to assemble or organize parts into one whole. Impairments in this domain are reflected in free drawing and copy tests. Non-verbal topographic or visual memory is a complex process that relates to receiving, processing, storing, and recalling visual information.

The Rey-Osterrieth Complex Figure (ROCF) [8, 9] is widely used in assessing visuospatial abilities, construction praxia in two dimensions, and non-verbal memory [10], as well as in forming the strategy, planning, and organization. The performance on the ROCF can be assessed by quantitative and qualitative scoring. Successful copying of Rey's figure requires attention and concentration activation, the ability of visuospatial perception for identifying elements of the figure, and visuomotor coordination with the control of the executive system. All of this is associated with the activation of different brain zones, such as the right occipitoparietal lobe, the prefrontal lobe, the superior parietal lobule, and Brodmann's area V5 [11].

Although there are studies that do not report the importance of lateralization, visuospatial and visuoconstructive functions' impairments in stroke are mainly associated with lesions of the right hemisphere. Visuospatial and visuoconstructive deficits have been associated with infarction in the middle cerebral artery circulation, posterior lesions, occipital and parieto-occipital lesions, and bilateral posterior lesions.

All this points to the importance of strategic localization of ischemic lesions in the development of impairments of these cognitive functions, but so far there have been few reports of the differences between the VaD subtypes in relation to the mentioned neuropsychologic functions impairments.

The aim of this study was to examine whether MID and SSVD differed concerning the impairment of visuospatial and visuoconstructive abilities in two dimensions and visual memory using the ROCF test. This study also aimed to determine if there was a correlation between these impairments and the volume of ischemic lesion measured on magnetic resonance imaging (MRI).

Methods

The study included 60 patients aged 50 to 80 years, with probable VaD according to the NINDS-AIREN (National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l'Enseignement en Neurosciences) criteria [12], with 8 to 16 years of education. The study was prospective and randomized. The sample of patients with VaD was divided into two groups according to the operationalized NINDS-AIREN neuroradiologic criteria [13] for vascular dementia: MID comprising 28 patients (17 men and 11 women) and SSVD comprising 32 patients (23 men and 9 women). The study included patients with mild and moderate dementia according to the Mini-Mental State Examination Test (MMSE) [14] score [15–25]. The study did not include patients with deep paresis or plegia of the dominant hand, visual and hearing impairments, and patients who have aphasia, delirium, outpatients and inpatients treated at the Clinic for Neurology, Clinical Center of Vojvodina in Novi Sad.

The standard procedure for copying the Rey-Osterrieth Complex Figure (ROCF) was applied [8, 9]. Visuospatial and visuoconstructive abilities in two dimensions were evaluated using the ROCF copy, immediate recall after 3 minutes, using the ROCF immediate recall, and delayed recall after 45 minutes, using the ROCF delayed recall. All 18 ROCF elements in all three attempts to draw ROCF were scored as follows: 2 points for correct and well-placed figure; 1 point for correct and poorly placed figure; 1 point for deformed or incomplete figure, or recognizable and well placed; 0.5 points for deformed or incomplete, or recognizable and poorly placed figure; 0 points for missing or unrecognizable figure. The maximum score was 36. Lower scores indicated a lower performance. To evaluate the accuracy of the elements of the figure, Taylor descriptive criteria were used [15].

Visualization of cerebral ischemic lesions was done with the Siemens Avanto II apparatus (Erlangen, Germany), with the magnitude of the magnetic field of 1.5 Tesla, and the 3T Trio-Team in the interval of up to 3 months from the date of the neuropsychological testing. The study excluded patients who were in the acute phase of the stroke.

For determining the volume of ischemic lesions, the following protocol was used: FLAIR (Fluid attenuation inversion recovery) sequence in the sagittal plane, slice thickness of 1 mm; between sequences 144 and 191, 1 mm thick slices were used, depending on the volume of the cranium; diffusion sequences (B = 0; 500; 1000) in the transversal plane with calculated ADC map (apparent diffusion coefficients), 5 mm thick, in order to exclude the presence of acute infarction.

Neuroradiological criteria and volume calculation were made by a neuroradiologist who was ignorant of the information on the neurological status or neuropsychological profile of the patient.

Calculating the lesion volume on MRI slices was done by a semi-automated method, using the non-commercial software program MIPAV (Medical Image Processing Analysis and Visualization) [16]. The MIPAV program was used to analyze each individual FLAIR sagittal MRI slice in the DICOM (Digital Imaging and Communications in Medicine) format.

The MIPAV is designed to automatically isolate the ischemic area from the surrounding, intact parenchyma, based on the difference in the signals of the changed and unchanged brain parenchyma. This is made possible by using the FLAIR (Fluid-attenuated inversion recovery) MRI sequence that optimally displays changed parenchyma in the form of a high signal (ischemia, gliosis, myelin destruction) and preserved parenchyma, which has an intermediary signal. Figure 1 shows an example of mapping ischemic parenchyma in a patient with leukoaraiosis.

Fig. 1 – Mapping ischemic parenchyma in a patient with leukoaraiosis.

The volume of ischemic lesions was calculated by multiplying the surface of the ischemic area. The volume was automatically calculated with the MIPAV program using the 1 mm MRI slices with the obtained volume of the ischemic lesion in mm ^{3}. By dividing the product by 1,000, the volume of the lesion in milliliters was obtained. For both study groups, the following parameters were calculated: the volume of right-sided lesions (MRI right), the volume of left-sided lesions (MRI left), the volume of anterior or prerolandic lesions (MRI anterior), the volume of posterior or postrolandic lesions (MRI posterior), the volume of basal ganglia on the right (MRI BG right) and the volume of basal ganglia on the left (MRI BG left).

The research was conducted in accordance with the Ethical Principles of Medical Research Involving Human Subjects – the World Medical Association Declaration of Helsinki and with the consent of the Ethics Committee of the Medical Faculty of the University of Novi Sad and the Ethics Committee of the Clinical Center of Vojvodina.

As part of the descriptive statistics, data were presented in the form of arithmetic mean, standard deviation, median, and range. At the level of inferential statistics, the significance of the difference between the investigated groups was tested with the Student *t*-test. In the case of disturbed normality of distribution, Mann-Whitney *U*-test

was used to determine the differences between the groups. The correlation between the tested parameters (performance on the ROCF and volumetric measures of brain damage) was determined by Spearman's rank correlation coefficient since the distribution of the volume variables significantly deviated from the normal distribution. Statistical data were processed using the statistical software package SPSS (SPSS 17.00 for Windows).

Results

There was a statistically significant difference in all three subtests of the ROCF test (copy, immediate recall, and delayed recall) between MID and SSVD patients (Table 1). Additionally, the SSVD group had a statistically significantly higher average ROCF score in all three subtests than the MID group.

Regarding the descriptive parameters of brain injury volume on MRI (Table 2), there was a higher average volume of the ischemic lesion in the right cerebral hemisphere compared to the left one in MID patients and posterior compared to anterior parts. On average, SSVD patients had a higher volume of lesions in the right cerebral hemisphere compared to the left one, and anterior compared to posterior parts. On average, SSVD patients had the smallest lesion volumes in the left-sided basal ganglia.

There was a statistically significant moderate negative correlation between MRI total brain lesion volume with ROCF copy score (-0.484) and MRI volume of posterior lesions with ROCF copy score (-0.455) in MID patients (Table 3). No other correlations in MID patients were statistically significant. In SSVD patients, there was a statistically significant moderate positive correlation between MRI total brain lesion volume with ROCF immediate recall score (0.490) and MRI posterior lesion volume with ROCF immediate recall score (0.424) (Table 4). No other correlations in SSVD patients were statistically significant.

Table 1

Mean score differences on the Rey-Osterrieth Complex Figure (ROCF) test between patients with multi-infarct dementia (MID) and subcortical small vessel disease dementia (SSVD)

Test recall	MID (n = 28) mean ± SD	SSVD (n = 32) mean ± SD	*p*
ROCF copy*	8.27 ± 7.60	13.31 ± 6.69	0.008
ROCF immediate recall†	2.61 ± 3.10	4.59 ± 3.00	0.005
ROCF delayed recall*	1.95 ± 1.82	4.30 ± 3.07	0.001

SD – standard deviation; *Student's *t*-test; †Mann-Whitney *U-test.*

Table 2

Descriptive parameters of magnetic resonance imaging (MRI) ischemic brain injury volume in patients with multi-infarct dementia (MID) and subcortical small vessel disease dementia (SSVD)

Parameter	MID (n = 24)			SSVD (n = 28)		
	range	median	mean ± SD	range	median	mean ± SD
MRI total	1.3–146.1	53.0	59.8 ± 43.3	0.3–42669.0	17.0	3948.6 ± 9271.9
MRI anterior	0.2–121.7	21.7	26.0 ± 30.1	0.6–30247.0	66.0	2758.0 ± 6672.0
MRI posterior	0.0–98.7	24.7	28.6 ± 28.3	0.0–17282.0	496.5	2685.5 ± 4116.8
MRI left	0.0–141.2	13.5	25.8 ± 35.9	0.0–13989.0	1407.5	3050.5 ± 3914.8
MRI right	0.0–145.6	22.4	33.6 ± 42.1	0.0–32610.0	656.0	3360.6 ± 6472.4
MRI BG left	0.0–313.0	0.0	13.8 ± 63.8	0.0–1784.0	1.0	214.1 ± 399.6
MRI BG right	0.0–1.3	0.0	0.1 ± 0.3	0.0–4353.0	1.4	297.5 ± 884.6

BG – basal ganglia; SD – standard deviation.

Table 3

Spearman's rank correlation coefficient between the Rey-Osterreith Complex Figure (ROCF) score and magnetic resonance imaging (MRI) volumetric brain lesions in patients with multi-infarct dementia (MID)

MRI	ROCF copy	ROCF immediate recall	ROCF delayed recall
MRI total	-0.484*	-0.245	-0.228
MRI anterior	-0.198	0.007	0.088
MRI posterior	-0.455*	-0.387	-0.262
MRI left	-0.091	-0.188	-0.305
MRI right	-0.378	-0.140	-0.027
MRI BG left	-0.131	-0.058	0.158
MRI BG right	-0.117	-0.005	0.033

BG – basal ganglia; *$p < 0.05$.

Table 4

Spearman's rank correlation coefficient between the Rey-Osterreith Complex Figure (ROCF) score and magnetic resonance imaging (MRI) volumetric brain lesions in patients with subcortical small vessel disease dementia (SSVD)

MRI	ROCF copy	ROCF immediate recall	ROCF delayed recall
MRI total	-0.005	0.490†	0.298
MRI anterior	0.006	0.283	0.313
MRI posterior	0.054	0.424*	0.354
MRI left	0.123	0.198	0.205
MRI right	-0.012	0.294	0.132
MRI BG left	0.235	0.092	0.140
MRI BG right	0.177	0.275	0.048

BG – basal ganglia;*$p < 0.05$; †$p < 0.01$.

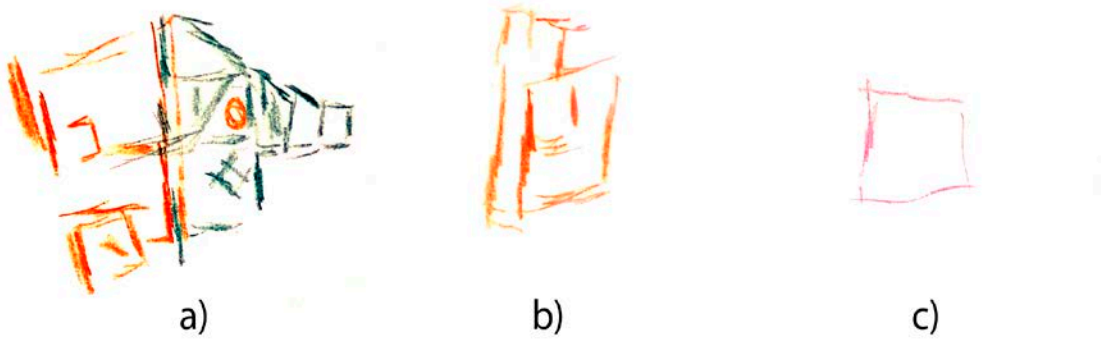

Fig. 2 – An example of a copy (a), immediate recall (b), and delayed recall (c) of Rey-Osterreith Complex Figure (ROCF) in subcortical ischaemic small vessel disaese dementia (SSVD) patient

Figure 2 represents an example of severe impairment in the visuospatial domain, the deficit of visuoconstructional praxia with perseverations, as well as the deficit of immediate and delayed recall ROCF in patients with SSVD.

Discussion

The aim of this study was to compare whether there were differences in the performance on the ROCF test between patients with MID and those with SSVD, matched for gender, age structure, education, and severity of dementia. The study also aimed to assess whether there was a correlation between ROCF performance and the volume of brain ischemic lesions.

The ROCF test is recommended for assessing visuospatial abilities as a part of the 60-minute protocol in examining cognitive functions in vascular cognitive impairment [17, 18].

Our results confirmed that patients with VaD had deficits in visuospatial and visuoconstructive abilities and visual memory [19].

Our data showed that both the MID and SVDD groups had low scores on the ROCF copy, which means that both groups had problems with visual perception, organization, assembling the whole, and data processing. However, since the scores on immediate and delayed recall were low as well, it indicated a problem with coding and storing visual information.

Although vascular dementia is the second most common among dementias, the results of neuropsychological studies are not unambiguous in terms of specifying a clear neuropsychological profile associated with vascular brain damage [19]. Considering that the differences in the deficits of numerous cognitive functions [20] have not yet been clearly defined between VaD subtypes, the characteristics of the visuospatial impairment are not sufficiently defined either, nor is the deficit of constructional praxis in VaD subtypes.

The heterogeneity of VaD [21, 22], multiple classifications, and diagnostic criteria influence interpreting the results of neuropsychological studies in VaD. However, it was observed that between VaD subtypes, executive functions were more frequently impaired in small vessel dementia compared to large vessel and mixed dementia and that visuospatial and language deficits were more commonly expressed in large vessel dementia (37.1% versus 15.5%) [20].

Our study indicated that patients with MID have more severe impairment of visuospatial and visuoconstructive abilities, but also a more severe deficit of visual memory, compared to those with SSVD.

The lesion volumetry in the MID group on MRI showed a higher lesion volume in the right cerebral hemisphere than in the left, as well as in the posterior regions compared to the anterior ones. Even though the volume threshold was not the subject of the research in this study, patients with very small lesion volumes were also analyzed. This can indirectly confirm the results of earlier studies [23, 24], which in the

context of association between cognition and imaging parameters in VaD, emphasize greater importance of localization than the volume of ischemic lesions. Here, the strategic localizations include the dominant angular gyrus, the territory of the anterior cerebral artery and posterior cerebral artery, the territory of the upper-middle cerebral artery, left anterior corona radiata artery, basal ganglia, bilateral medial thalamus, dominant nucleus caudatus, anterior capsula interna, hippocampus, amygdala, and basal forebrain.

However, some imaging studies also showed contradictory results in the correlation between the infarct location and dementia [24, 25]. The stated result of our study may indirectly indicate the importance of other parameters, such as the total number of lesions, lesion size, and bilaterality of infarction.

The association between the volume of total and posterior ischemic lesions and performance on the ROCF copying test was found in the MID group. However, in all other investigated domains in this group, as well as in SSVD, no statistically significant negative correlation was found between the performance on the ROCF and volumetric measures. A possible reason for the absence of the correlations in the present study is the insufficient sensitivity of standard MRI techniques since studies using advanced neuroimaging techniques have shown significant correlations with cognitive impairment in VaD, especially in SSVD.

In agreement with earlier studies [26, 27] , our results found an association between cognitive impairment and the volume of ischemic lesions. However, it should be taken into account that the volume of functional loss may be more important because it involves the effect of deafferentation of the cortex.

The association between visuospatial and visuoconstructive deficits with the right hemispheric infarction and posterior lesions [28] was confirmed, but our study also anticipated the importance of MRI posterior ischemic volumes. Lower performance in the MID group on the ROCF copy was associated with MRI posterior volumes and the total lesion load, indicating the association between diffuse lesions and the visuospatial and visuoconstructive deficits in MID.

The low ROCF performance in SSVD in our study is in accordance with the published data that have shown that visuoconstructive deficits occur in subcortical white matter lesions, as well as in diffuse brain lesions and small infarctions [29, 30]. Although it was not included in our study, the qualitative analysis is important in assessing the copying, immediate, or delayed recall of ROCF. Nevertheless, indicative low ROCF scores in SSVD, as our results present, are also important and are most likely a part of the dysexecutive syndrome, which is the leading deficit in SSVD. It may occur as a feature of interruption of the frontal–subcortical circles, within diffuse changes of the white matter and lacunae with a predilection for subcortical frontal regions.

A moderate positive correlation was found between the total lesion load and ROCF immediate recall in the SSVD group, as well as between the posterior lesion volume and ROCF immediate recall. This result could generally reduce the significance of the ischemic lesions' volume on MRI in terms of visual memory deficits in patients with SSVD.

Study limitations encompass insufficient sensitivity of volumetric measurements with a standard MRI technique and lack of CSF and imaging biomarkers of amyloid pathology. Therefore, patients with mixed pathology could not have been excluded.

Conclusion

In patients suffering from multi-infarct dementia of mild to moderate severity, there is a more severe impairment of visuospatial and visuoconstructive abilities in two dimensions, as well as a more severe impairment of immediate and delayed visual memory, compared to patients with mild to moderate subcortical ischemic small vessel disease dementia. In patients with multi-infarct dementia, there is a correlation between lower ROCF copy scores with a higher total lesion load and a larger volume of posterior lesions.

R E F E R E N C E S

1. *Roman GC, Sachdev P, Royall DR, Bullock RA, Orgogozo JM, Lopez-Pousa S*, et al. Vascular cognitive disorder: A new diagnostic category updating vascular cognitive impairment and vascular dementia. J Neurol Sci 2014; 226(1–2): 81–7.
2. *Gorelick PB, Scuteri A, Black SE, DeCarli C, Greenberg SM, Iadecola C,* et al. Vascular contributions to cognitive impairment and dementia: A statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2011; 42(9): 2672–713.
3. *Skrobot OA, Black SE, Chen C, DeCarli C, Erkinjuntti T, Ford GA*, et al. Progress toward standardized diagnosis of vascular cognitive impairment: Guidelines from the Vascular Impairment of Cognition Classification Consensus Study. Alzheimers Dement 2018; 14(3): 280–92.
4. *Skrobot OA, O'Brien J, Black S, Chen C, DeCarli C, Erkinjuntti T*, et al. The Vascular Impairment of Cognition Classification Consensus Study. Alzheimerz Dement 2017; 13(6): 624–33.
5. *Kalaria RN*. Neuropathological diagnosis of vas- cular cognitive impairment and vascular dementia with implications for Alzheimer's disease. Acta Neuropathol 2016; 131: 659–85.
6. *Gorelick PB, Counts SE, Nyenhuis D*. Vascular cognitive impairment and dementia. Biochim Biophys Acta 2015; 1862(5): 860–8.
7. *Khan A, Kalaria RN, Corbett A, Ballard C*. Update on Vascular Dementia. J Geriatr Psychiatry Neurol 2016; 29(5): 281–301.
8. *Rey A*. L'examen psychologique dans les cas d'encephalopathie traumatique. Arch Psychol 1941; 28: 286–340. (French)
9. *Osterrieth PA*. Le test de copie d'une figure complexe. Arch Psychol 1944; 30: 206–356. (French)
10. *Carone DA, Strauss E, Sherman MS, Spreen O*. A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary. Appl Neuropsychol 2007; 14(1): 62–3.
11. *Thiyagesh SN, Farrow TF, Parks RW, Accosta-Mesa H, Young C, Wilkinson ID*, et al. The neural basis of visuospatial perception in Alzheimer's Neuroimaging 2009; 172(2): 109–16.

12. *Roman GC, Tatemichi TK, Erkinjuntti T, Cummings JL, Masdeu JC, Garcia JH*, et al. Vascular dementia: Diagnostic criteria for research studies. Report of the NINDS-AIREN international workshop. Neurology 1993; 43(2): 250–60.
13. *van Straaten EC, Scheltens P, Knol DL, van Buchem MA, van Dijk EJ, Hofman PA*, et al. Operational definitions for the NINDS-AIREN criteria for vascular dementia an interobserver study. Stroke 2003; 34(8): 1907–12.
14. *Folstein MF, Folstein SE, McHugh PR*. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12(3): 189–98.
15. *Taylor LB*. Scoring criteria for the ROCF. In: *Spreen O, Strauss E,* editor. A compendium of neuropsychological tests: Administration, norms, and commentary. New York: Oxford University Press; 1991. p. 350–1.
16. *McAuliffe MJ, Lalonde FM, McGarry D, Gandler W, Csaky K, Trus BL*. Medical image processing, analysis and visualization in clinical research. In: CBMS 2001. Proceedings of the 14th IEEE Symposium on Computer-Based Medical Systems; Bethesda; 2001 July 26-27; Bethesda, MD, USA, USA: IEEE; 2001. p. 381–6.
17. *Hachinski V, Iadecola C, Petersen RC, Breteler MN, Nyenhuis DL, Black SE*, et al. National Institute of Neurological Disorders and Stroke—Canadian Stroke Network vascular cognitive impairment harmonization standards. Stroke 2006; 37(9): 2220– 41.
18. *Gorelick PB, Counts SE, Nyenhuis D*. Vascular cognitive impairment and dementia. Biochim Biophys Acta 2015; 1862(5): 860–8.
19. *Graham NL, Emery T, Hodges JR*. Distinctive cognitive profiles in Alzheimer's disease and subcortical vascular dementia. J Neurol Neurosurg Psychiatry 2004; 75: 61–71.
20. *Ying H, Jianping C, Jianqing Y, Shanquan Z*. Cognitive variations among vascular dementia subtypes caused by small-, large-, or mixed-vessel disease. Arch Med Sci 2016; 12(4): 747–53.
21. *van Straaten EC, Scheltens P, Knol DL, van Buchem MA, van Dijk EJ, Hofman PA*, et al. Operational defini- tions for the NINDS-AIREN criteria for vascular dementia: an interobserver study. Stroke 2003; 34(8): 1907-12.
22. *Jellinger KA*. Morphologic diagnosis of "vascular dementia" – a critical update. J Neurol Sci 2008; 270(1–2): 1–12
23. Pohjasvaara T, Erkinjuntti T, Ylikoski R, Hietanen M, Vataja R, Kaste M. Clinical determinants of poststroke dementia. Stroke 1998; 29(1): 75–81.
24. *Erkinjuntti T, Bowler JV, DeCarli CS, Fazekas F, Inzitari D, O'Brien JT,* et al. Imaging of static brain lesions in vascular dementia: implications for clinical trials. Alzheimer Dis Assoc Disord 1999; 13 Suppl 3: S81–90.
25. *Bastos-Leite AJ, van der Flier WM, van Straaten EC*. The Contribution of Medial Temporal Lobe Atrophy and Vascular Pathology to Cognitive Impairment in Vascular Dementia. Stroke 2007; 38(12): 3182–5.
26. *Tomlinson BE, Blessed G, Roth M*. Obsrevations on the brains of demented old peopled. J Neurol Sci 1970; 11(3): 205–42.
27. *Erkinjuntti T, Pantoni L, Scheltens P*. Cooperation and networking on white matter disorders: the European Task Force on Age-Related White Matter Changes. Dement Geriatr Cogn Disord1998; 9(1): 44–5.
28. *Arena R, Gainotti G*. Constructional apraxia and visuoperceptive disabilities in relation to laterality of cerebral lesions. Cortex 1978; 14(4): 463–73.
29. *Fushimi H, Inoue T, Yamada Y, Udaka, F, Kameyama M*. Asymptomatic cerebral small infarcts (lacunae), their risk factors and intellectual disturbances. Diabetes 1996; 45(3): 98–100.
30. *van Rooden S, van den Berg-Huysmans AA, Croll PH, Labadie G, Hayes JM, Viviano R,* et al. Subjective Cognitive Decline Is Associated with Greater White Matter Hyperintensity Volume. J Alzheimers Dis 2018; 66(3): 1283–94.

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The effects of acute and chronic Red Bull® consumption on cardiodynamics and oxidative stress in coronary effluent of trained rats

Efekti akutne i hronične konzumacije Red Bull®-a na kardiodinamiku i oksidativni stres u koronarnom efluentu treniranih pacova

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Abstract

Background/Aim. Energy drinks (EDs) are widely used by athletes as ergogenic agents and Red Bull® is one of the most consumed EDs among them. The aim of this study was to determine the acute and chronic effects of Red Bull® on cardiodynamics and parameters of oxidative stress in physically trained rats. **Methods.** Rats were subjected to a swimming practice (1h a day, 5 days a week, for 4 weeks). They were divided into 4 groups: rats that did not consume ED either before swimming or prior to sacrificing; rats that did not consume ED before swimming but did consume ED 30 min prior to sacrificing; rats that consumed ED 30 min before every swimming training but did not consume ED prior to sacrificing; rats that consumed ED 30 min before every swimming training and 30 min before sacrificing. After sacrificing, the hearts of the rats were isolated and perfused according to the Langendorff technique. The parameters of cardiac function were recorded, and also the levels of prooxidants were measured in the coronary effluent during coronary autoregulation. **Results.** Acute administration of the ED had a positive inotropic effect (manifested as a significantly higher level of the maximum and minimum rate of pressure development in the left ventricle), while chronic administration affected the isolated increase in systolic left ventricular pressure. The prooxidative effect of the ED was observed, which was more pronounced in chronic consumption. **Conclusion.** The main conclusion of our study is that chronic consumption of ED changes the cardiovascular response and redox status in acute consumption ED.

Key words:
energy drinks; exercise; cardiovascular system; oxidative stress; rats; swimming.

Apstrakt

Uvod/Cilj. Energetska pića (EP) se često koriste kao ergogena sredstva od strane sportista, a Red Bull® je jedno od najčešće konzumiranih EP. Cilj rada bio je da se utvrde akutni i hronični efekti Red Bull®-a na kardiodinamiku i parametre oksidativnog stresa kod fizički treniranih pacova. **Metode.** Pacovi su bili podvrgnuti plivanju (1 h dnevno, 5 dana u nedelji, tokom 4 nedelje) i podeljeni u 4 grupe: pacovi koji nisu konzumirali EP ni pre plivanja, ni pre žrtvovanja; pacovi koji nisu konzumirali EP pre plivanja, ali jesu 30 min pre žrtvovanja; pacovi koji su konzumirali EP 30 min pre svakog plivanja, ali ga nisu konzumirali pre žrtvovanja; pacovi koji su konzumirali EP 30 min pre svakog plivanja i 30 min pre žrtvovanja. Nakon žrtvovanja, srca pacova su bila izolovana i perfundovana prema tehnici po Langendorff-u. Određivani su parametri funkcije srca, kao i nivo prooksidantnih vrsta u koronarnom efluentu tokom koronarne autoregulacije. **Rezultati.** U poređenju sa kontrolnom grupom, akutna primena EP imala je pozitivan inotropni efekat, značajno povećanje maksimalne i minimalne stope promene pritiska u levoj komori, dok je hronična konzumacija uticala na izolovano povećanje sistolnog arterijskog pritiska. Zapaženi su prooksidativni efekti EP, što je bilo izraženije kod hronične konzumacije EP. **Zaključak.** Glavni zaključak ove studije jeste da hronična konzumacija EP menja kardiovaskularni odgovor i redoks status prilikom akutne primene EP.

Ključne reči:
energetski napici; vežbanje; kardiovaskularni sistem; stres, oksidativni; pacovi; plivanje.

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Introduction

Energy drinks (EDs) are beverages with stimulating effects due to a combination of specific ingredients [1, 2]. The main active ingredient of these drinks is caffeine but it has been shown that other components also contribute to the changes in the work of the cardiovascular system [3–7]. EDs are consumed to provide additional energy, increasing cognitive and physical performance, prolonging alertness, and improving mood [8]. Due to the positive inotropic effect, they should induce some benefit to exercising individuals by improving skeletal muscle oxygenation and increasing aerobic metabolism and muscular performance [9]. Thus, EDs are widely used by athletes as ergogenic agents [10].

Red Bull® (RB®) is considered to be one of the most consumed EDs [4, 11]. It has been shown that 355 mL of RB® leads to a significant increase in the systolic and diastolic blood pressure, heart rate, stroke volume, and a double product, which is an indirect indicator of oxygen consumption in the myocardium [12]. Because of the significant increase in myocardial function of the right and left ventricles (LVs), a positive inotropic effect of EDs has been suggested [13]. EDs affect the increase in glycemia, cholesterol, and triglycerides [14]. Thus the overall effect of EDs represents an increase in cardiovascular risk [15]. Acute cardiovascular adverse effects of EDs include the effect on hemodynamics and electrophysiological changes, the effect on endothelial function, and the association with vascular pathology [16]. The association between ED consumption and cardiovascular changes includes supraventricular and ventricular arrhythmias, ischemia and myocardial infarction, QT interval prolongation, aortic dissection, and death [7]. Although moderate consumption of EDs is considered relatively safe in healthy population [10], their consumption is not recommended in sports and during exercise, and special caution is recommended for people with cardiovascular diseases [17, 18].

The occurrence of oxidative stress in a blood vessel and damage to the endothelium-dependent vasodilation is associated with a reduction in the production of nitric oxide (NO) or the increased production of the reactive oxygen species (ROS), in particular of superoxide anion (O_2^-) [19]. The effect of caffeine on blood vessel regulation is manifested through the balance of vasoconstrictor and vasodilatory effect [20]. At rest, caffeine either improves or does no alteration [21] to the endothelial function [20]. However, during physical activity, this function is reduced [21]. Current data suggest that EDs decrease endothelial function at rest [22, 23]. In mice, it is shown that not only does the application of EDs affect the reduction of peri-intestinal fat tissue, but it also increases the pericardial fat tissue, which represents a significantly greater source of chemokines and cytokines with proinflammatory properties, compared to the subcutaneous fat tissue [24], which further implies a larger production of ROS [25]. Particular importance is attributed to oxidative stress because it plays an important role in pathogenesis and the development of cardiovascular diseases.

There are very few preclinical studies in the literature examining the influence of EDs on the cardiovascular system. The aim of this study was to determine the acute and chronic effects of RB® on cardiodynamics and oxidative stress in the coronary effluent in physically trained rats. The study also aimed to determine the effect of chronic RB® consumption on the changing of the indicated parameters (cardiodynamics and oxidative stress) in the presence of an acute RB® consumption.

Methods

The study was conducted in the Laboratory for Cardiovascular Physiology at the Faculty of Medical Sciences, University of Kragujevac. It was approved by the Ethics Committee of the Faculty. Good Laboratory Practice and the European Council Directive (86/609EEC) were followed during the conception, design and performance of the study.

Subjects

The study was performed using the Wistar albino rats. The sample size calculation, based on a study published by Barcelos et al. [26], revealed that 24 rats were requisited to perform the study. At the beginning of the study, the rats were eight weeks old and weighed 200–250 g. They were kept in cages (8 rats in one cage), fed with commercial rat food (20% protein food, Veterinary Institute Subotica, Serbia), and watered ad libitum. The temperature in the room was set to 25°C, and 12 hours of light were provided.

Training protocol

The study lasted for 4 weeks. All rats were subjected to a swimming practice (1h a day, 5 days a week) in an 80 × 60 × 100 cm pool for experimental animals. An electric heater was used to keep the water temperature at 34°C. During swimming, the pump installed in the pool made constant waves, in order to prevent the rats from floating. Rats were constantly monitored during swimming.

ED consumption

Initially, rats were divided into two groups based on ED consumption during the study period (rats that did and did not consume ED 30 min before swimming). Later those two groups were further divided into groups based on ED consumption before the sacrificing (rats that did and did not consume ED before they were sacrificed). Thus, groups were formed as follows: control group (C-T) – rats that did not consume ED either before swimming or prior to sacrificing (n = 6); acute ED group (acED-T) – rats that did not consume ED before swimming, but did consume ED 30 min prior to sacrificing (n = 6); chronic ED group (chED-T) – rats that consumed ED 30 min before every swimming training, but did not consume ED prior to sacrificing (n = 6); chronic + acute group (ch + acED-T) – rats that consumed

ED 30 min before every swimming training and 30 min before sacrificing (n = 6).

The ED administration was performed by an intragastric gavage (p.o.). RB® was used in the amount of 3.75 mL/kg, as determined on the basis of the previously published studies [26, 27]. The indicated dose corresponds to a dose of caffeine close to the maximum recommended (about 6 mg/kg). A standard of 250 mL RB® contains the following: 80 mg of caffeine, 1,000 mg of taurine, 21.5 g of sucrose, 5.25 g of glucose, 600 mg of glucuronolactone, 20 mg of vitamin B3 (niacinamide), 5 mg of vitamin B5 (calcium pantothenate), 5 mg of vitamin B6 (pyridoxine hydrochloride), 50 mg of inositol, 5 μg of vitamin B12 (cyanocobalamin), 100 mg of sodium citrate, as well as natural and artificial flavors and colors (caramel, riboflavin) [6, 28, 29].

Cardiodynamic parameters

After short ketamine/xylazine narcosis, rats were sacrificed, and their hearts were excised and attached to the Langendorff apparatus via aortic cannula. Krebs-Henseleit buffer was used during the performance of the retrograde perfusion according to the Langendorff technique. The equilibration period, during which coronary perfusion pressure (CPP) was kept at 70 cm H_2O, was performed first. After that, CPP was changed in the following order: 1) 60 cm H_2O, 2) 80 cm H_2O, 3) 100 cm H_2O, 4) 120 cm H_2O, and 5) 40 cm H_2O.

Parameters of myocardial function were measured using the pressure sensor (transducer BS4 73-0184, Experimentria Ltd, Hungary) attached to the latex balloon, filled with bubble-free saline, which was inserted into the left chamber [30]. Cardiodynamic parameters were continuously measured. The following parameters of myocardial function were recorded: 1) the maximum and minimum rate of pressure development in LV (dP/dt max and dP/dt min), 2) systolic LV pressure (SLVP) and diastolic LV pressure (DLVP), and 3) heart rate (HR). Furthermore, coronary flow (CF) was measured flowmetrically.

Oxidative stress

CF collected during each CPP was used to measure the levels of oxidative stress in coronary venous effluent. A spectrophotometer (Analytic Jena Specord S 600, UK) was used to determine the levels of the following: 1) superoxide anion radical (O_2^-), 2) hydrogen peroxide (H_2O_2), 3) nitrogen monoxide (NO), and 4) index of lipid peroxidation (TBARS). The exact protocols for measurement of those prooxidative species may be found in our previously published papers [31] or in the original sources [32–35].

Statistics

SPSS 23.0 was used to perform the statistical analysis. Comparison of groups was performed using the parametric (*t*-test for independent samples) or nonparametric tests (Mann-Whitney *U* test), depending on the results of the Shapiro-Wilk test for data distribution. The results in the figures are shown as the mean ± standard error (SE) of the mean.

Results

Cardiodynamics

Cardiodynamic parameters of isolated rat hearts in four groups (C-T, acED-T, chED-T, ch + acED-T) are shown in Figures 1–6.

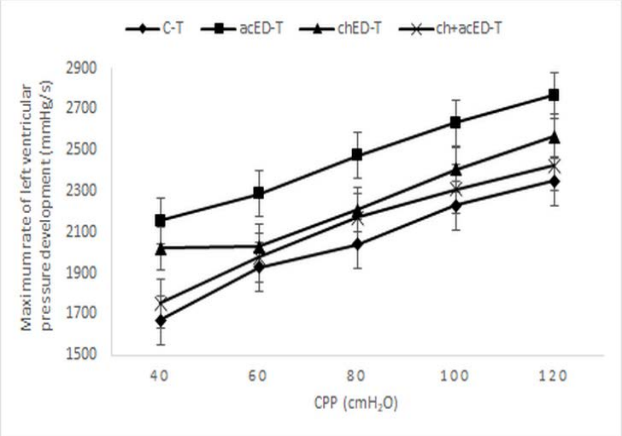

Fig. 1 − Values of the maximum rate of left ventricular pressure development during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T (data are given as means ± standard error).
C-T – control group; acED-T – acute energy drinks (ED-T) group; chED-T – chronic ED-T group; ch + acED-T – chronic + acute ED-T groups; CPP – coronary perfusion pressure.

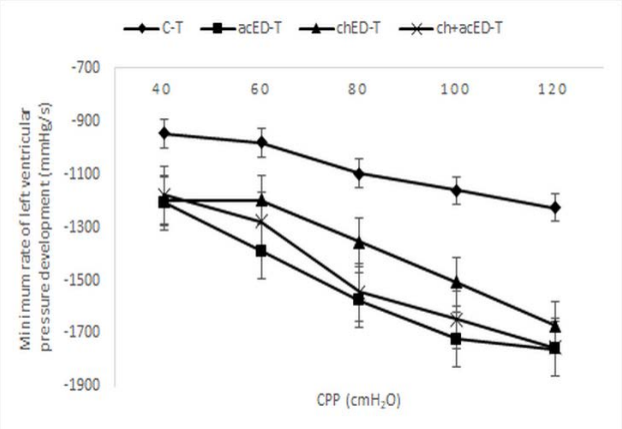

Fig. 2 − Values of the minimum rate of left ventricular pressure development during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure (data are given as means ± standard error).
For abbreviations see under Figure 1.

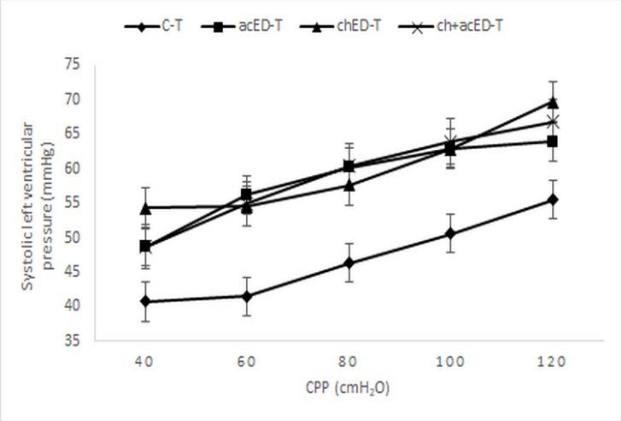

Fig. 3 − Values of systolic left ventricular pressure during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure (data are given as means ± standard error).
For abbreviations see under Figure 1.

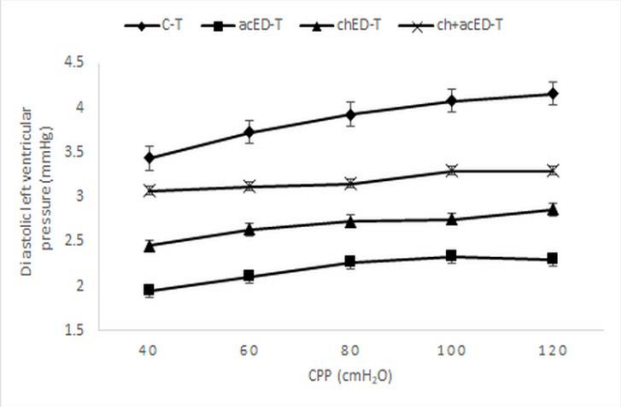

Fig. 4 − Values of diastolic left ventricular pressure during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure (data are given as means ± standard error).
For abbreviations see under Figure 1.

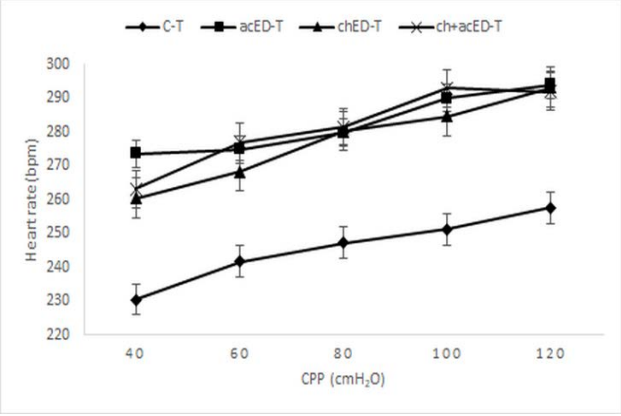

Fig. 5 − Values of heart rate during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure (data are given as means ± standard error).
For abbreviations see under Figure 1.

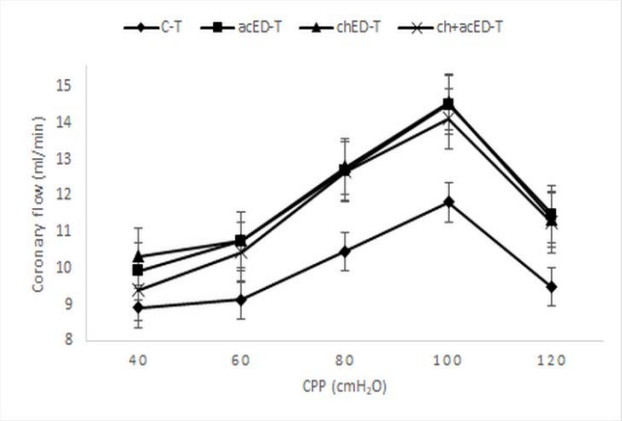

Fig. 6 − Values of coronary flow during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure (data are given as means ± standard error).
For abbreviations see under Figure 1.

Concerning the C-T group, the following results were recorded in the acED-T group: 1) at all CPPs, statistically significantly higher level of dP/dt max ($p < 0.05$; *t*-test for independent samples), significantly higher level of HR ($p < 0.05$; Mann-Whitney test) and a significantly lower level of DLVP ($p < 0.05$; *t*-test for independent samples); 2) at CPP 60–100 cm H_2O, statistically significantly higher level of SLVP ($p < 0.05$; *t*-test for independent samples); 3) at CPP 60–120 cm H_2O, statistically significantly higher levels of dP/dt min and CF ($p < 0.05$; *t*-test for independent samples).

Concerning the C-T group, the following results were recorded in the chED-T group: 1) at all CPPs, statistically significantly higher level of HR ($p < 0.05$; *t*-test for independent samples) and significantly lower level of DLVP ($p < 0.05$ Mann-Whitney test); 2) at CPP 60–120 cm H_2O, statistically significantly higher level of CF ($p < 0.05$; Mann-Whitney test); 3) at all CPP, higher level of SLVP, but only statistically significantly higher at CPP 60, 100 and 120 cm H_2O ($p < 0.05$; *t*-test for independent samples) and higher level of dP/dt min, but statistically significant only at CPP 100–120 cm H_2O ($p < 0.05$; *t*-test for independent samples); 4) at all CPP, higher level of dP/dt max, but without statistical significance ($p > 0.05$; *t*-test for independent samples).

Concerning the acED-T group, the following results were recorded in the ch + acED-T group: 1) at all CPPs, lower level of dP/dt max and higher level of DLVP, but statistically significant for both parameters only CPP 40 cm H_2O ($p < 0.05$; *t*-test for independent samples); 2) at all CPP, lower level of dP/dt min and CF, but without statistical significance ($p > 0.05$; *t*-test for independent samples). There were no statistically significant differences in the levels of SLVP and HR between these two groups ($p > 0.05$; Mann-Whitney test).

Concerning the chED-T group, the ch + acED-T group recorded a lower level of dP/dt max and CF, as well as a higher level of DLVP, but without statistical significance (at all CPPs) ($p > 0.05$; *t*-test for independent samples; Mann-

Whitney test). There were no statistically significant differences in the levels of SLVP, dP/dt min, and HR between these two groups ($p > 0.05$; Mann-Whitney test; *t*-test for independent samples).

Concerning the C-T group, the following results were recorded in the ch+acED-T group: 1) at all CPPs, a significantly higher level of HR ($p < 0.05$; *t*-test for independent samples), higher level of SLVP, but only at CPP 60 and 80 cm H_2O statistically significantly higher ($p < 0.05$; Mann-Whitney test) and higher level of dP/dt min, but at CPP 60–120 cm H_2O statistically significantly higher ($p < 0.05$; *t*-test for independent samples); 2) at all CPPs, higher level of dP/dt max and CF, and lower level of DLVP, but without statistical significance ($p > 0.05$; *t*-test for independent samples).

Oxidative stress

Prooxidative parameters in the effluent during coronary autoregulation of isolated rat hearts in four groups (C-T, acED-T, chED-T, ch + acED-T) are shown in Figures 7–10.

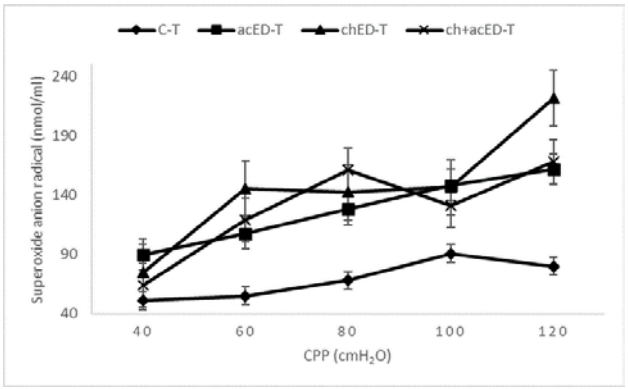

Fig. 7 − Values of superoxide anion radical in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T (data are given as means ± standard error). For abbreviations see under Figure 1.

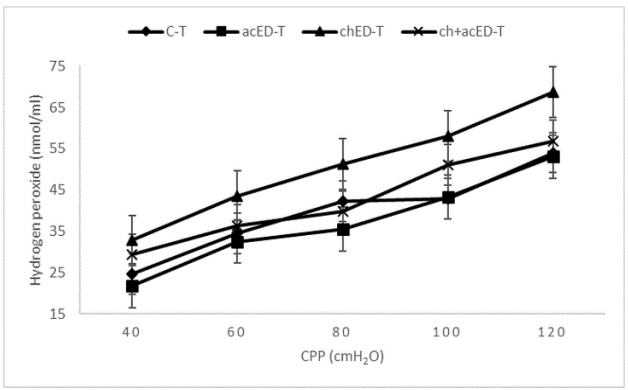

Fig. 8 − Values of hydrogen peroxide in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T. CPP, coronary perfusion pressure (data are given as means ± standard error). For abbreviations see under Figure 1.

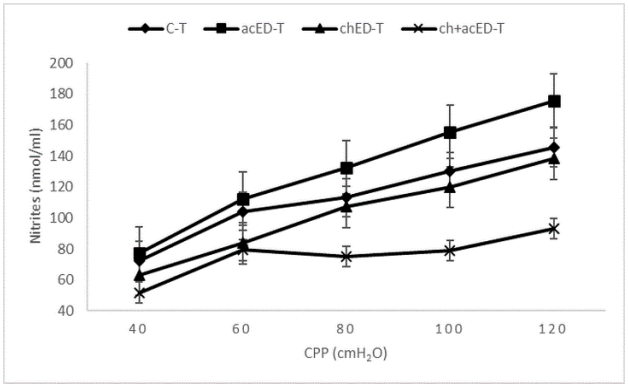

Fig. 9 − Values of nitrites in effluent, during coronary autoregulation of the isolated trained rat hearts in the following groups: C-T, acED-T, chED-T, ch + acED-T (data are given as means ± standard error). For abbreviations see under Figure 1.

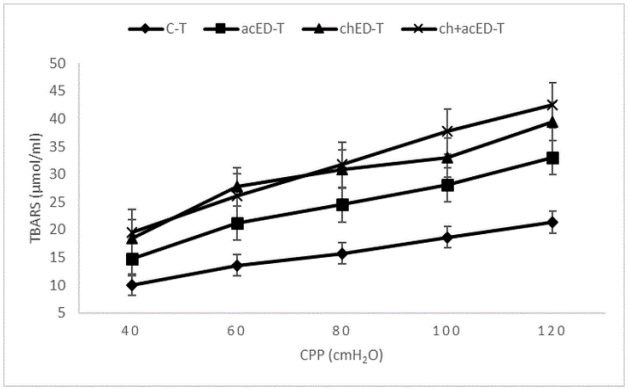

Fig. 10 − Values of index of lipid peroxidation (TBARS − thiobarbituric acid reactive substances) in effluent during coronary autoregulation of the isolated trained rat heart in the following groups: C-T, acED-T, chED-T, ch + acED-T (data are given as means ± standard error). For abbreviations see under Figure 1.

In relation to the C-T group, the following results were recorded in the acED-T group: 1) at all CPP, the level of TBARS (lipid peroxidation index) was statistically significantly higher ($p < 0.05$; Mann-Whitney test); 2) at all CPP, levels of O_2^- and nitrites (NO) were higher, but without statistical significance ($p > 0.05$; Mann-Whitney test; $p > 0.05$; *t*-test for independent samples, respectively). There was no statistically significant difference in the level of H_2O_2 between these two groups ($p > 0.05$; Mann-Whitney test).

In relation to the C-T group, the following results were recorded in the chED-T group: 1) at all CPP, the level of TBARS was statistically significantly higher ($p < 0.05$; Mann-Whitney test); 2) at CPP 60, 80, and 120 cm H_2O, the level of O_2^- was statistically significantly higher ($p < 0.05$; Mann-Whitney test); 3) at all CPP, level of H_2O_2 was higher but statistically significant only at CPP 60 cm H_2O ($p < 0.05$; Mann-Whitney test); 4) at all CPP, levels of nitrites (NO) were lower, but without statistical significance ($p > 0.05$; Mann-Whitney).

In relation to the acED-T group, the following results were recorded in the ch + acED-T group: 1) at all CPPs, the

level of TBARS was statistically significantly higher ($p < 0.05$; *t*-test for unbound samples); 2) at CPP 40, 80, 100 and 120 cm H_2O, the levels of NO were statistically significantly lower ($p < 0.05$; Mann-Whitney test); 3) at all CPP, level of H_2O_2 was higher, but without statistical significance ($p > 0.05$; Mann-Whitney test). There was no statistically significant difference in the level of O_2^- between these two groups ($p > 0.05$; Mann-Whitney test).

In relation to the chED-T group, the following results were recorded in the ch + acED-T group: 1) at all CPPs, the levels of NO were lower, but statistically significant only at CPP 80–120 cm H_2O ($p < 0.05$; Mann-Whitney test); 2) at all CPPs, the level of H_2O_2 was lower, but without statistical significance ($p > 0.05$; Mann-Whitney test). There were no statistically significant differences in the levels of O_2^- and TBARS between these two groups (p > 0.05; Mann-Whitney test; $p > 0.05$; *t*-test for independent samples).

In relation to the C-T group, the following results were recorded in the ch + acED-T group: 1) at all CPPs, the level of TBARS was statistically significantly higher ($p < 0.05$; Mann-Whitney test), while the level of O_2^- was higher, but statistically significant only at CPP 60 and 80 cm H_2O ($p < 0.05$; Mann-Whitney test); 2) at all CPPs, the levels of nitrites (NO) were lower, but statistically significant at CPP 40, 80–120 cm H_2O ($p < 0.05$; Mann-Whitney test). There was no statistically significant difference in the level of H_2O_2 between these two groups ($p > 0.05$; *t*-test for independent samples).

Discussion

In this research, we studied the effect of RB® on cardiovascular parameters and coronary autoregulation in the isolated heart of trained rats, as well as the level of prooxidative parameters in the coronary venous effluent. The training included swimming for four weeks (1 hour/day, 5 days/week), which is considered to be a moderate-intensity exercise. The specificity of swimming as an exercise is in engaging the muscles of the entire body, which improves the capacity of the cardiovascular system. The influence of RB® was evaluated at three levels: acute consumption, chronic consumption, as well as a combination of chronic and acute consumption.

Coronary autoregulation implies an intrinsic cardiac ability to maintain a relatively constant blood flow in response to a change in perfusion pressure when myocardial oxygen demand is constant [36]. The maximum rate of pressure change in the LV (dP/dt max) occurs at the end of the isovolumetric contraction and is used to estimate the inotropic properties of the myocardium [37], while the minimum rate of pressure change in the LV (dP/dt min) represents the relaxation rate (lusitropic properties of the myocardium) and reflects the maximum rate of pressure drop in the LV [38]. In our study, the increase in the level of dP/dt max and dP/dt min within the acED-T group was registered, when compared to the C-T group. In line with that, acute consumption of EDs contributed to an increase in SLVP, HR, and CF, while DLVP was lower in the acED-T group.

As for the impact of RB® and other EDs on cardiodynamics, the majority of previously published papers have focused on acute consumption and have been conducted on young, healthy people in a state of rest. As far as athletes are concerned, after consuming RB® and during their recovery phase following physical exercise, a significant increase in contractility of the left atrium and ventricle was registered [3]. It is believed that most of the biological effects of EDs are mediated by a positive inotropic effect [15], which is in line with our results. As for humans, it has been demonstrated that the acute consumption of 250 mL of RB® affects the increase in mean arterial pressure [22], but there are also studies that have shown no effect on systolic and diastolic arterial pressure at rest [28]. Additionally, in terms of the effect of EDs on the HR, non-homogeneous results were obtained (mostly with no effect or increase in the HR) [22, 28], but there was also a study published, showing how EDs influenced the reduction of the HR [39]. It has been shown that a higher dose of RB® (355 mL) affects the increase in systolic and diastolic arterial pressure and the increase in the HR [40], as well as that RB® at a dose of 500 mL affects the increase in the activity of the sympathetic nervous system [41]. The aforementioned can be explained by the effect of EDs on the increase in norepinephrine levels [42], which increases the HR and blood pressure, triggers the release of glucose from energy stores, and increases blood flow to skeletal muscles [16].

The dP/dt min level within the chED-T group was higher when compared to the C-T group, indicating a positive lusitropic effect of the EDs, while a decrease in DLVP was also registered. As it is the case with acute ED consumption, chronic consumption has also affected the increase in HR and CF. The fact that there was no significant difference in the dP/dt max level between the chED-T and the C-T group, as well as that SLVP was significantly higher in the chED-T group, can be interpreted as a negative influence of chronic ED consumption. Hypertension can cause LV hypertrophy, which is a risk factor for future cardiovascular events [43]. In pre-clinical studies, chronic use of EDs has mainly been evaluated through their effect on heart metabolism and, following our results, a negative effect on the heart of the rats has been registered [11, 44, 45]. Regular moderate exercise has beneficial effects on the heart [46], but our results show the chronic consumption of EDs can disrupt that effect.

Compared to acED-T, significantly lower levels of dP/dt max were observed in the ch + acED-T group, which may indicate slight depression in cardiac contractile force and systolic function. Moreover, when compared to the acED-T group, the lower values of dP/dt min (less negative) and CF were registered in the ch + acED-T group and, although they were not statistically significant, in combination with a significantly higher level of DLVP, they may be interpreted as mild changes in diastolic function. The obtained results suggest that EDs have a different effect on the cardiovascular system in chronic consumers when they acutely consume EDs and when it comes to occasional acute consumption. Given that the consumption of EDs by athletes is still a controversial topic, in terms of whether the benefits for improving the

performance are greater than the potential health hazards, the results describing how chronic consumption of EDs affects cardiovascular response in acute consumption can be useful for further research on this topic.

In acED-T and chED-T groups, when compared to the C-T, there was a significant increase in lipid peroxidation index level (estimated through the TBARS level), which indicates the deterioration of redox status. Furthermore, a significant increase in TBARS level was observed in the ch + acED-T group, when compared to the acED-T group, which suggests that in chronic consumers acute ED consumption continues to deteriorate redox status. The intense lipid peroxidation in biological membranes leads to the loss of fluidity, a decrease in the membrane potential, the increased permeability for H^+ and other ions and, in the end, a membrane rupture may also occur with the release of cellular content into extracellular space [47]. Our results are consistent with the increase in lipid peroxidation observed in the liver and brain of rats, after 14 days of using another commercially available ED [48]. In the chED-T group, an increased level of prooxidative species, O_2^- and H_2O_2, was also registered, when compared to the C-T. It is known that O_2^- and H_2O_2 affect the activation of the mitochondrial permeability transition pores, which leads to the loss of cytochrome C from mitochondria and the activation of caspases with the development of apoptosis [31, 49]. Generally, a larger amount of O_2^- reacts with NO, reducing its bioavailability and damaging endothelium-dependent vasodilatation [50]. A moderate-intensity training leads to a reduction in TBARS, O_2^-, and H_2O_2 [31] while, in our study, chronic ED consumption, in combination with moderate-intensity training, had the opposite effect and caused an increase in TBARS, O_2^-, and H_2O_2.

In the ch + acED-T group, when compared with the acED-T group, a significant decrease in the NO level was registered (estimated through the level of nitrite). It is known that atherosclerosis occurs due to the mechanism of vascular inflammation, which is defined by the increased production of ROS and due to the fact that endothelial dysfunction is characterized by the reduced production of NO [51]. NO is produced from L arginine and represents an important endogenous basal coronary tone regulator, while reactive hyperaemia and shear stress are a stimulus for the release of NO from the endothelium and the formation of vasodilatation. NO leads to the relaxation of the smooth muscles of coronary vessels, inhibits adhesion and platelet aggregation, inhibits leukocyte activation, and reduces the consumption of oxygen in the myocardium [52]. High glucose levels in EDs can be a factor that contributes to the platelet function damaging and the occurrence of endothelial dysfunction [22]. Hyperglycemia contributes to an increase in oxidative stress markers, and lipid peroxidation in erythrocytes is directly proportional to *in vitro* glucose concentration [53].

As it was already mentioned, EDs reduce endothelial function in humans at rest [22, 23, 54], and our results in rats show that this also applies to the physical activity of chronic consumers when they consume EDs acutely. On the other hand, acute administration of RB® at a dose of 250 mL and 355 mL has been shown to improve the endothelial function [12, 55], and this topic is an open field for further studies. Due to endothelial dysfunction, ED consumption is associated with an increased risk of myocardial ischaemia [56]. Previous evidence linking ED consumption with myocardial ischaemia is mainly based on case reports. The lack of randomized and prospective research is a major obstacle to the impossibility of establishing an unambiguous connection between excessive ED consumption and ischemia or myocardial infarction.

Conclusion

While the acute effects of EDs on the cardiovascular system are fairly clarified, chronic effects are much less studied and further research is suggested. The conclusion of our study is that acute administration of EDs had a positive inotropic effect, while chronic administration affected the isolated increase in SLVP, which could be considered the potentially negative impact of EDs. Chronic administration of EDs changed the cardiovascular response in acute consumption. Moreover, the prooxidative effect of EDs was observed. Due to the potential association of ED consumption with the onset of endothelial dysfunction and potential morbidity combined with physical exercise, further research is needed to clarify action mechanisms and significance of their effects, i.e. the correlations with clinical outcomes.

Acknowledgement

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REFERENCES

1. *Mubarak R.* Effect of red bull energy drink on Rat's submandibular salivary glands (Light and Electron microscopic study). J Am Sci 2012; 8(1): 366–72.
2. *Ayuob N, ElBeshbeishy R.* Impact of an Energy Drink on the Structure of Stomach and Pancreas of Albino Rat: Can Omega-3 Provide a Protection? PLoS One 2016; 11(2): e0149191.
3. *Baum M, Weiss M.* The influence of a taurine containing drink on cardiac parameters before and after exercise measured by echocardiography. Amino Acids 2001; 20(1): 75–82.
4. *Franks AM, Schmidt JM, McCain KR, Fraer M.* Comparison of the effects of energy drink versus caffeine supplementation on indices of 24-hour ambulatory blood pressure. Ann Pharmacother 2012; 46(2): 192–9.

5. *Fletcher EA, Lacey CS, Shah SA.* Impact of high volume energy drink consumption of electrocardiographic and blood pressure parameters. Circulation 2014; 130(Suppl 2): A15885.
6. *Miles-Chan JL, Charriere N, Grasser EK, Montani JP, Dulloo AG.* The blood pressure-elevating effect of Red Bull energy drink is mimicked by caffeine but through different hemodybamic pathways. Physiol Rep 2015; 3(2). pii: e12290.
7. *Mangi MA, Rehman H, Rafique M, Illovsky M.* Energy Drinks and the Risk of Cardiovascular Disease: A Review of Current Literature. Cureus 2017; 9(6): e1322.
8. *Ishak WW, Ugochukwu C, Bagot K, Khalili D, Zaky C.* Energy drinks: psychological effects and impact on well-being and quality of life-a literature review. Innov Clin Neurosci 2012; 9(1): 25–34.
9. *Schaffer SW, Shimada K, Jong CJ, Ito T, Azuma J, Takahashi K.* Effect of taurine and potential interactions with caffeine on cardiovascular function. Amino Acids 2014; 46(5): 1147–57.
10. *Salinero JJ, Lara B, Abian-Vicen J, Gonzalez-Millán C, Areces F, Gallo-Salazar C,* et al. The use of energy drinks in sport: perceived ergogenicity and side effects in male and female athletes. Br J Nutr 2014; 112(9): 1494–502.
11. *Crisan M, Munteanu C, Jula C, Lang C, Rosioru C.* Effects of Red Bull on cardiac muscle in physically trained and untrained Wistar rats. Ann Rom Soc Cell Biol 2014; 19(1): 37–41.
12. *Grasser EK, Yepuri G, Dulloo AG, Montani JP.* Cardio- and cerebrovascular responses to the energy drink Red Bull in young adults: a randomized cross-over study. Eur J Nutr 2014; 53(7): 1561–71.
13. *Menci D, Righini FM, Cameli M, Lisi M, Benincasa S, Focardi M,* et al. Acute effects of an energy drink on myocardial function assessed by conventional echo-Doppler analysis and by speckle tracking echocardiography on young healthy subjects. J Amino Acids 2013; 2013: 646703.
14. *Ebuehi OA, Ajayl OE, Onyeulor AL, Awelimobor D.* Effects of oral administration of energy drinks on blood chemistry, tissue histology and brain acetylcholine in rabbits. Nig Q J Hosp Med 2011; 21(1): 29–34.
15. *Lippi G, Cervellin G, Sanchis-Gomar F.* Energy Drinks and Myocardial Ischemia: A Review of Case Reports. Cardiovasc Toxicol 2016; 16(3): 207–12.
16. *Higgins JP, Babu K, Deuster PA, Shearer J.* Energy Drinks: A Contemporary Issues Paper. Curr Sports Med Rep 2018; 17(2): 65–72.
17. *Sanchis-Gomar F, Pareja-Galeano H, Cervellin G, Lippi G, Earnest CP.* Energy drink overconsumption in adolescents: implications for arrhythmias and other cardiovascular events. Can J Cardiol 2015; 31(5): 572–5.
18. *Finnegan D.* The health effects of stimulant drinks. Br Nutr Found Nut Bull 2003; 28(2): 147–55.
19. *Higashi Y, Noma K, Yoshizumi M, Kihara Y.* Endothelial function and oxidative stress in cardiovascular diseases. Circ J 2009; 73(3): 411–8.
20. *Umemura T, Ueda K, Nishioka K, Hidaka T, Takemoto H, Nakamura S,* et al. Effects of acute administration of caffeine on vascular function. Am J Cardiol 2006; 98(11): 1538–41.
21. *Higgins JP, Babu KM.* Caffeine reduces myocardial blood flow during exercise. Am J Med 2013; 126(8): 730.e1–8.
22. *Worthley MI, Prabhu A, De Sciscio P, Schultz C, Sanders P, Willoughby SR.* Detrimental effects of energy drink consumption on platelet and endothelial function. Am J Med 2010; 123(2): 184–7.
23. *Higgins JP.* Endothelial function acutely worse after drinking energy beverage. Int J Cardiol 2013; 168(2): e47–9.
24. *Sadowska J.* Evaluation of the effect of consuming an energy drink on the concentration of glucose and triacylglycerols and on fatty tissue deposition. A model study. Acta Sci Pol Technol Aliment 2012; 11(3): 311–8.
25. *Salgado-Somoza A, Teijeira-Fernández E, Fernández AL, González-Juanatey JR, Eiras S.* Proteomic analysis of epicardial and subcutaneous adipose tissue reveals differences in proteins involved in oxidative stress. Am J Physiol Heart Circ Physiol 2010; 299(1): H202–9.
26. *Barcelos RP, Souza MA, Amaral GP, Stefanello ST, Bresciani G, Figbera MR,* et al. Caffeine supplementation modulates oxidative stress markers in the liver of trained rats. Life Sci 2014; 96(1–2): 40–5.
27. *Ugwuja E.* Biochemical effects of energy drinks alone or in combination with alcohol in normal albino rats. Adv Pharm Bull 2014; 4(1): 69–74.
28. *Alford C, Cox H, Wescott R.* The effects of red bull energy drink on human performance and mood. Amino Acids 2001; 21(2): 139–50.
29. *Higgins JP, Tuttle TD, Higgins CL.* Energy beverages: content and safety. Mayo Clin Proc 2010; 85(11): 1033–41.
30. *Nikolic TR, Zivkovic VI, Srejovic IM, Radovanovic DS, Jeremic NS, Jevdjevic MD,* et al. Acute effects of nandrolone decanoate on cardiodynamic parameters in isolated rat heart. Can J Physiol Pharmacol 2016; 94(10): 1048–57.
31. *Stanojevic D, Jakovljevic V, Barudzic N, Zivkovic V, Srejovic I, Parezanovic Ilic K,* et al. Overtraining does not induce oxidative stress and inflammation in blood and heart of rats. Physiol Res 2016; 65(1): 81–90.
32. *Auclair C, Voisin E.* Nitroblue tetrazolium reduction. In: *Greenwald RA,* editor. Handbook of methods for oxygen radical research. Boca Raton: CRC Press; 1985. p. 123–32.
33. *Pick E, Keisari Y.* A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. J Immunol Methods 1980; 38(1–2): 161–70.
34. *Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR.* Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem 1982; 126(1): 131–8.
35. *Ohkawa H, Ohishi N, Yagi K.* Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95(2): 351–8.
36. *Dole WP.* Autoregulation of the coronary circulation. Prog Cardiovasc Dis 1987; 29(4): 293–323.
37. *Hamlin RL, del Rio C.* dP/dt(max)--a measure of 'baroinometr y'. J Pharmacol Toxicol Methods 2012; 66(2): 63–5.
38. *Leite-Moreira AF.* Current perspectives in diastolic dysfunction and diastolic heart failure. Heart 2006; 92(5): 712–8.
39. *Hajsadeghi S, Mohammadpour F, Manteghi MJ, Kordshakeri K, Tokazebani M, Rahmani E,* et al. Effects of energy drinks on blood pressure, heart rate, and electrocardiographic parameters: An experimental study on healthy young adults. Anatol J Cardiol 2016; 16(2): 94–9.
40. *Elitok A, Öz F, Panc C, Sarıkaya R, Sezikli S, Pala Y,* et al. Acute effects of Red Bull energy drink on ventricular repolarization in healthy young volunteers: a prospective study. Anatol J Cardiol 2015; 15(11): 919–22.
41. *Cavka A, Stupin M, Panduric A, Plazibat A, Cosic A, Rasic L,* et al. Adrenergic System Activation Mediates Changes in Cardiovascular and Psychomotoric Reactions in Young Individuals after Red Bull (®) Energy Drink Consumption. Int J Endocrinol 2015; 2015: 751530.
42. *Svatikova A, Covassin N, Somers KR, Somers KV, Soucek F, Kara T,* et al. A Randomized Trial of Cardiovascular Responses to Energy Drink Consumption in Healthy Adults. JAMA 2015; 314(19): 2079–82.
43. *Kahan T, Bergfeldt L.* Left ventricular hypertrophy in hypertension: its arrhythmogenic potential. Heart 2005; 91(2): 250–6.
44. *Backer W, Baeissa H.* Effect of Different Energy Drinks on Liver and Heart Enzymes in Rats. Int J Biotechnol 2014; 3(1): 1–11.

45. *Chimezie OS*. Effects of Bullet Energy Drink on Creatininephosphokinase (CPK) and Lactate Dehydrogenaase (LDH) Level of Albino Rat. J Nat Sci Res 2013; 3(3): 15–7.
46. *Stojanovic Tosic JT, Jakovljevic VLj, Zivkovic VV, Srejovic IM, Valdevit ZJ, Radovanovic DS*, et al. Biphasic response of cardiodynamic adaptations to swimming exercise in rats. Gen Physiol Biophys 2015; 34(3): 301–10.
47. *Gutteridge JM*. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995; 41(12 Pt 2): 1819–28.
48. *Reis R, Charehsaz M, Sipahi H, Ekici AI, Macit Ç, Akkaya H*, et al. Energy Drink Induced Lipid Peroxidation and Oxidative Damage in Rat Liver and Brain When Used Alone or Combined with Alcohol. J Food Sci 2017; 82(4): 1037–43.
49. *Cai J, Jones DP*. Superoxide in apoptosis. Mitochondrial generation triggered by cytochrome c loss. J Biol Chem 1998; 273(19): 11401–4.
50. *Cai H, Harrison DG*. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 2000; 87(10): 840–4.
51. *Barudzic N, Turjacanin-Pantelic D, Zivkovic V, Selakovic D, Srejovic I, Joksimovic J*, et al. The effects of cyclooxygenase and nitric oxide synthase inhibition on oxidative stress in isolated rat heart. Mol Cell Biochem 2013; 381(1–2): 301–11.
52. *Jakovljevic VLj, Canovic PS, Andjelkovic NV, Djuric DM*. The effects of nimodipine and L-NAME on coronary flow and oxidative stress parameters in isolated rat heart. Acta Physiol Hung 2006; 93(4): 251–61.
53. *Rains JL, Jain SK*. Oxidative stress, insulin signaling, and diabetes. Free Radic Biol Med 2011; 50(5): 567–75.
54. *DeSciscio P, Prabhu A, Worthley M, Roberts-Thomson R, Sanders P, Willoughby S*. Acute Effects of Red Bull on Platelet and Endothelial Function. Heart Lung Circulation 2008; 17(Suppl 3): S23–4.
55. *Molnar J, Somberg JC*. Evaluation of the Effects of Different Energy Drinks and Coffee on Endothelial Function. Am J Cardiol 2015; 116(9): 1457–60.
56. *Higgins JP, Ortiz BL*. Energy drink ingredients and their effect on endothelial function: A Review. Int J Clin Cardiol 2014; 1: 1–6.

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Clinical presentation of incidentally discovered adrenal tumors – our experience

Klinička prezentacija slučajno otkrivenih tumora nadbubrega – naša iskustva

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Abstract

Background/Aim. Incidentalomas of the adrenal gland are adrenal masses commonly discovered by chance on imaging not performed for suspected adrenal disease. The aim of this study was to analyze clinical, hormonal and histopathological characteristics of adrenal incidentalomas. **Methods.** This retrospective study included 85 patients (32 men and 53 women) examined for adrenal incidentalomas at the Clinic for Endocrinology, Military Medical Academy in Belgrade, from January 2013 to December 2017. The age of the patients, gender, size, and localization of adrenal tumors, functional activity, as well as the presence of comorbidities were analyzed. Adrenalectomy was performed in 35 patients due to its size and functional activity, and histological findings were analyzed. **Results.** The largest number (56.4%) of the adrenal tumors, was detected by ultrasound examination of the abdomen, 23 (27.2%) by abdominal computed tomography (CT) scan, 13 (15.2%) by chest CT scan and 1 (1.2%) by magnetic resonance (MR) imaging of the abdomen. The average tumor size was 3.8 ± 2.3 cm (range from 1 to 15 cm). Adrenal tumors were bilateral in 20 (23%) patients, in 39 (46%) patients, the tumors were localized in the right adrenal gland, and in 26 (31%) in the left gland. Subclinical hypercortisolism, defined as insufficient cortisol suppression during overnight dexamethasone test (1 mg), was observed in 34 (40%) patients, while the absence of cortisol suppression (autonomous cortisol secretion) was found in 4 (4.7%) patients. In the remaining 47 (55.3%) patients, complete overnight suppression of cortisol secretion was achieved. Thirty-five (41%) patients underwent adrenalectomy; among them, in 4 (11.4%) cases, adrenocortical carcinoma was found, 15 (42.9%) were adenomas, pheochromocytoma was found in 4 (11.4%) cases, nodular hyperplasia in 5 (14.3%) cases, distant metastasis in one (2.8%) case and the remaining were different benign masses. **Conclusion.** For patients with adrenal incidentalomas, two fundamental questions on determining the functionality of the tumor and/or the presence of malignancy need to be clarified. All patients with adrenal incidentaloma should undergo hormonal evaluation for autonomous or possible autonomous cortisol secretion, as well as for autonomous, adrenergic, and mineralocorticoid excess. In patients with autonomous adrenal secretion, surgery is indicated even if the typical clinical manifestation is absent.

Key words:
adrenal gland neoplasms; adrenal incidentaloma; diagnosis; surgical procedures, operative; histological techniques; ultrasonography.

Apstrakt

Uvod/Cilj. Incidentalomi nadbubrega su tumori nadbubrežne regije koji su slučajno otkriveni različitim radiološkim ispitivanjima, u toku dijagnostike oboljenja koja nisu povezana sa adrenalnom patologijom. Cilj rada je bio da se analiziraju kliničke, biohumoralne i histopatološke karakteristike adrenalnih incidentaloma. **Metode**. Retrospektivnom analizom bilo je obuhvaćeno 85 bolesnika (32 muškarca i 53 žena), koji su ispitivani na Klinici za endokrinologiju Vojnomedcinske akademije u Beogradu, u periodu od januara 2013. do decembra 2017. godine zbog incidentaloma nadbubrega. Ispitivani su životno doba bolesnika, veličina lokalizacija i funkcionalna aktivnost tumora, kao i postojanje komorbiditeta. Adrenalektomija je učinjena kod 35 bolesnika na osnovu tumorske veličine, hormonske aktivnosti i pridruženih bolesti. **Rezultati**. Najveći broj (56,4%) adrenalnih tumora otkriven je ultrazvučnim pregledom abdomena, kod 23 (27,2%) bolesnika putem kompjuterizovane tomo

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grafije (CT) abdomena, kod 13 (15,2%) bolesnika putem CT pregleda grudnog koša i kod 1 (1,2%) bolesnika pomoću magnetne rezonance abdomena. Prosečna veličina tumora iznosila je 3,8 ± 2,3 cm (raspon od 1 do 15 cm). Adrenalni tumori su bili bilateralni kod 23% bolesnika, kod 46% bolesnika su bili lokalizovani u desnom, a u 31% slučajeva u levom nadbubregu. Supklinički hiperkorticizam, definisan kao neadekvatna kortizolska supresija prekonoćnim deksametazonskim testom (1 mg) uočena je kod 34 (40%) bolesnika, dok je odsustvo kortizolske supresije (autonomna kortizolska sekrecija) pronađena kod 4 (4.7%) bolesnika. U preostalih 47 (55.3%) bolesnika ostvarena je kompletna prekonoćna supresija deksametazonom. Adrenelektomija je učinjena kod 35 (41%) bolesnika, među kojima je kod njih 4 (11,4%) dijagnostikovan adrenokortikalni karcinom, kod 15 (42,9%) adenomi, feohromocitom kod 4 (11,4%), nodularna hiperplazija kod 5 (14,3%), udaljena metastaza kod jednog (2,8%), dok se kod preostalih bolesnika radilo o različitim benignim masama. **Zaključak.** Kod bolesnika sa incidentalno uočenim tumorima nadbubrežne regije trebalo bi razjasniti dva osnovna pitanja: funkcionalni status tumora i/ili prisustvo malignoteta. Kod svih bolesnika bi trebalo sprovesti biohumoralno ispitivanje u smislu autonomne ili moguće autonomne kortizolske sekrecije, kao i ispitivanje autonomnog adrenergičnog i mineralokortikoidnog ekscesa. Kod svih bolesnika kod kojih postoji dokaz o autonomnoj sekreciji bilo kojeg hormona, neophodno je sprovesti radikalno lečenje, nezavisno od prisustva ili odsustva tipične kliničke manifestacije.

Ključne reči:
nadbubrežne žlezde, neoplazme; nadbubrežna žlezda, incidentalom; dijagnoza; hirurgija, operativne procedure; histološke tehnike; ultrasonografija.

Introduction

Adrenal incidentalomas are frequent endocrine disorders considered as the disease of modern technology, which lead to a marked increase of accidentally discovered tumors. By definition, these are asymptomatic adrenal masses detected on imaging not performed for suspected adrenal disease [1]. Adrenal incidentaloma is not a single clinical entity; this term includes a range of different pathological states common to being discovered by chance. Incidentalomas of the adrenal gland include a large number of histological diagnoses that originate from the adrenal medulla, cortex, or extra-adrenal tissues. According to the clinical features, these are most often nonfunctioning tumors, while a small number of patients exhibit a clinical presentation of enhanced secretion of one or more adrenal hormones. The initial diagnostic evaluation is aimed at revealing the functional status of the mass and the possibility of malignancy. For this reason, all patients with incidentally discovered adrenal masses must undergo a detailed clinical, biochemical and radiological assessment [2, 3].

The treatment of these patients depends on the tumor's functional activity, size, radiological characteristics and growth rate. The quality of life is another vital factor that influences the decision, but our knowledge about the impact of these tumors on quality of life is insufficient. According to the current guidelines, surgical treatment is recommended for adrenal masses larger than 4 cm in diameter, except in the case of clear benign lesions such as cysts or myelolipomas. Tumors smaller than 4 cm should be regularly laboratory and radiologically followed [1, 2].

The aim of this study was to analyze clinical and hormonal characteristics of adrenal masses, incidentally discovered and treated in our clinic during the five-year period, as well as the relationship with histopathological diagnosis in operated patients.

Methods

This retrospective study included 85 patients (32 men and 53 women), examined for incidentally discovered adrenal masses at the Clinic for Endocrinology, Military Medical Academy in Belgrade, from January 2013 to December 2017. The examination excluded patients with previously suspected adrenal functional adenomas or concurrent history of primary malignancies. We analyzed the age of the patients, gender, size and localization of adrenal tumors, previous examinations that led to the diagnosis of incidentaloma, functional activity, as well as the presence of concomitant arterial hypertension, diabetes mellitus, osteoporosis, dyslipidemia and obesity.

For assessing hormonal activity, we analyzed basal plasma adrenocorticotropic hormone and cortisol plasma values, overnight dexamethasone suppression test, serum dehydroepiandrosterone sulphate (DHEAS), serum testosterone and plasma concentrations of 17-beta estradiol, metanephrine and normetanephrine. Due to some technical incapacities, plasma aldosterone concentrations and plasma renin activity were performed only in patients with hypertension and accompanied hypokalemia, while daily urinary free cortisol and urinary 17-ketosteroids were not performed at all. The diagnosis of subclinical Cushing's syndrome (SCS) (silent Cushing's syndrome or possible autonomous cortisol secretion) was based on plasma cortisol levels after an overnight dexamethasone test (values between 50 nmol/L and 138 nmol/L). Patients with cortisol levels over 138 nmol/L after the overnight dexamethasone suppression were considered to have autonomous cortisol secretion. In patients with suspected mineralocorticoid excess, if the

aldosterone/renin ratio was greater than 20, autonomous aldosterone secretion was confirmed.

Adrenalectomy was performed in 35 patients due to tumor size (over 4 cm). Functional activities, comorbidities, and histological findings were analyzed.

Results

Eighty-five patients, 32 (37.5%) men and 53 (62.4%) women, were hospitalized for a five-year period for examining incidentally discovered adrenal masses. The average age of these patients was 59 ± 30 years (range from 28 to 79 years of age). Sixty-four (75%) patients were over 50 years old, with a peak in the seventh decade in 32 (38.5%) patients.

In 48 (56.4%) patients with adrenal incidentaloma, tumors were detected by an ultrasound examination of the abdomen, in 23 (27.2%) patients by abdominal computed tomography (CT) scan, in 13 (15.2%) patients by chest CT scan and in 1 (1.2%) patient by magnetic resonance (MR) imaging of the abdomen. The average tumor size was 3.8 ± 2.3 cm (range from 1 to 15 cm).

Adrenal tumors were bilateral in 20 (23%) patients, in 39 (46%) patients, adrenal tumors were localized in the right adrenal gland, and in 26 (31%) patients in the left gland.

The way of detecting adrenal tumors is shown in Table 1.

Table 1

Detection of adrenal incidentalomas

Indications for medical examination	Patients n (%)
Medical checkup (non-endocrine disorders)	21 (24.7)
Gastrointestinal symptoms	12 (14.1)
Pulmonary symptoms	12 (14.1)
Urinary symptoms	11 (12.9)
Stomach ache	13 (15.3)
Cardiovascular symptoms	7 (8.2)
Lumbar syndrome	5 (5.9)
Gynecology symptoms	2 (2.4)
Traumatic injury	2 (2.4)
Total	85 (100)

Subclinical hypercortisolism (SCS), defined as an insufficient cortisol suppression during the low dose overnight dexamethasone test (1 mg) (plasma cortisol between 50 and 138 nmol/L) was observed in 34 (40%) patients, while the absence of cortisol suppression (autonomous cortisol secretion) was found in 4 (4.7%) patients. In the remaining 47 (55.3%) patients, complete overnight suppression of cortisol secretion was achieved (plasma cortisol less than 50 nmol/L) (Table 2). In the group of patients with SCS, nearly 23 (74%) patients had accompanied arterial hypertension, 7 (20%) patients had type 2 diabetes, 5 (14.70%) had dyslipidemia, another 5 (14.7%) had osteoporosis and 20 (58.8%) were overweight or obese – body mass index > 25 kg/m^2. Normal values of basal plasma cortisol levels were found in 63 (53.6%) patients, while the values were elevated in the remaining 22 patients. Among them, 10 patients had SCS, 4 had autonomous cortisol secretion, and in 8 patients, complete suppression during the overnight dexamethasone test was found.

Table 2

Frequency of different types of adrenal incidentalomas (n = 85)

Type of adrenal incidentaloma	Patients n (%)
Nonfunctioning	41 (48.2)
Functioning	44 (51.8)
SCS	34 (40)
ACS	4 (4.7)
AAS	2 (2.4)
pheochromocytoma	4 (4.7)

SCS – subclinical Cushing´s syndrome;
ACS – autonomous cortisol secretion;
AAS – autonomous aldosterone secretion.

Cut-off values (more than 20) were reached in two patients, who underwent surgical adrenalectomy, and histopathologic examination confirmed adrenal cortical adenoma. After the surgical treatment, both patients remained normotensive and normokalemic.

Thirty-five (41%) patients underwent adrenalectomy; among them, adrenocortical carcinomas were found in 4 (11.4%) cases, 15 (42.9%) were adenomas, pheochromocytoma was found in 4 (11.4%) cases, nodular hyperplasia in 5 (14.3%) cases, distant metastasis (2.8%) in one case and the remaining were different benign masses (Figure 1). Of the 15 patients with adenomas, 2 had aldosteronoma, 7 had SCS, 3 had autonomous cortisol secretion and the rest had nonfunctioning adenomas.

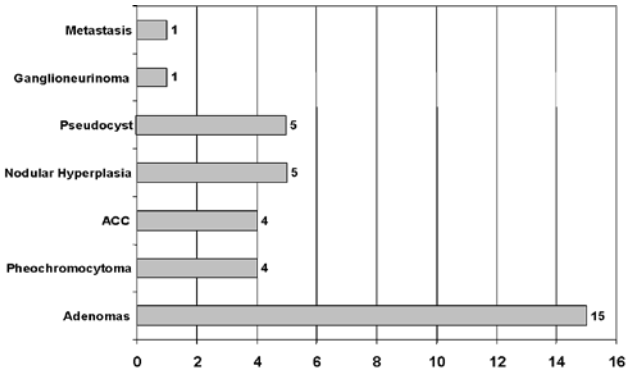

Fig 1. - Histological findings in operated patients.

ACC – adrenocortical carcinoma.

The surgical treatment was performed in 8 patients with SCS due to the tumor size (over 4 cm) and the presence of at least two comorbidities. In one operated patient, the histological analysis confirmed adrenocortical carcinoma; the rest were functional adenomas. Interestingly enough, 5

(75%) patients with SCS and mild arterial hypertension became normotensive after adrenalectomy, with no need for any further antihypertensive treatment.

Pheochromocytoma was found in 4 patients (1 male and 3 female) with an average age of 53 ± 13 years and average tumor size of 4.5 ± 3.1 cm (range from 1.8 to 6.1 cm). All of the patients had biochemical confirmation of autonomous adrenergic hypersecretion expressed by elevated plasma levels of metanephrine and normetanephrine. Hypertension was present in 75% of the patients. Adrenocortical carcinoma (ACC) was observed in 4 patients (2 male and 2 female), with an average age of 53 ± 9 years (range from 44–60 years of age) and an average tumor size of 7.9 ± 5.7 cm (range from 3.5 to 15 cm). Two patients had arterial hypertension, one was diabetic and one patient had autonomous cortisol secretion. Bilateral adrenal masses were found in 19 cases (22.3%). Of these patients, 10 had SCS, while the rest were nonfunctioning tumors. Among these, adrenalectomy of tumors over 4 cm in diameter was performed in 4 cases.

The results of histological analysis of patients operated on due to incidentally discovered adrenal tumors are shown in Figure 1.

Due to some technical inabilities, we performed measurement of aldosterone/plasma renin activity ratio in only three patients who had associated hypertension and hypokalemia. Cut-off values (more than 20) were reached in two patients who underwent surgical adrenalectomy and histopathologic examination confirmed adrenal cortical adenoma. After surgical treatment, both patients remained normotensive and normokalemic.

Discussion

The prevalence of randomly detected adrenal masses during the CT imaging is 3%–4%, with slightly greater incidence in women [4]. In autopsy studies, the frequency of these tumors varies depending on the patient's age and ranges from 1%–7.8% [5]. These tumors are rarely seen in people under 30 years of age (0.3%), with the highest incidence in people between 50 and 70 years of age. Adrenal incidentalomas are most often found in the right adrenal gland, 50%–60%, in the left adrenal gland, 30%–40%, and in 10%–15% of patients in both glands. The average size of these changes is 3–3.5 cm and ranges from 0.5 cm to 25 cm [4–7]. Similarly, in our investigated group 75.3% of the patients were over 50 years old, with a peak in the seventh decade in 38.5% of the patients. The average size of incidentaloma in our patient group was 3.8 cm ranging up to 15 cm in diameter and was most often localized in the right adrenal gland. A possible explanation for the higher incidence in older age is the more frequent use of radiological diagnostic imaging, as part of the evaluation of various diseases, or possible compensatory hyperplasia in response to local ischemia due to atherosclerotic changes in blood vessels [8].

Clinical evaluation of adrenal incidentaloma implies detailed history and physical examination in order to access clinical symptoms and signs of adrenal hormonal excess. Biohumoral investigation should focus on determining the existence of hypercortisolism, hyperaldosteronism, or autonomous sympathoadrenal hypersecretion. Radiological evaluation should be done to determine whether the incidentally revealed mass is benign or malignant. Most authors suggest that MR imaging has several advantages over the CT scan because of its lack of radiation exposure, iodine-based contrast media, and the superior tissue contrast resolution [5–8]. Yet, the current recommendations of the European Clinical Practice Guideline primarily suggest the use of non-contrast CT imaging in order to determine if the mass is homogenous [Hounsfield units (HU) ≤ 10] and lipid-rich and therefore benign[1]. Regardless of the way the incidentalomas were found, all patients in this series underwent adrenal non-contrast or contrast CT imaging.

According to clinical features, adrenal incidentalomas are most often nonfunctioning tumors, while in a small percentage of patients, the hypersecretion of one or more adrenal hormones is present. Interestingly, in this group of incidentalomas, there was a slightly higher frequency of functioning tumors (51.8%), while the patients with SCS were the most frequent. Literature data report a prevalence of 5%–24% for SCS in patients with adrenal incidentaloma [7–10]. This wide range could be partly explained by different diagnostic criteria for SCS. In our group of patients, the presence of SCS in 34 (47.3%) patients was observed, while the autonomous cortisol secretion was found in 4 (5.3%) cases. In the remaining 47 (47.4%) cases, complete overnight suppression of cortisol secretion was achieved. The risk of developing autonomous cortisol secretion without signs of overt Cushing's syndrome varies between 0%–11% of patients with adrenal incidentaloma [9, 10]. All four patients with autonomous cortisol secretion in this group had no convincing clinical signs of Cushing's syndrome.

Normal values of basal plasma cortisol levels were found in 63 (53.6%) patients, while the values were elevated in the remaining 22 patients. Among the patients with elevated cortisol levels at basal state, 12 patients had SCS, 4 had autonomous cortisol secretion, while in 4 patients, complete overnight suppressions were found. Normal values of basal cortisol levels do not exclude the presence of cortisol excess. In the group of patients with normal basal cortisol levels, SCS was observed in 10 cases, while the complete suppression of cortisol secretion was achieved in the remaining patients.

Patients with SCS should be considered individually. When deciding on further treatment, patients' age, general condition, presence of comorbidities and the degree of cortisol excess should be taken into account.

Adrenocortical carcinoma (ACC) is a rare malignancy with an incidence that ranges from 0.7–2 cases per million habitans/year, with the peak of occurrence between 40 and 50 years of age. In some series, malignancy was significantly associated with age, weight loss and increased tumor size [11]. Although steroid hormone excess is present in most ACC (40% to 60%), in 20% of cases, it is diagnosed incidentally [12]. Traditional imaging is able to correctly

diagnose an adrenal mass as ACC in most cases. The risk of ACC rises with age and tumor size. Index of suspicion increases for tumors over 4 cm in diameter (sensitivity 97%, specificity 52%) and over 6 cm (sensitivity 91%, specificity 80%). Most of the ACC are larger heterogeneous tumors due to the areas of necrosis, hemorrhage and calcification, usually with irregular margins [13]. Currently, a non-contrast CT scan is recommended as a mandatory imaging technique in suspicion of ACC. The threshold of ≤ 10 Hounsfield units (HU) for benign adrenal masses on non-contrast CT has been established by many studies. At the same time, the risk of malignancy in homogenous adrenal masses with 5 cm in diameter and non-contrast attenuation values of ≤ 10 HU is almost zero. When the basal density is more than 10 HU, contrast CT imaging should be performed; since the malignant adrenal lesions demonstrate a slower washout of contrast medium, an absolute washout of over 50% suggests a benign adrenal mass [13–15].

The benefit of MR imaging in the differential diagnosis of adrenal masses is less clear.

If the CT scan cannot perfectly differentiate the origin of adrenal masses, MR imaging could be useful in diagnosing ACC by the presence of isointense to hypointense signal on T1-weighted images; the hyperintense signal on T2-weighted images, and a heterogeneous signal drop on chemical shift [12].

According to the current guidelines, surgical treatment is recommended for adrenal masses larger than 4 cm in diameter, except in the case of clear benign lesions, such as cysts or myelolipomas. Tumors of less than 4 cm should be regularly laboratory and radiologically followed [1, 2].

In this series of adrenal incidentaloma, ACC was observed in 4 patients (2 male and 2 female), with the average age of 53 ± 9 years (range 44–60 years of age) and the average size of 7.9 ± 5.7 cm (range 3.5–15cm). Deciding upon the operative treatment was based on the tumor size in three patients (more than 4 cm) and on the typical radiological characteristics observed in contrast CT imaging in all four cases. Two patients with ACC were hypertensive, one was diabetic, and one patient had autonomous cortisol secretion.

The presence of pheochromocytoma should be excluded in all patients with adrenal incidentaloma, including those with normal blood pressure. About 30% of all pheochromocytoma are detected incidentally and their prevalence is increasing. Prevalence of pheochromocytoma in adrenal incidentalomas varies from 1% to 11%, according to different authors [4–7, 16]. In this study, there were 4 patients (11.4%) with pheochromocytoma with an average tumor size of 4.5 ± 3.1 cm (range 1.8–6.1 cm). Currently, there is a lack of consensus on the best initial diagnostic test for evaluating pheochromocytoma. Most authors recommend using plasma metanephrine as the initial diagnostic test due to its high diagnostic sensitivity. Plasma metanephrine levels 3 times higher than normal are highly diagnostic for pheochromocytoma (sensitivity 96%–100% and specificity 85%–89%) [16–18].

On contrast-enhanced CT imaging, pheochromocytoma may show homogenous or variable enhancement due to areas of cystic changes and hemorrhage. Contrast washout in pheochromocytoma may be variable and may overlap both benign or malignant lesions such as ACC, although a non-contrast CT of less than 10 HU is extremely rare in these tumors [19, 20]. Pheochromocytoma typically shows avid gadolinium enhancement in the MR imaging, but this could be variable depending on the presence of cystic or necrotic areas [21].

All our patients had biochemical confirmation of autonomous adrenergic hypersecretion expressed by elevated plasma levels of metanephrine and normetanephrine. All cases were confirmed by pathohistological examination. It is not unusual that incidentally discovered pheochromocytoma show no hypertension in clinical presentation [4, 16, 18]. The prevalence of normotensive pheochromocytoma in a series of adrenal incidentalomas ranges up to 50% [22, 23]. Deciding upon the surgical treatment was based on the radiographic characteristics of the tumor and the results of biochemical analysis, even though one patient had no hypertension.

The estimated prevalence of primary hyperaldosteronism in adrenal incidentaloma is less than 1%. In the examined group, there were two patients (2.4%) with autonomous mineralocorticoid excess. It is known that some of these patients can be normokalemic. Because of that, it is recommended that the screening for primary hyperaldosteronism be performed in all hypertensive patients with adrenal incidentalomas [1, 2, 24, 25].

Bilateral adrenal masses were found in 19 (22.3%) cases; of these, 10 patients had SCS, while the rest were nonfunctioning tumors. Unilateral adrenalectomy of larger tumors (over 4 cm in diameter) was performed in 4 cases. Since none of the SCS patients and bilateral adrenal masses had no clinical signs of overt Cushing´s syndrome, deciding upon the operative treatment was based on the tumor size and the presence of comorbidities. Among the operated masses, the histopathological analysis showed the presence of adenoma in all 4 patients.

In our study group, 35 patients underwent adrenalectomy. Surgical treatment was performed in all functioning tumors, those larger than 4 cm in diameter and those with suspicious radiological characteristics. In patients with SCS, surgical treatment was performed on large tumors, or if at least two comorbidities were present. Adrenalectomy was performed using laparoscopic surgery or open laparotomy. For adrenal masses that were suspected of malignancy, larger than 6 cm, and with signs of local invasion, open adrenalectomy was performed; for tumors without evidence of local invasion and less than 6 cm in diameter, laparoscopic surgery was performed. All patients with autonomous cortisol secretion and those with SCS received preoperative and postoperative glucocorticoid treatment for at least 6 months. Follow-up of operated patients is ongoing according to actual recommendations [1, 2, 26].

Conclusion

For patients with incidentally detected adrenal tumors, two questions on determining the tumor functionality and/or the presence of malignancy need to be clarified. The existence of typical clinical manifestation of hormonal excess is not

necessary for hormonal testing. All patients with adrenal incidentaloma should undergo hormonal evaluation for autonomous or possible autonomous cortisol secretion, as well as for autonomous, adrenergic, and mineralocorticoid excess. The radiologic evaluation most often refers to contrast medium washout on CT scan or tumor density on non-contrast CT imaging. Adrenal masses with suspicious radiologic characteristics, functional tumors, and those with more than 4 cm in diameter should undergo adrenalectomy. In patients suspected of having autonomous adrenal secretion, surgery is indicated even if the typical clinical manifestation is absent.

R E F E R E N C E S

1. *Fassnacht M, Arlt W, Bancos I, Dralle H, Newell-Price J, Sahdev A,* et al. Management of adrenal incidentalomas: European Society of Endocrinology Clinical Practice Guideline in collaboration with the European Network for the Study of Adrenal Tumors. Eur J Endocrinol 2016; 175(2): G1–G34.
2. *Zeiger MA, Thompson GB, Duh QY, Hamrahian AH, Angelos P, Elaraj D,* et al. American Association of Clinical Endocrinologists; American Association of Endocrine Surgeons. American Association of Clinical Endocrinologists and American Association of Endocrine Surgeons Medical Guidelines for the Management of Adrenal Incidentalomas: executive summary of recommendations. Endocr Pract 2009; 15(5): 450–3.
3. *Barzon L, Sonino N, Fallo F, Palu G, Boscaro M.* Prevalence and natural history of adrenal incidentalomas. Eur J Endocrinol 2003; 149(4): 273–85.
4. *Zeiger MA, Siegelman SS, Hamrahian AH.* Medical and surgical evaluation and treatment of adrenal incidentalomas. J Clin Endocrinol Metab 2011; 96(7): 2004–15.
5. *Erdem H, Çetinkünar S, Kuyucu F, Erçil H, Görür M, Sözen S.* Surgical approach in adrenal incidentalomas: Report of thirteen cases and review of the literature. Ulus Cerrahi Derg 2015; 32(2): 103–6.
6. *Porcaro AB, Novella G, Ficarra V, Curti P, Antoniolli SZ, Suangwoua HS,* et al. Adrenal incidentalomas: surgical treatment in 28 patients and update of the literature. Int Urol Nephrol 2001; 32(3): 295–302.
7. *Luton JP, Martinez M, Coste J, Bertherat J.* Outcome in patients with adrenal incidentaloma selected for surgery: an analysis of 88 cases investigated in a single clinical center. Eur J Endocrinol 2000; 143(1): 111–7.
8. *Kim BY, Chun AR, Kim KJ, Jung CH, Kang SK, Mok JO,* et al. Clinical Characteristics and Metabolic Features of Patients with Adrenal Incidentalomas with or without Subclinical Cushing's Syndrome. Endocrinol Metab (Seoul) 2014; 29(4): 457–63.
9. *Terzolo M, Bovio S, Pia A, Osella G, Borretta G, Angeli A*, et al. Subclinical Cushing's syndrome. Arq Bras Endocrinol Metabol 2007; 51(8): 1272–9.
10. *Reincke M.* Subclinical Cushing's syndrome. Endocrinol Metab Clin North Am 2000; 29(1): 43–56.
11. *Lumachi F, Borsato S, Tregnaghi A, Marino F, Fassina A, Zucchetta P*, et al. High risk of malignancy in patients with incidentally discovered adrenal masses: accuracy of adrenal imaging and image guided fine-needle aspiration cytology. Tumori 2007; 93(3): 269–74.
12. *Libe R.* Adrenocortical carcinoma (ACC): diagnosis, prognosis and treatment. Front Cell Dev Biol 2015; 3: 45.
13. *Schteingart DE, Doherty GM, Gauger PG, Giordano TJ, Hammer GD, Korobkin M,* et al. Management of patients with adrenal cancer: recommendations of an international consensus conference. Endocr Relat Cancer 2005; 12(3): 667–80.
14. *Hussain S, Belldegrun A, Seltzer SE, Richie JP, Gittes RF, Abrams HL.* Differentiation of malignant from benign adrenal masses: predictive indices on computed tomography. AJR Am J Roentgenol 1985; 144(1): 61–5.
15. *van Erkel AR, van Gils AP, Lequin M, Kruitwagen C, Bloem JL, Falke TH.* CT and MR distinction of adenomas and nonadenomas of the adrenal gland. J Comput Assist Tomogr 1994; 18(3): 432–8.
16. *Leung K, Stamm M, Raja A, Low G.* Pheochromocytoma: the range of appearances on ultrasound, CT, MRI, and functional imaging. AJR Am J Roentgenol 2013; 200(2): 370–8.
17. *Chen H, Sippel RS, O'Dorisio MS, Vinik AI, Lloyd RV, Pacak K.* North American Neuroendocrine Tumor Society (NANETS). The North American Neuroendocrine Tumor Society consensus guideline for the diagnosis and management of neuroendocrine tumors: pheochromocytoma, paraganglioma, and medullary thyroid cancer. Pancreas 2010; 39(6): 775–83.
18. *Kopetschke R, Slisko M, Kilisli A, Tuschy U, Wallaschofski H, Fassnacht M,* et al. Frequent incidental discovery of phaeochromocytoma: data from a German cohort of 201 phaeochromocytoma. Eur J Endocrinol 2009; 161(2): 355–61.
19. *Hamrahian AH, Ioachimescu AG, Remer EM, Motta-Ramirez G, Bogabathina H, Levin HS,* et al. Clinical utility of noncontrast computed tomography attenuation value (hounsfield units) to differentiate adrenal adenomas/hyperplasias from nonadenomas: Cleveland Clinic experience. J Clin Endocrinol Metab 2005; 90(2): 871–7.
20. *Szolar DH, Kammerhuber F.* Quantitative CT evaluation of adrenal gland masses: a step forward in the differentiation between adenomas and nonadenomas? Radiology 1997; 202(2): 517–21.
21. *Lumachi F, Tregnaghi A, Zucchetta P, Cristina Marzola M, Cecchin D, Grassetto G,* et al. Sensitivity and positive predictive value of CT, MRI and 123I-MIBG scintigraphy in localizing pheochromocytomas: a prospective study. Nucl Med Commun 2006; 27(7): 583–7.
22. *Mansmann G, Lau J, Balk E, Rothberg M, Miyachi Y, Bornstein SR.* The clinically inapparent adrenal mass: update in diagnosis and management. Endocr Rev 2004; 25(2): 309–40.
23. *Grumbach MM, Biller BM, Braunstein GD, Campbell KK, Carney JA, Godley PA*, et al. Management of the clinically inapparent adrenal mass ("incidentaloma"). Ann Intern Med 2003; 138(5): 424–9.
24. *McAlister FA, Lewanczuk RZ.* Primary hyperaldosteronism and adrenal incidentaloma: an argument for physiologic testing before adrenalectomy. Can J Surg 1998; 41(4): 299–305.
25. *Fuller PJ.* Adrenal diagnostics: An endocrinologist's perspective focused on hyperaldosteronism. Clin Biochem Rev 2013; 34(3): 111–6.
26. *Lee JM, Kim MK, Ko SH, Koh JM, Kim BY, Kim SW*, et al. For the the Korean Endocrine Society, Committee for Clinical Practice Guidelines. Clinical Guidelines for the Management of Adrenal Incidentaloma. Endocrinol Metab (Seoul) 2017; 32(2): 200–18.

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The effect of dental caries and restorative biomaterials on IL-1 β and TNF-α levels in the gingival crevicular fluid

Uticaj karijesa i zubnih ispuna na nivoe IL-1 β i TNF-α u gingivalnoj tečnosti

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Abstract

Background/Aim. In the spirit of personalized medicine, determining caries biomarkers in the saliva and gingival crevicular fluid (GCF) attracts great attention in the current dental research. The concentration of GCF cytokines is illustrative in depicting the processes in tooth structures. Their relevance must be inspected with aspects of tooth position and caries lesion level. Different impacts of dental restoration materials on GCF IL-1β and TNF-α could be used as a parameter for estimating local inflammation. This paper aimed to estimate the concentrations of the proinflammatory cytokines (IL-1β and TNF-α) in the GCF and to correlate them with caries extension, tooth position, and different restorative biomaterials. **Methods.** GCF samples were collected from 90 periodontally healthy patients demonstrating at least one tooth with proximal caries and one intact tooth, at the baseline, 7 and 30-days post-treatment. The biomarkers' profile was investigated in relation to different levels of caries extension (superficial, pulpitis, gangrenous, root affection), defect size, and restorative biomaterial. **Results.** Before therapy, caries level was significantly associated with GCF IL-1β concentration, demonstrating the lowest level in gangrenous (C4) and superficial caries (C2). Thirty days after therapy, root affection (C5) was characterized by the highest IL-1β concentration. Different dental fillings showed various GCF cytokine changes. CPC induced a significant IL-1β increase in more than 70% of treated patients. Caries lesion size was insignificantly associated with GCF levels of these proinflammatory cytokines, where larger defects were followed by an average cytokine increase. Considering the tooth position before therapy, IL-1β had the highest level in GCF samples from caries-affected canines and second molars, while TNF-α showed the highest levels from canines GCF. Dental restoration induced cytokine increase in canines (IL-1β and TNF-α), 1st and 2nd molars GCF (IL-1β). **Conclusion.** Inflammation intensity of tooth structures was directly reflected in IL-1β and TNF-α concentrations. Dental restoration significantly affects IL-1β and TNF-α levels, depending on the used dental filling-type material. The profile of these cytokines varied in GCF samples of the tooth with different anatomical positions, where canines and molars demonstrated the highest level. An increase of these proinflammatory cytokines in the absence of any symptomatic manifestation of the inflammatory response can be considered as a possible tooth reparation parameter.

Key words:
dental caries; dental restoration, permanent; dental materials; gingival crevicular fluid; interleukin-1; tumor necrosis factor-alpha.

Apstrakt

Uvod/Cilj. U duhu personalizovane medicine, određivanje biomarkera za karijes u pljuvački i gingivalnoj crevikularnoj tečnosti (*gingival creviculr fluid* – GCF) privlači veliko interesovanje u novijim stomatološkim istraživanjima. Koncentracija citokina u GCF reflektuje procese u zubnim strukturama. U smislu interpretacije njihovih koncentracija, treba uzeti u obzir uticaj položaja zuba i obim karijesne destrukcije. Nivoi IL-1β i TNF-α u GCF mogu poslužiti kao indikator zapaljenskog odgovora na biomaterijale koji se koriste za zubne ispune. Cilj rada bio je određivanje proinflamatornih citokina IL-1 β i TNF-α u GCF poreklom od karijesom zahvaćenih i intaktnih zuba i njihovo

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korelisanje sa obimom karijesa, položajem zuba i različitim zubnim ispunima. **Metode.** Uzorci GCF sakupljeni su od 90 parodontološki zdravih osoba koji su imali najmanje jedan zub sa proksimalnim karijesom i jedan intaktni zub, na početku terapije, kao i 7 i 30 dana nakon terapije. Profil biomarkera ispitivan je u odnosu na obim karijesnih lezija: površne (C2), pulpitis (C3), gangrena (C4) i karijes korena (C5), veličinu defekta i vrstu biomaterijala. **Rezultati.** Obim karijesnih lezija značajno je bio udružen sa IL-1β, čije su koncentracije bile najniže u grupi gangrenoznih zuba (C4) i površnog karijesa (C2). Trideset dana nakon terapije grupa C5 pokazala je najviše vrednosti IL-1β. Različiti materijali za zubne ispune pokazali su različiti profil citokina u GCF. CPC je uzrokovao porast IL-1β kod više od 70% bolesnika. Obim karijesne lezije nije pokazao značajnu korelaciju sa merenim citokinima, dok su veći defekti bili udruženi sa povećanjem srednjih vrednosti citokina. U pogledu položaja zuba, IL-1β je pokazivao najviše vrednosti kod karijesom zahvaćenih očnjaka i drugih molara, dok je TNF-α imao najveće vrednosti kod očnjaka. Nakon terapije, povećanje koncentracije citokina je utvrđeno kod očnjaka (IL-1β i TNF-α), prvog i drugog molara (IL-1β). **Zaključak.** Intenzitet zapaljenja zubnih struktura se direktno reflektovao na koncentraciju IL-1β i TNF-α. Zubni ispuni su značajno uticali na nivoe IL-1β i TNF-α u odnosu na vrstu korišćenog biomaterijala. Profil merenih citokina je varirao u odnosu na različiti položaj zuba, pri čemu su očnjaci i molari pokazali najviše vrednosti. Uočeni porast merenih pro-inflamatornih citokina u odsustvu klinički manifestne patologije može ukazivati na reparatorne efekte.

Ključne reči:
zub, karijes; zub, trajni ispuni; stomatološki materijali; gingivalna sulkusna tečnost; interleukin-1; faktor nekroze tumora.

Introduction

Dental caries is the most frequent health problem in population [1, 2]. It is caused by bacterial biofilms whose maturation is associated with an anaerobic shift in microflora [3], while the subsequent acidification leads to demineralization of the dental enamel representing the pathognomonic sign of the disease. Despite outstanding prophylactic strategies, dental caries and related complications are still highly prevalent in the population and provide a negative impact on oral and systemic health [4]. Therefore, many efforts are invested in a better understanding of caries pathogenesis in order to improve respective preventive strategies, diagnostic approaches, and predictive treatment protocols with decreased complication rates. In the spirit of personalized medicine, the search for caries biomarkers in the saliva and gingival crevicular fluid (GCF) attracts great attention in the current dental research.

The cariogenic microorganisms and their byproducts, following the initial invasion of tooth enamel, reach the dental tubules and get in contact with dental odontoblasts' cellular extensions. Odontoblasts are specialized cells that, apart from producing the dentin, express many metabolical functions and play an important part in the local immune response against infective threats [5, 6]. They express numerous pathogen recognition receptors that bind di- and/or tri-acetylated lipoproteins, lipopolysaccharides (LPS), flagellin, viral dsRNA, and unmethylated CpG motif-containing DNA [7–10]. As a response to toll-like receptors (TLR) and nucleotide-binding oligomerization domain (NOD), stimulation odontoblasts secrete numerous mediators, such as cytokines and chemokines (IL-6, IL-8, IL-10, IL-1β, TNF-α, CCL2, CCL20, CXCL10) [6, 11–14], and defensins [6, 15, 16]. This inflammatory reaction is directed to eliminate or attenuate cariogenic pathogens in odontoblasts' proximity. In the case of low-intensity inflammation, this reaction is usually sufficient to control the tooth infection and to induce the regenerative process that finally results in the formation of reactionary dentin. Indeed, more intensive or prolonged inflammation interrupts the regeneration processes and results in intensive mediator response from odontoblasts, dental pulp resident cells, and infiltrating immune cells [17, 18]. Further progression of bacterial invasion through the odontoblast barrier generates an immune response in the dental pulp complex, resulting in pulpitis and progression of the inflammatory process toward periodontium [19, 20]. Moreover, the *in vitro* stimulation of the tooth crown odontoblasts with TLR2 or TLR4 agonists resulted in a completely different profile of IL-1β, TNF-α, IL-8 CCL20, and β-defensin-2 production, indicating a differential response to aerobic or anaerobic bacteria. The inflamed dental pulp is a significant source of IL-1β and IL-8 [21]. On the other hand, locally produced IL-1β and TNF-α exert significant influence on odontoblast functions, inducing further β-defensin production [22], production of dental matrix protein-1, and inducing proliferation of odontoblast-like cells derived from stem cells [23]. Moreover, the studies that investigated the cytokine profile in the GCF samples following dental restoration reported controversial data [24–30].

The present study hypothesized that IL-1β and TNF-α profile in the GCF from caries-affected and intact teeth are different, while the caries extension, tooth position, and different restorative biomaterials alter the biomarker profile as well.

The aim of the study was to investigate GCF IL-1β and TNF-α profile between caries-affected and healthy teeth, and estimate the effect of caries destruction, restorative material and tooth position on their respective concentrations.

Methods

Study design

The study was designed as a short-controlled prospective study, longitudinally assessing the effect of caries and its respective treatment on the local levels of the IL-1β and TNF-α in the split mouth-design.

Study population and inclusion criteria

The study population was comprised of 90 outpatients attending the Clinic for Stomatology at the Military Medical Academy, Belgrade, Serbia, in the period between January 2015 until June 2018. The population consisted of younger participants (mean age of 31 ± 6.15 years) with similar distribution in gender. The study was conducted in accordance with the International Ethical Guidelines and Declaration of Helsinki (1964/1975) and was approved by the Institutional Ethics Committee (reference number VMA/10-12/A.1). The participants were informed about the study characteristics and the scheduled procedures and accepted to participate by signing informed consent.

The enrolled participants had to be systemically healthy non-smokers, presenting at least one caries-affected and one intact tooth from the same morphological group of teeth, with intact periodontal tissues. The exclusion criteria were as follows: active periodontal disease; subgingival periodontal treatment in less than 6 months; antibiotic and anti-inflammatory intake in the last 3 months; health conditions and chronic diseases affecting the inflammatory status and/or bone metabolism; unsatisfying oral hygiene.

Caries lesions were diagnosed using a visual-tactile technique combined with the radiological exam and according to the Black's Classification [31], while the periodontal condition was assessed using a combination of clinical parameters and panoramic radiographs according to the recent Classification of periodontal and peri-implant diseases and conditions [32, 33]. Based on the progression levels, caries lesions were classified as superficial (C2), pulp involvement (C3), gangrene (C4), and root involvement (C5).

Restorative biomaterials

Six different restorative materials were used for dental filling – two temporary materials: zinc-phosphate cement (ZPhC-Cegal NV, Galenika, Serbia) and carboxylate cement (ZPoC-Harvard, USA); two permanent restorations: amalgam (Amg-Extracap D caps, Galenika, Serbia); nanohybrid composites: BF (the mixture of bisphenol-A-glycidyl-dimethacrylate (BisGMA) 15–25%, triethylene glycol dimethacrylate (TEGDMA) 12–14%, aluminofluoroborosilicate glass 50–60% [aluminium trioxide (Al_2O_3) 1–2%, and DL-camphorquinone, Shofu, Japan] and TEC (Tetric EvoCeram), the mixture of 2.5–10% of BisGMA and 2.5–10% of urethane-dimethacrylate (UEDMA) and nonhazardous additions (Ivoclar Vivadent, USA); GIC (glass ionomer cement, GIC Fuji PLUS®, Green Circle, USA) was used for both settings, standalone restorations and the base for nanohybrid composites (BF and TEC). Dental fillings (temporary and permanent) were sealed in one session while the placed mass counted between 0.07–2.03 g (Table 1).

Table 1

Percentage of patients with IL-1β and TNF-α gingival crevicular fluid (GCF) increase at time points (at least 20% up increase comparing to 0 time point, before dental filling)

Parameters		IL-1β				TNF-α			
		7 days		30 days		7 days		30 days	
		n/total	%	n/total	%	n/total	%	n/total	%
All		39/86	45	33/74	45	35/84	42	25/74	34
Dental filling type	TEC	9/17	53	8/17	47	6/17	35	9/17	53
	AMA	2/14	14	4/11	36	4/13	31	2/11	18
	BEA	8/15	53	3/15	20	6/15	40	2/15	13
	CFC	6/14	43	4/9	44	6/13	46	3/ 9	33
	GJC	7/13	54	6/11	55	6/13	46	4/11	36
	CPC	7/13	54	8/11	73	7/13	54	5/11	45
Caries level	C2	31/58	53	19/51	37	31/58	53	19/51	37
	C3	1/9	11	5/9	55	1/ 9	11	4/7	57
	C4	2/6	33	3/6	50	2/ 6	33	2/5	40
	C5	7/13	54	6/11	55	7/13	54	6/11	55
Dental filling volume	< 0.5 g	35/69	51	23/62	37	30/69	44	22/62	35
	< 1.0 g	4/9	44	1/7	14	4/9	44	1/7	14
	> 1.0 g	2/8	25	4/8	50	1/8	13	3/8	38
Tooth position	1	3/5	60	2/4	50	2/5	40	3/4	75
	2	5/9	56	4/9	44	6/9	66	3/9	33
	3	5/7	71	4/7	57	4/7	57	4/7	57
	4	6/ 11	55	2/10	20	3/11	27	2/10	20
	5	6/ 24	25	6/18	33	9/24	38	6/18	33
	6	7/ 12	58	5/11	45	6/12	50	4/11	36
	7	7/ 16	44	6/14	32	7/16	44	6/14	32

Biomarker measurement

The GCF sampling was performed using the filter paper technique as previously described [34]. Strips contaminated with blood or saliva were discarded. The GCF volume was measured using Periotron 6000 (Interstate Drug Exchange, Amityville, NY, USA), calibrated prior to each set of measurements. Following that, the paper strips were placed into microcentrifuge plastic tubes, and elution was performed with 500 μL phosphate-buffered saline by vortexing for 10 seconds and centrifugation at 3,000 g for 5 min, in order to remove plaque and cellular detritus. The supernatants were stored in plastic tubes at -70°C until further analysis. The biomarker estimation was performed using flow cytometry (Beckman FC500; Beckman, USA) with commercial assays BioLegend's LEGENDplex™, Human Inflammation Panel (Cat No 740118, USA). Detection limits: TNF-α (1.0 pg/mL), IL1-β (1.0 pg/mL).

Statistical analysis

Inter-group comparisons of the parameters were tested with the ANOVA test, with Bonferroni *post hoc* test comparison of selected groups. The 0 time point before therapy, was the control value for every individual investigated tooth, with the 7th and 30th-day values compared to the initial level. The differences between the two selected groups were evaluated using the Mann-Whitney test. Thereafter, the *p*-values lower than 0.05 were considered significant. The correlations between the variables were tested with Spearman's rank correlation test. The average concentrations of IL-1β and TNF-α were expressed as pg of biomarker/μLof GCF, mean ± standard deviation (SD). The statistical analysis was performed using commercial software (GraphPad Prism, USA).

Results

The average concentration of IL-1β and TNF-α in GCF samples of patients according to different time points

The IL-1β and TNF-α concentrations between caries-affected and healthy teeth are depicted in Table 2. At the baseline, IL-1β showed significantly increased levels in caries-affected teeth when compared to the healthy controls (HC), while 30-days post-treatment, TNFa levels were significantly higher in the treated sites than in HC (Table 3).

Table 2

Average concentration of IL-1β and TNF-α in GCF samples of patients according to different time points

Parameters		IL-1β (pg) mean ± SD			TNF-α (pg) mean ± SD		
		0 days	7 days	30 days	0 days	7 days	30 days
Dental filling type	*TEC*	111 ± 211	92 ± 122	[a] 158 ± 188	24 ± 24	19 ± 17	28 ± 23
	AMA	155 ± 197	126 ± 177	187 ± 210	34 ± 37	24 ± 44	27 ± 25
	BEA	54 ± 84	88 ± 106	[b] 55 ± 107	12 ± 21	25 ± 46	18 ± 43
	CFC	31 ± 26	54 ± 104	[c] 65 ± 100	4 ± 5	7 ± 7	4 ± 5
	GJC	103 ± 104	153 ± 170	192 ± 265	33 ± 33	37 ± 39	37 ± 28
	CPC	243 ± 269	235 ± 245	[a,b,c] 427 ± 331	29 ± 38	44 ± 50	49 ± 57
Caries level	C2	76 ± 104	94 ± 105	[d] 115 ± 161	20 ± 26	24 ± 39	29 ± 62
	C3	172 ± 243	51 ± 92	182 ± 182	27 ± 44	47 ± 66	42 ± 52
	C4	49 ± 59	113 ± 144	[e] 104 ± 119	5 ± 6	10 ± 9	12 ± 12
	C5	[f] 101 ± 103	156 ± 169	[d,e,f] 286 ± 226	36 ± 35	30 ± 23	35 ± 23
Dental filling volume	< 0.5 g	107 ± 183	114 ± 187	167 ± 253	36 ± 93	18 ± 17	33 ± 30
	< 1.0 g	118 ± 174	148 ± 213	56 ± 61	33 ± 63	19 ± 24	25 ± 22
	> 1.0 g	137 ± 204	113 ± 121	236 ± 229	35 ± 55	11 ± 13	30 ± 26
Tooth position	1	59 ± 74	43 ± 25	141 ± 190	2 ± 2	5 ± 9	14 ± 12
	2	46 ± 48	44 ± 54	149 ± 205	15 ± 12	15 ± 17	15 ± 20
	3	117 ± 152	135 ± 93	253 ± 227	49 ± 62	35 ± 30	47 ± 62
	4	44 ± 37	65 ± 58	77 ± 75	17 ± 17	20 ± 29	18 ± 20
	5	107 ± 111	70 ± 79	103 ± 149	31 ± 40	34 ± 56	29 ± 35
	6	[g,h] 59 ± 41	[g] 166 ± 164	[h] 214 ± 198	24 ± 27	32 ± 45	34 ± 44
	7	133 ± 225	175 ± 171	223 ± 222	20 ± 19	26 ± 24	23 ± 22

[a] IL-1β, dental filling type, TEC/CPC, 30 days, *; [b] IL-1β, dental filling type, BEA/CPC, 30 days, **; [c] IL-1β, dental filling type, CFC / CPC, 30days, *; [d] IL-1β, caries level, C2 / C5, 30 days, *; [e] IL-1β, caries level, C4 / C5, 30 days, *; [f] IL-1β, caries level C5, 0d / 30 days, *; [g] IL-1β, tooth position 6, 0/7days, *; [h] IL-1β, tooth position 6, 0/30 days, *; SD − standard deviation.**

Table 3

The IL-1β and TNF-α concentration between caries affected and healthy teeth

Biomarker	Control (C), mean ± SD	Caries affected teeth, mean ± SD					
		Baseline	Day 7	Day 30	Baseline vs. C	Day 7 vs. C	Day 30 vs. C
IL-1β	78.23 ± 90.53	245.67 ± 750.10	79.02 ± 84.00	148.39 ± 290.12	$p = 0.012$		
TNF-α	24.05 ± 47.67	41.45 ± 109.34	84.01 ± 356.50	88.14 ± 361.21			$p = 0.010$

SD – standard deviation

Biomarker levels between sites with different restorative materials

The analysis of average GCF IL-1β level before dental restoration demonstrated a significant variation, with the lowest values in patient samples later treated with BEA and CFC fillings. After restoration, all materials, except BEA, demonstrated GCF IL-1β increase, with the maximal level at a 30-day time interval (Table 2). Temporary dental filling materials (CFC, GIC, CPC) demonstrated a much more intensive local IL-1β increase (from +75 to 210 %) compared to the materials for permanent (TEC, AMA, BEA) dental filling (from -37 to +42 %).

As shown for IL-1β concentration, GCF TNF-α level before dental restoration was the lowest in the patient samples later treated with BEA and CFC fillings. Again, the used dental filling materials induced the increase of GCF TNF-α. The highest average GCF TNF-a was recorded in the samples of GIC and CPC treated patients 30 days after. Temporary dental filling materials (CFC, GJC, CPC) demonstrated again a much more intensive local TNF-α increase (from +12 to 78 %) compared to the materials for permanent (TEC, AMA, BEA) dental filling (from -23 to +17 %).

Association of caries destruction extension with GCF IL-1β and TNF-α concentration

In our study, caries lesion is associated with significant GCF IL-1β concentration even in the initial stage, as a superficial dental change (C2) (Table 2). Before therapy, patients with the gangrenous process (C4) demonstrated the lowest average GCF IL-1β value, while those with pulpitis (C3) had the highest recorded GCF IL-1β concentration. On day 30 after therapy, all patients demonstrated an increase in average GCF IL-1β concentration. This increase was minimal for patients with pulpitis, due to the high initial concentration, but was maximal for patients with the process in the root canal.

Before therapy, GCF TNF-α showed the lowest concentration in the C4 group. However, after dental restoration, the highest average TNF-α concentration was demonstrated in the pulpitis group (C3).

Size of the caries lesion

The size of the caries lesion was determined indirectly, according to the volume of dental filling material needed for restoration. Before therapy, the concentration of GCF IL-1β was the highest in the group with the largest tooth defect caused by caries (> 1.0 g). Interestingly, 30 days after dental restoration, the average concentration increased in the samples of groups with small and very large caries defects, while it decreased in the group with intermediate fillings (0.5–1.0 g) (Table 3). Before therapy, GCF TNF-α demonstrated almost similar values in all groups divided according to caries tooth defect. Contrary to IL-1β findings, dental restoration induced decrement on day 30 in all groups.

Association of tooth position with GCF IL-1β and TNF-α concentration

Tooth position was significantly associated with GCF IL-1β concentration (Table 2). Before therapy, the average concentration was the highest in samples from a canine, second premolar, and second molar. After therapy, GCF IL-1β concentration increased in samples from all teeth except the second molar. The highest average concentration on day 30 was demonstrated in GCF of a canine and second molar.

The concentration of TNF-α before therapy was the highest in samples from canine and second premolar. Dental restoration therapy on day 30 demonstrated an increase of TNF-α in GCF of the first incisor and I and II molars, and contrary to IL-1β showed unchanged or decreased value in GCF of the second incisor, canine, and both molars.

Level of GCF IL-1β and TNF-α after dental restoration varies according to caries extensity, type and volume of dental restoration filling, and tooth position

Seven days after therapy, GCF IL-1β showed an increased value in samples of more than half of the patients treated with both temporary and permanent filling materials, except for those treated with amalgam (AMA) (Table 1). However, after 30 days, GCF IL-1β concentration demonstrated a further decrease in all patients treated with a permanent type of filling (TEC, AMA, BEA), while an increase was demonstrated in all of those treated with a temporary type of filling. This was especially evident for CPC, where almost 75% of treated patients demonstrated a significant GCF IL-1β rise compared to the level before therapy.

On the 7th day, GCF IL-1β was increased in more than half of the patients with superficial caries (C2) or those with the affected root canal (C5). On day 30, a further increase was evident in more than 50% of patients from the more

profound caries lesion (C3, C4, C5), with a documented decrease only in the C2 group.

Interestingly, the filling volume of less than 1 g was associated with an increase in 44–50%, while a larger filling volume was associated with a decrease of GCF IL-1β in 75% of treated patients. Conversely, on the 30th day, a smaller filling volume was associated with a local IL-1β increase in minor frequency (14–37%).

According to the tooth position, on the 7th day, GCF IL-1β was increased in more than 50% of patients in both incisors, canines, first premolar, and first molar. The 30th day was associated with an IL-1β decrement in GCF of all treated teeth, except the second premolar.

Seven days after dental restoration, the GCF TNF-α value increased in less than half of the patients, both treated with temporary and permanent filling materials. After 30 days, a further decrease of patients percent with documented TNF-α increase was documented in all groups except in those treated with TEC.

As for IL-1β, on the 7th day, GCF TNF-α was increased in more than 50% of C2 and C5 groups. Identically, on day 30, a further increase was evident in more than 50% of patients from the more profound caries lesion (C3, C4, C5), with a documented decrease only in the C2 group.

Again, identically as IL-1β, although in smaller frequency, on the 7th day, GCF TNF-α demonstrated an increase in samples where the filling volume was less than 1 g and a decrease in more than 85% of those treated with a larger filling volume. Conversely, on the 30th day, a smaller filling volume was associated with a local TNF-α increase in minor frequency (14–35%).

Seven days after therapy, GCF TNF-α demonstrated an increase in 57–66% of samples from canines and second incisors. On day 30, there was a TNF-α decrement in GCF of all investigated teeth except the first incisor.

Dental restoration is associated and correlated with IL-1β and TNF-α values in GCF of teeth with superficial caries, small caries extensivity, and specific tooth position

After therapy, coordinated local secretion/liberation of GCF IL-1β and TNF-α was demonstrated in the teeth treated with amalgam (7th day), BEA, and CFC (30th day) (Table 4).

According to the caries level before therapy, only patients with the gangrenous process (C4) did not show a significant correlation of GCF IL-1β and TNF-α. After dental restoration, a significant correlation of GCF IL-1β and TNF-α was demonstrated only in the group with superficial caries lesion, both on the 7th and 30th day.

Caries lesions that needed fillings of less than 1 g were characterized by a significant correlation of GCF IL-1β and TNF-α, both before and after dental restoration.

The specific position of a caries tooth is associated with the correlated production of GCF IL-1β and TNF-α both before and after dental restoration. A significant correlation between IL-1β and TNF-α was demonstrated before and after restoration in GCF of second incisors (7th day), second premolar (7th day), and second molar (7th and 30th day).

Table 4

Correlation of IL-1β and TNF-α concentration in gingival crevicular fluid (GCF) samples in the different time points

Parameters	IL-1β + TNF-α	
	7 days	30 days
Caries destruction level		
C2	0.0004	0.0030
C3	ns	ns
C4	ns	ns
C5	ns	ns
Restorative biomaterial		
TEC	ns	ns
AMA	0.0030	ns
BEA	ns	0.0002
CFC	ns	0.0170
GJC	ns	ns
CPC	ns	ns
Biomaterial amount (g)		
< 0.5	0.0007	0.0003
< 1.0	0.0140	ns
> 1.0	ns	ns

ns – not significant.

Discussion

Inflammation in the tooth structures is unequivocally associated with the presence of inflammatory mediators, especially inflammatory cytokines IL-1β and TNF-α. The concentration of GCF IL-1β and TNF-α were extensively studied in local inflammatory conditions as periodontitis [35–38] and periimplantitis [39–44] or even as a systemic inflammatory condition like diabetes [45, 46] or connective tissue disease [47–50]. Compared to these inflammatory conditions, cytokines were infrequently investigated in dental caries, especially in GCF of caries teeth [51–53].

Caries is associated with increased local IL-1β and TNF-α levels. Couglo et al. [52] demonstrated that children with high *Streptococcus mutans* numbers had high salivary IL-1β concentration and low IL1RA. They found that IL-1β was slightly elevated in the saliva and serum of children with caries but was not significantly associated with the caries lesion severity [54]. They also showed that IL-1β, IL1RA, and IL-10 gene polymorphism were not significantly associated with dental caries. Eslami et al. [53] demonstrated higher average IL-6 and IL-1β concentrations locally in the inflamed pulpal tissues of subjects with dental caries compared with intact pulpal tissue samples. This increase was significantly associated with *S. mutans* infection. McLachlan et al. [54] documented a significant expression of genes for S100A8, S100A9, S100A10, S100A12, S100A13, TNF-α, IL-1β, IL-8, IL-6, and ENA-78 in the pulp of caries teeth, close to the lesion. Pulp inflammation resulting from carious lesions is characterized by a strong increase in the production of proinflammatory cytokines, including TNF-α, IFN-γ, IL-1β, IL-6, CXCL8, and IL-18 [55–57]. Therefore, pulpitis intensity is significantly associated with intensive

local inflammatory mediators production. Additionally, our patients with pulpitis (C3 group) and the largest caries defect demonstrated the highest average IL-1β and TNF-α levels before therapy.

IL-1 seems to be of extreme importance in the pathophysiology of caries lesion. Horst et al. [56] investigated gene expression of inflammatory mediators in the odontoblast layer of extirpated caries teeth. Both the pulp and the odontoblast layers demonstrated a significant mRNA increase of CCR2, CCR4, CCR5, CCR9, CCL3, CCL23, IL-1β, and TNF-α. More importantly, they showed that TNF-α and especially IL-1β induced an *in vitro* increase of a human b-defensin 2 (HBD2) mRNA expression in odontoblasts, up to 100 times more intensive than LPS/TLR4 agonist. The only limitation of their study is the selection of teeth because all 32 samples were third molars, with caries lesion reaching 50 to 75% of dentin thickness. Additionally, the authors did not provide data on whether these teeth were previously treated or not. We have demonstrated that GCF IL-1β and TNF-α concentrations vary dramatically according to the tooth position, caries lesion extensivity. It has also been demonstrated that dental restoration material significantly alters its level further. Different groups of teeth are exposed to a different intensity of occlusal forces depending on their anatomical position and primary function, subsequently followed by a different profile of biochemical markers around different teeth. Briefly, the stimulation of periodontal mechanoreceptors is followed by the local release of neuropeptides, growth factors, and cytokines that accordingly regulate the remodeling of periodontal tissues [58–60].

He et al. [57] investigated pulpitis in the experimental model of pulp exposure to oral cavity microorganisms. They succeeded in documenting all the stages of pulpitis, from initial inflammatory cells recrutation to the exposed pulp and initial secretion of IL-1β and TNF-α, to chronic-like inflammation, the disappearance of dental odontoblasts and pulpal necrosis. This elegant study was performed with the micro computed tomography (CT) analysis, histopathological description of the local cell population, as well as RT PCR verification of IL-1β and TNF-α local presence in the time interval from 0h to 72h after pulp exposure. Although in their experimental model caries progressed from the initial lesion to pulpal necrosis in less than 3 days, some parallels could be drawn between caries lesions of the different levels seen in patients. Before therapy, IL-1β GCF increased from the initial C2 caries (enamel + dentin lesion) to pulpitis (C3) and root inflammation (C5), with a modest increase in gangrenous pulp (C4). Similarly, He et al. [57] demonstrated a local pulp IL-1β increase from the initial inflammation to the maximal presence in irreversible pulpitis until the beginning of the necrosis process, after which the value decreased. In our study, only the C2 group had noticeably the smallest increase rate compared to the level before therapy.

Surprisingly, at both control points, on the 7th and 30th day, the average concentration of GCF IL-1β and TNF-α were increased compared to the level before dental restoration practically in all investigated samples. Conclusively, Ilday et al. [27] demonstrated that silorane composite dental restoration after dental caries is associated with a significant increase of average TNF-α, IL-6, and IL-8, while Geraldeli et al. [58] found that amalgam dental restoration induced an increase of local TNF-α but a slight decrease of IL-1β in coronal occlusal dentine of trimmed molars. Since the restored teeth were without any clinical and/or radiological signs, this increase could not be attributed to further progression of caries lesion or any other inflammatory process.

According to one group of studies, proinflammatory cytokines are just indispensable in dental regeneration processes. Bone regeneration itself is critically connected to proinflammatory cytokines. The regeneration of bone fracture is associated with biphasic TNF-α and IL-1β increase, with a peak during the initiation of fracture repair, followed by a second peak at the transition from chondrogenesis to osteogenesis during endochondral maturation [61, 62]. The balanced immune response appears to be essential for a successful bone healing process [63, 64]. The absence of TNF-α delays fracture healing, while prolonged exposure to TNF-α destroys the bone [65, 66]. Our study in children with long bone fractures (unpublished results), showed significantly lower IL-1β and MCP-1 serum concentrations in children with insufficient callus formation and minor fragment dislocation (angulation and dislocation less than 1 cm). Therefore, newer studies demonstrated that IL-1β and TNF-α influence the biological behavior of dental stem cells. In a way, they are needed for tooth tissue regeneration. The study from Yang et al. [67] demonstrated that IL-1β and TNF-α have synergistic effects on odontogenic differentiation of isolated dental pulp stem cell population. The *in vitro* treatment with both IL-1β and TNF-α compared to a single treatment with either cytokine demonstrated a significantly faster stem cell proliferation, increased alkaline phosphatase (ALP) activity, increased osteocalcin and bone sialoprotein expression, augmented mRNA expression of ALP, osteocalcin, bone sialoprotein, dentin sialophosphoprotein, and dentin matrix protein-1. Both cytokines synergistically induced significant morphologic dental stem cell changes on the 3rd day at the surfaces of the HA/TCP ceramic scaffolds. The *in vivo* experiments with dental stem cell implants, pretreated with IL-1 and TNF-2, showed a significant level of hard bone formation, with even bone marrow like hematopoietic tissue.

Goldberg et al. [68] stated that inflammatory processes are very important not only for defense but also for pulp regeneration. Therefore, it seems that local inflammation is overseen only as an unwanted and harmful process, leading only to necrosis in the undesirable outcome. Migration and odontoblastic differentiation of dental stem cells is a crucial step in dental regeneration after caries lesion [69–72]. Leprince et al.[73] concluded that dental pulp stem cells and mesenchymal stem cells have identical characteristics, and are needed for dental pulp regeneration. According to this aspect, after initial response to local microbiota agents mediated by inflammatory cytokines, after their elimination

and dental restoration, local stem cells are activated and induced to differentiate into cells that produce reactionary and reparative dentin [74]. Another inflammatory wave could regulate transdifferentiation of fibroblast-like pulp cells to stem cells [75], or inflammatory monocytes itself could be converged to odonto-progenitor cells.

The balance between the inflammatory process as a defense mechanism and an inflammatory initiated reparation seems to be influenced by the severity and presence of infection. Controlled, acute production of inflammatory mediators and clearing of microorganisms is associated with tissue repair, while chronic, uncontrolled inflammation is destructive [20].

Restorative dental materials significantly influence GCF mediators concentration [29]. Celik et al. [25] and Ilday et al. [26, 27] reported that different dental restorative materials induce the various local response, inducing a significant variation of GCF IL-6, IL-8, and TNF-α profile after dental therapy. Sakallioğlu et al. [77] investigated the concentration of substance-P, calcitonin gene-related peptide, neurokinin-A, IL-1α, IL-1β, and PGE2 in GCF samples of teeth restored with ceramic, metal, composite, opposite-composite, amalgam, opposite-amalgam, or enamel. Although the study was performed only on 14 patients without any data before therapy or tooth position, they noted significant inter-group variations 4 weeks after restoration. They found the highest level of substance-P in amalgam restored teeth, PGE2 in composite restored, while IL-1α and IL-1β were highly present after metal-based restoration. Similarly, Björkman et al. [78] reported that the removal of amalgam restoration resulted in the normalization of GCF Th1 cytokine levels. We also demonstrated that dental restorative material (both permanent and temporary) induce a significant change in GCF IL-1α and IL-1β levels.

There are several explanations for the increases of GCF IL-1α and IL-1β levels after restoration. Local inflammatory mediators could be induced from dental cells with chemical content liberated from the restorative material, and/or by mediators generated from *de novo* plaque accumulation. Since there were no clinical signs of any inflammatory process or plaque accumulation after restoration either in our or previous studies [29, 25, 26], inflammatory mediator increase could be attributed to a healing or reparation process. Calcium hydroxide and mineral trioxide aggregate (MTA) are known to stimulate dentinogenesis and cementogenesis, together with the early inflammation [79], while MTA, at least *in vitro*, demonstrated significant IL-1β stimulating capacity [80]. Hydroxyl ions derived from these restorative materials change the oxido-reductive balance at lesion site [28], ultimately inducing chemical tissue irritation and cellular necrosis. Necrotic cells release low levels of cytokines and other damage signals to facilitate the removal of the dead or dying cells, leading to the inflammation without microorganisms in the lesion itself [81, 82].

Conclusion

The significant presence of inflammatory mediators in GCF of the restored teeth without signs of the inflammatory process could be associated with the reparative process. Different influences of various types of dental fillings on GCF IL-1α and IL-1β levels could represent the ground for selecting the optimal restorative material.

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Conflict of interest

None.

R E F E R E N C E S

1. *Jin L, Lamster I, Greenspan J, Pitts N Scully C, Warnakulasuriya S.* Global burden of oral diseases: emerging concepts, management and interplay with systemic health. Oral Dis 2016; 22(7): 609–19.
2. *Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C.* The global burden of oral diseases and risks to oral health. Bull World Health Organ 2005; 83(9): 661–9.
3. *Jang JH, Shin HW, Lee JM, Lee HW, Kim EC, Park SH.* An Overview of Pathogen Recognition Receptors for Innate Immunity in Dental Pulp. Mediators Inflamm 2015; 2015: 794143.
4. *Sanz M, Beighton D, Curtis MA, Cury JA, Dige I, Dommisch H*, et al. Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. J Clin Periodontol 2017; 44 Suppl 18: S5–S11.
5. *Cooper PR, Takahashi Y, Graham LW, Simon S, Imazato S, Smith AJ.* Inflammation-regeneration interplay in the dentine-pulp complex. J Dent 2010; 38(9): 687–97.
6. *Veerayutthwilai O, Byers MR, Pham TT, Darveau RP, Dale BA.* Differential regulation of immune responses by odontoblasts. Oral Microbiol Immunol 2007; 22(1): 5–13.
7. *Durand SH, Flacher V, Roméas A, Carrouel F, Colomb E, Vincent C*, et al. Lipoteichoic acid increases TLR and functional chemokine expression while reducing dentin formation in in vitro differentiated human odontoblasts. J Immunol 2006; 176(5): 2880–7.
8. *Staquet MJ, Durand SH, Colomb E, Roméas A, Vincent C, Bleicher F*, et al. Different roles of odontoblasts and fibroblasts in immunity. J Dent Res 2008; 87(3): 256–61.
9. *Jiang HW, Zhang W, Ren BP, Zeng JF, Ling JQ.* Expression of toll like receptor 4 in normal human odontoblasts and dental pulp tissue. J Endod 2006; 32(8): 747–51.
10. *Staquet MJ, Carrouel F, Keller JF, Baudouin C, Msika P, Bleicher F*, et al. Pattern-recognition receptors in pulp defense. Adv Dent Res 2011; 23(3): 296–301.
11. *He W, Yu Q, Zhou, Wang P.* CpG oligonucleotides induce an immune response of odontoblasts through the TLR9, MyD88 and NF-kappaB pathways. Biochem Biophys Res Commun 2010; 399(2): 274–8.

12. *Keller JF, Carrouel F, Colomb E, Durand SH, Baudouin C, Msika P*, et al. Toll-like receptor 2 activation by lipoteichoic acid induces differential production of pro-inflammatory cytokines in human odontoblasts, dental pulp fibroblasts and immature dendritic cells. Immunobiology 2010; 215(1): 53–9.
13. *Farges JC, Carrouel F, Keller JF, Baudouin C, Msika P, Bleicher F,* et al. 2010 Cytokine production by human odontoblast-like cells upon Toll-like receptor-2 engagement. Immunobiology 2011; 216(4): 513–7.
14. *He W, Zhang Y, Zhang J, Yu Q, Wang P, Wang Z,* et al. Cytidine-phosphate-guanosine oligonucleotides induce interleukin-8 production through activation of TLR9, MyD88, NF-κB, and ERK pathways in odontoblast cells. J Endod 2012; 38(6): 780–5.
15. *Paris S, Wolgin M, Kielbassa AM, Pries A, Zakrzewicz A*. Gene expression of human beta-defensins in healthy and inflamed human dental pulps. J Endod 2009; 35(4): 520–3.
16. *Dommisch H, Winter J, Açil Y, Dunsche A, Tiemann M, Jepsen S*. Human beta-defensin (hBD-1, -2) expression in dental pulp. Oral Microbiol Immunol 2005; 20(3): 163–6.
17. *Farges JC, Alliot-Licht B, Renard E, Ducret M, Gaudin A, Smith AJ,* et al. Dental Pulp Defence and Repair Mechanisms in Dental Caries. Mediators Inflamm. 2015; 2015: 230251.
18. *Turner MD, Nedjai B, Hurst T, Pennington DJ*. (2014) Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochim Biophys Acta 2014; 1843(11): 2563–82.
19. *Hirao K, Yumoto H, Takahashi K, Mukai K, Nakanishi T, Matsuo T*. Roles of TLR2, TLR4, NOD2, and NOD1 in pulp fibroblasts. J Dent Res 2009; 88(8): 762–7.
20. *Cooper PR, Holder MJ, Smith AJ*. Inflammation and regeneration in the dentin-pulp complex: a double-edged sword. J Endod 2014; 40(4 Suppl): S46–51.
21. *Silva AC, Faria MR, Fontes A, Campos MS, Cavalcanti BN*. Interleukin-1 beta and interleukin-8 in healthy and inflamed dental pulps. J Appl Oral Sci 2009; 17(5): 527–32.
22. *Kim YS, Min KS, Lee SI, Shin SJ, Shin KS, Kim EC*. Effect of proinflammatory cytokines on the expression and regulation of human beta-defensin 2 in human dental pulp cells. J Endod 2010; 36(1): 64–9.
23. *Hase N, Ozeki N, Hiyama T, Yamaguchi H, Kawai R, Kondo A,* et al. Products of dentin matrix protein-1 degradation by interleukin-1β-induced matrix metalloproteinase-3 promote proliferation of odontoblastic cells. Biosci Trends 2015; 9(4): 228–36.
24. *Huang TH, Yang CC, Ding SJ, Yeng M, Kao CT, Chou MY*. Inflammatory cytokines reaction elicited by root-end filling materials. J Biomed Mater Res B Appl Biomater 2005; 73(1): 123–8.
25. *Celik, N, Askın, S, Gul MA, Seven N*. The effect of restorative materials on cytokines in gingival crevicular fluid. Arch Oral Biol 2017; 84: 139–44.
26. *Ilday NO, Celik N, Dilsiz A, Alp HH, Aydin T, Seven N,* et al. The effects of overhang amalgam restoration on levels of cytokines, gingival crevicular fluid volume and some periodontal parameters. Am J Dent 2016; 29(5): 266–70.
27. *Ilday NO, Celik N, Dilsiz A, Alp HH, Aydin T, Seven N*, et al. The effects of silorane composites on levels of cytokines and periodontal parameters. Contemp Clin Dent 2013; 4(4): 437–42.
28. *Taso E, Stefanovic V, Stevanovic I, Vojvodic D, Topic A, Petkovic-Curcin A*, et al. Influence of Dental Restorations on Oxidative Stress in Gingival Crevicular Fluid. Oxid Med Cell Longev 2018; 2018: 1823189.
29. *Stefanović V, Taso E, Petković-Ćurčin A, Đukić M, Gardašević M, Rakić M,* et al. Influence of dental filling material type on the concentration of interleukin 9 in the samples of gingival crevicular fluid. Vojnosanit Pregl 2016; 73(8): 728–34.
30. *Ariaans K, Heussen N, Schiffer H, Wienert AL, Plümäkers B, Rink L,* et al. Use of molecular indicators of inflammation to assess the biocompatibility of all-ceramic restorations. J Clin Periodontol 2016; 43(2): 173–9.
31. *Fisher J, Glick M*. FDI World Dental Federation Science Committee. A new model for caries classification and management: the FDI World Dental Federation caries matrix. J Am Dent Assoc 2012; 143(6): 546–51.
32. *Caton JG, Armitage G, Berglundh T, Chapple ILC, Jepsen S, Kornman KS*, et al. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. J Clin Periodontol 2018; 45 Suppl 20: S1–S8.
33. *Lang NP, Bartold PM*. Periodontal health. J Clin Periodontol 2018; 45 Suppl 20: S9–S16.
34. *Rakic M, Lekovic V, Nikolic-Jakoba N, Vojvodic D, Petkovic-Curcin A, Sanz M*. Bone loss biomarkers associated with peri-implantitis. A cross-sectional study. Clin Oral Implants Res 2013; 24(10): 1110–6.
35. *Almehmadi AH, Alghamdi F*. Biomarkers of alveolar bone resorption in gingival crevicular fluid: A systematic review. Arch Oral Biol 2018; 93: 12–21.
36. *Hokari T, Morozumi T, Komatsu Y, Shimizu T, Yoshino T, Tanaka M*, et al. Effects of Antimicrobial Photodynamic Therapy and Local Administration of Minocycline on Clinical, Microbiological, and Inflammatory Markers of Periodontal Pockets: A Pilot Study. Int J Dent 2018; 2018: 1748584.
37. *Zhang J, Zhang AM, Zhang ZM, Jia JL, Sui XX, Yu LR,* et al. Efficacy of combined orthodontic-periodontic treatment for patients with periodontitis and its effect on inflammatory cytokines: A comparative study. Am J Orthod Dentofacial Orthop 2017; 152(4): 494–500.
38. *Cicek Ari V, Ilarslan YD, Erman B, Sarkarati B, Tezcan I, Karabulut E,* et al. Statins and IL-1β, IL-10, and MPO Levels in Gingival Crevicular Fluid: Preliminary Results. Inflammation 2016; 39(4): 1547–57.
39. *Luo L, Xie P, Gong P, Tang XH, Ding Y, Deng LX*. Expression of HMGB1 and HMGN2 in gingival tissues, GCF and PICF of periodontitis patients and peri-implantitis. Arch Oral Biol 2011; 56(10): 1106–11.
40. *Abduljabbar T, Akram Z, Vohra F, Warnakulasuriya S, Javed F*. Assessment of interleukin-1β, interleukin-6, and tumor necrosis factor-A levels in the peri-implant sulcular fluid among water-pipe (narghile) smokers and never-smokers with peri-implantitis. Clin Implant Dent Relat Res 2018; 20(2): 144–50.
41. *Petković AB, Matić SM, Stamatović NV, Vojvodić DV, Todorović TM, Lazić ZR,* et al. Proinflammatory cytokines (IL-1beta and TNF-alpha) and chemokines (IL-8 and MIP-1alpha) as markers of peri-implant tissue condition. Int J Oral Maxillofac Surg 2010; 39(5): 478–85.
42. *Che C, Liu J, Ma L, Xu H, Bai N, Zhang Q*. LOX-1 is involved in IL-1β production and extracellular matrix breakdown in dental peri-implantitis. Int Immunopharmacol 2017; 52: 127–35.
43. *Schincaglia GP, Hong BY, Rosania A, Barasz J, Thompson A, Sobue T,* et al. Clinical, Immune, and Microbiome Traits of Gingivitis and Peri-implant Mucositis J Dent Res 2017; 96(1): 47–55.
44. *Renvert S, Widén C, Persson GR*. Cytokine expression in peri-implant crevicular fluid in relation to bacterial presence. J Clin Periodontol 2015; 42(7): 697–702.
45. *Doğan ŞB, Kurtiş MB, Tüter G, Serdar M, Watanabe K, Karakış S*. Evaluation of Clinical Parameters and Levels of Proinflammatory Cytokines in the Crevicular Fluid Around Dental Implants in Patients with Type 2 Diabetes Mellitus. Int J Oral Maxillofac Implants 2015; 30(5): 1119–27.
46. *Raslan SA, Cortelli JR, Costa FO, Aquino DR, Franco GC, Cota LO*, et al.) Clinical, microbial, and immune responses observed in patients with diabetes after treatment for gingivitis: a three-month randomized clinical trial. J Periodontol 2015; 86(4): 516–26.

47. *Özçaka Ö, Alpöz E, Nalbantsoy A, Karabulut G, Kabasakal Y.* Clinical periodontal status and inflammatory cytokines in primary Sjögren syndrome and rheumatoid arthritis. J Periodontol 2018; 89(8): 959–65.
48. *Cetinkaya B, Guzeldemir E, Ogus E, Bulut S.* Proinflammatory and anti-inflammatory cytokines in gingival crevicular fluid and serum of patients with rheumatoid arthritis and patients with chronic periodontitis. J Periodontol 2013; 84(1): 84–93.
49. *Javed F, Ahmed HB, Mikami T, Almas K, Romanos GE, Al-Hezaimi K.* Cytokine profile in the gingival crevicular fluid of rheumatoid arthritis patients with chronic periodontitis. J Investig Clin Dent 2014; 5(1): 1–8.
50. *Buyukoğlu B, Buduneli N, Aksu K, Nalbantsoy A, Lappin DF, Evrenosoğlu E,* et al. Periodontal therapy in chronic periodontitis lowers gingival crevicular fluid interleukin-1beta and DAS28 in rheumatoid arthritis patients. Rheumatol Int 2013; 33(10): 2607–16.
51. *Kumar NK, Reddy VK, Padakandla P, Togaru H, Kalagatla S, Chandra SN.* Evaluation of chemokines in gingival crevicular fluid in children with dental caries and stainless steel crowns: A clinicobiochemical study. J Indian Soc Pedod Prev Dent 2016; 34(3): 273–9.
52. *Couglo D, Onay H, Ozdemer Y, Aslan G, Ozkinay F, Kutukculer N,* et al. Associations of interleukin (IL)-1β, IL-1 receptor antagonist, and IL-10 with dental caries. J Oral Sci 2015; 57(1): 31–6.
53. *Eslami H, Pouralibabai F, Sepan R, Zerandis A.* Evaluation of Relationship between *Streptococcus mutans*, Dental Caries and IL-1α and IL-6. J Periodontal Implant Dent 2016; 8(1): 33–6.
54. *McLachlan JL, Sloan AJ, Smith AJ, Landini G, Cooper PR.* S100 and cytokine expression in caries. Infect Immun 2004; 72(7): 4102–8.
55. *Farges JC, Alliot-Licht B, Baudouin C, Msika P, Bleicher F, Carrouel F.* Odontoblast control of dental pulp inflammation triggered by cariogenic bacteria. Front Physiol 2013;4: 326.
56. *Horst OV, Horst JA, Samudrala R, Dale BA.* Caries induced cytokine network in the odontoblast layer of human teeth. BMC Immunol 2011; 12: 9.
57. *He Y, Gan Y, Lu J, Feng Q, Wang H, Guan H,* et al. Pulpal Tissue Inflammatory Reactions after Experimental Pulpal Exposure in Mice. J Endod 2017; 43(1): 90–5.
58. *Geraldeli S, Li Y, Hogan MM, Tjaderhane LS, Pashley DH, Morgan TA,* et al. Inflammatory mediators in fluid extracted from the coronal occlusal dentine of trimmed teeth. Arch Oral Biol 2012; 57(3): 264–70.
59. *Iwasaki LR, Crouch LD, Tutor A, Gibson S, Hukmani N, Marx DB,* et al. Tooth movement and cytokines in gingival crevicular fluid and whole blood in growing and adult subjects. Am J Orthod Dentofacial Orthop 2005; 128(4): 483–91.
60. *George A, Evans CA.* Detection of root resorption using dentin and bone markers. Orthod Craniofac Res 2009; 12(3): 229–35.
61. *Kon T, Cho TJ, Aizawa T, Yamazaki M, Nooh N, Graves D,* et al. Expression of osteoprotegerin, receptor activator of NF-kappaB ligand (osteoprotegerin ligand) and related proinflam-matory cytokines during fracture healing. J Bone Miner Res 2001; 16(6): 1004–14.
62. *Lehmann W, Edgar CM, Wang K, Cho TJ, Barnes GL, Kakar S,* et al. Tumor necrosis factor alpha (TNF-alpha) coordinately regulates the expression of specific matrix metalloproteinases (MMPS) and angiogenic factors during fracture healing. Bone 2005; 36(2): 300–10.
63. *Kolar P, Schmidt-Bleek K, Schell H, Gaber T, Toben D, Schmidmaier G,* et al. The early fracture hematoma and its potential role in fracture healing. Tissue Eng Part B Rev 2010; 16(4): 427–34.
64. *Schmidt-Bleek K, Schell H, Lienau J, Schulz N, Hoff P, Pfaff M,* et al. Initial immune reaction and angiogenesis in bone healing. J Tissue Eng Regen Med 2014; 8(2): 120–30.
65. *Karnes J, Daffner S, Watkins C.* Multiple Roles of Tumor Necrosis Factor - Alpha in Fracture Healing. Bone 2015; 78: 87–93.
66. *Mountziaris PM, Spicer PP, Kasper FK, Mikos AG.* Harnessing and modulating inflammation in strategies for bone regenera-tion. Tissue Eng Part B Rev 2011; 17(6): 393-402.
67. *Yang X, Han G, Pang X.* Chitosan/collagen scaffold containing bone morphogenetic protein-7 DNA supports dental pulp stem cell differentiation in vitro and in vivo J Biomed Mater Res A 2012; doi: 10.1002/jbm.a.34064
68. *Goldberg M, Njeh A, Uzunoglu E.* Is Pulp Inflammation a Prerequisite for Pulp Healing and Regeneration? Mediators In-flamm 2015; 2015: 347649.
69. *Louvrier A, Euvrard E, Nicod L, Rolin G, Gindraux F, Pazart L,* et al. Odontoblastic differentiation of dental pulp stem cells from healthy and carious teeth on an original PCL-based 3D scaffold. Int Endod J 2018; 51 Suppl 4: e252–e263.
70. *Werle S, Lindemann D, Steffens D, Demarco F, de Araujo F, Pranke P,* et al. Carious deciduous teeth are a potential source for dental pulp stem cells. Clin Oral Investig 2016; 20(1): 75–81.
71. *Rombouts C, Jeanneau C, Bakopolou A, About I.* Dental Pulp Stem Cell Recruitment Signals within Injured Dental Pulp Tissue. Dent J (Basel) 2016; 4(2): 8.
72. *Nakashima M, Iohara K, Murakami M, Nakamura H, Sato Y, Ariji Y,* et al. Pulp regeneration by transplantation of dental pulp stem cells in pulpitis: a pilot clinical study. Stem Cell Res Ther 2017; 8(1): 61.
73. *Leprince JG, Zeitlin BD, Tolar M, Peters OA.* Interactions be-tween immune system and mesenchymal stem cells in dental pulp and periapical tissues. Int Endod J 2012; 45(8): 689–701.
74. *Goldberg M, Opsahl S, Aubin I, Septier D, Chaussain-Miller C, Boskey A,* et al. Sphingomyelin degradation is a key factor in dentin and bone mineralization: lessons from the fro/fro mouse. The chemistry and histochemistry of dentin lipids. J Dent Res 2008; 87(1): 9–13.
75. *Yang G, Crawford RC, Wang JH.* Proliferation and collagen production of human patellar tendon fibroblasts in response to cyclic uniaxial stretching in serum-free conditions. J Biomech 2004; 37(10): 1543–50.
76. *Kuwana M, Okazaki Y, Kodama H, Izumi K, Yasuoka H, Ogawa Y,* et al. Human circulating CD14+ monocytes as a source of progenitors that exhibit mesenchymal cell differentiation. J Leukoc Biol 2003; 74(5): 833–45.
77. *Sakallioğlu EE, Lütfioğlu M, Sakallioğlu U, Ceylan GK, Pamuk F, Dede FÖ,* et al. Gingival crevicular fluid levels of neuropeptides following dental restorations. J Appl Biomater Funct Mater 2015; 13(2): e186–93.
78. *Björkman L, Brokstad KA, Moen K, Jonsson R.* Minor changes in serum levels of cytokines after removal of amalgam restora-tions. Toxicol Lett 2012; 211(2): 120–5.
79. *Reyes-Carmona JF, Santos AR, Figueiredo CP, Felippe MS, Felippe WT, Cordeiro MM.* In vivo host interactions with mineral tri-oxide aggregate and calcium hydroxide: inflammatory molecu-lar signaling assessment. J Endod 2011; 37(9): 1225–35.
80. *Cavalcanti D.* A deployment strategy for Internet Exchange Points as part of a National Broadband Plan. Proceedings of the 5th ACORN-REDECOM Conference, Lima; 19–21 May 2011. Available from: www.acorn-redecom.org/papers/2011Cavalcanti_English.pdf
81. *Luheshi NM, McColl BW, Brough D.* Nuclear retention of interleukin-1α by necrotic cells: a mechanism to dampen sterile inflammation. Eur J Immunol 2009; 39(11): 2973–80.
82. *Acosta-Pérez G, Maximina Bertha Moreno-Altamirano M, Rodríguez-Luna G, Javier Sánchez-García F.* Differential dependence of the ingestion of necrotic cells and TNF-alpha / IL-1beta production by murine macrophages on lipid rafts. Scand J Immunol 2008; 68(4): 423–9.

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Database analysis of oral atropine treatment of infantile hypertrophic pyloric stenosis. A ten-year single-center experience

Analiza baze podataka o lečenju infantilne hipertrofične stenoze pilorusa oralnom primenom atropina. Desetogodišnje iskustvo jednog centra

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Abstract

Background/Aim. Infantile hypertrophic pyloric stenosis (IHPS) is the most common cause of surgery in newborns and young infants. Conservative treatment of IHPS is of great importance because it spares the newborn from stress caused by surgery and general anesthesia. The aim of this study was to evaluate the impact of various oral administration regimens of atropine on its efficacy in treating IHPS. **Methods.** The study included 45 patients with IHPS, conservatively treated by atropine sulfate in the period from 2006 to 2016. Clinical examination, laboratory analysis, and ultrasonography were performed on all patients on admission. The efficacy of treatment with different oral dosage regimens was analyzed and potential predictive factors of the negative outcome were defined. The evaluation of the success of the treatment was statistically analyzed by the method of the multivariate logistic regression model. **Results.** Out of 45 patients, 36 (80%) were successfully cured (p = 0.0008, without the need for surgery and without any complications. Gender prevalence, age, birth weight, body weight on admission, duration of symptoms, pyloric muscle thickness, and length had no statistically significant individual effect on the success of the atropine treatment. Patients who received a progressively increased dose of atropine had an 18 times higher risk of surgery, patients with hypochloremic alkalosis (HCA) had a 15 times higher risk, while others, with more than 5 vomitings within the first three days of the therapy, were 9 times more likely to be surgically treated. **Conclusion.** High success rate and no side effects represent an orally administered atropine treatment as a valid alternative choice for non-operative management of IHPS. Administration of initially high doses was shown to be more effective in relation to gradually increased oral doses of atropine sulfate. HCA and continued vomiting are considered as potential predictive factors of negative outcomes of the atropine treatment.

Key words:
atropine; infant; muscarinic antagonists; pyloric stenosis, hypertrophic; risk factors; surgical procedures, operative; treatment outcome.

Apstrakt

Uvod/Cilj. Infantilna hipertrofična stenoza pilorusa (IHSP) je najčešći razlog za hiruršku intervenciju u uzrastu novorođenčeta i mladog odojčeta, a efikasan konzervativni tretman je od velikog značaja, jer se na taj način novorođeno dete ne izlaže stresu uzrokovanom hirurškom intervencijom i opštom anestezijom. Cilj rada bio je procena uticaja različitih režima oralne primene atropina na njegovu efikasnost u lečenju IHSP. **Metode.** Studijom je bilo obuhvaćeno 45 bolesnika konzervativno lečenih oralnom primenom atropin sulfata zbog IHSP, u periodu od 2006. do 2016. godine. Klinički pregled, laboratorijske analize i ehosonografija urađeni su kod svih ispitanika na prijemu. Analizirana je efikasnost konzervativnog tretmana, s posebnim naglaskom na efekat doziranja leka i definisanje potencijalnih prediktivnih faktora negativnog ishoda. Procena uspešnosti lečenja analizirana je korišćenjem modela multivarijantne logističke regresije. **Rezultati.** Konzervativno je lečeno 45 bolesnika, od kojih je 36 (80%) bilo uspešno izlečeno (p = 0,0008, bez potrebe za hirurškom intervencijom i bez komplikacija. Polna zastupljenost, uzrast, porođajna telesna masa, telesna masa na prijemu, trajanje simptoma, kao i dužina i debljina pilorusnog mišića nisu imali statistički značajan

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pojedinačni uticaj na uspeh medikamentnog lečenja. Ispitanici kod kojih je primenjeno progresivno povećanje doze atropina imali su 18 puta viši rizik da će biti operisani, ispitanici koji su na prijemu imali hipohloremijsku alkalozu 15 puta viši rizik, dok su oni sa više od 5 povraćanja u prva tri dana od početka primene atropina imali 9 puta viši rizik od primene hirurškog lečenja. **Zaključak.** Visoka stopa uspešnosti lečenja oralnom primenom atropin sulfata čini ga validnim alternativnim lekom za neoperativni tretman IHSP. Pokazalo se da je primena inicijalno visokih doza efikasnija u odnosu na postepeno povećavane oralne doze atropin sulfata.

Ključne reči:
atropin; odojče; antimuskarinici; pilorus, stenoza, hipertrofička; faktori rizika; hirurgija, operativne procedure; lečenje, ishod.

Introduction

Infantile hypertrophic pyloric stenosis (IHPS) is the most common cause of surgical procedures in newborns and young infants [1]. The inability of gastric emptying, due to progressive hypertrophy of the pyloric circular muscle, causes postprandial, missile, non-bilious vomiting, resulting in body weight loss and the development of metabolic alkalosis. It is reported with an incidence of 4/1,000 live-born children, 4 times more often in boys [2]. Prolonged spasm of the pyloric muscle leads to its hypertrophy, but whether the absence of relaxation is caused by a genetic factor, a smaller number of ganglion cells and/or a lower level of nitrogen-monoxide (NO) synthetase with the neutrality of the gastric contents in the newborn, is not completely clarified [3–5]. The diagnostic method of choice is ultrasonography and extramucosal pyloromyotomy is the superior method of treatment of IHPS, whose outcome is a rapid providing lifelong resolution of symptoms [6–9]. Pyloromyotomy by the laparoscopic approach is equal to an open surgical method, although it is followed by a slightly higher percentage of mucosal perforations and incomplete pyloromyotomies [10]. Medication treatment, which implies treatment with atropine sulfate, is accepted as an alternative to pyloromyotomy, primarily in children with comorbidity. In some countries, like Japan, it is the first line of therapy. Initially, according to literature data, atropine was administered intravenously with higher efficacy on the treatment of IHPS but with more side effects. Also, the harmful effects of orally administered atropine were not recorded [11].

The aim of the study was to evaluate the impact of various oral administration regimens of atropine on its efficacy in treating IHPS, as well as to define potential predictive factors of its negative outcome.

Methods

A retrospective and prospective nonrandomized study was conducted in a ten-year period (2006–2016). Forty-five patients with IHPS were treated by orally administered atropine sulfate. The study included patients with a missile, non-bilious vomiting in a typical age group, while the IHPS was confirmed after the clinical and ultrasound examination. The inclusion criteria were positive ultrasound findings of the hypertrophic pylorus, defined by the Haller and Cohen criteria that considered the thickness of the muscle wall ≥ 4 mm, the total diameter of pylorus ≥ 15 mm, and pyloric muscle length ≥ 18 mm. The data were obtained by processing existing medical documentation for the retrospective group and complete diagnosis on admission and during hospitalization for patients from the prospective part of the study. Gender distribution, age, birth weight, and body weight were analyzed on admission, whereby the body weight gain was considered poor if it was < 10 g/kg/day. The analysis also included symptom duration and its correlation with the ultrasound findings (pyloric muscle thickness and length) and the incidence of hypochloremic alkalosis (HCA) on the admission, which considered pH > 7.35, Cl^- < 95 mmol/L, and base excess (BE) > 2 mmol/L. Conservative treatment implied the placement of a nasogastric tube, aspiration of gastric content before administration of atropine and a meal, 20 min after administration of the drug. Episodes of vomiting > 5 in 3 days were indications of switching to surgical treatment. Successful conservative treatment involved discharging the child without surgery, with a milk intake of 120 mL/kg/day and body weight gain. The patients were divided into two subgroups upon the concentration of the drug they received: subgroup Ia received gradually increased doses of atropine from 0.05 mg/kg/day to 0.18 mg/kg/day, with a continuous increase of 0.02 mg/kg/day, for 7 days from the day of admission. Subgroup Ib received the maximum dose of atropine (0.18 mg/kg/day) from the beginning, with an evaluation of the effectiveness of the therapy after three days, based on the total number of projectile vomiting during that period. A successful treatment implied discharge without operation. A comparative analysis of the obtained results was made. The aforementioned analysis examined the influence of predictive factors on the outcome of conservative treatment, enabling the definition of a negative one that signifies a withdrawal from the medicament therapy.

Statistical analysis

The statistical analysis was performed using SPSS statistical package for Windows, version 22. The descriptive statistics, including mean, median, and standard deviation of numerical variables, and numbers and percentages of categorical variables were used to characterize the study sample. For categorical data, the Pearson χ^2 test or the Fisher exact test were employed, and

for numerical data, the independent samples *t*-test or the Mann-Whitney *U* test were used. Multiple logistic regression model, with atropine treatment outcome as the dependent variable, included all variables with $p < 0.05$ from univariate analysis. In all analyses, the significance levels were set at 0.05.

Results

A group of patients on atropine treatment included 45 patients, 34 (75.6%) boys and 11 (24.4%) girls. This means that there was a significantly higher number of male children in total ($p = 0.0054$), although there is no statistically significant effect of the gender distribution on the success of the medication treatment ($p = 0.416$) (Table 1).

undergo surgery had less than 5 episodes of vomiting in 3 days, which proved to be statistically significant compared to 4 out of 9 (44%) patients who were surgically treated ($p = 0.002$).

The subgroup Ia included 15 (33.3%) patients initially treated with gradually increased doses, and the subgroup Ib included 30 (66.7%) patients, who received the maximum dose of atropine ($p = 0.0702$). Twenty-eight out of 36 (78%) patients who did not undergo surgery were treated with an initially high dose of atropine, which was statistically significantly higher than 8 (22%) non-operated patients who were treated with a gradually increased dose of atropine ($p = 0.003$). Twenty-eight (93%) out of 30 patients from the subgroup Ib were successfully cured by atropine, while only 2 (7%) underwent surgery, with a very

Table 1

Clinical characteristics of patients with infantile hypertrophic pyloric stenosis treated by atropine sulfate

Parameter	Atropine sulfate only – no surgery required	Atropine sulfate followed by surgery	*p*
Gender, n (%)			
male	26 (72)	8 (89)	0.416
female	10 (28)	1 (11)	
Age (days), mean ± SD	31.9 ± 12.6	27.8 ± 13.4	0.392
Birth weight (kg), mean ± SD	3.3 ± 0.6	3.6 ± 0.2	0.197
Body weight on admission (kg), mean ± SD	3.9 ± 0.8	3.9 ± 0.7	0.869
Duration of symptoms (days), median (range)	4 (2–10)	4 (2–15)	0.542
Pyloric muscle length, mean ± SD	17.5 ± 1.8	18.2 ± 3.8	0.407
Pyloric muscle thickness, mean ± SD	4.2 ± 0.7	4.4 ± 0.7	0.385
Hypochloremic alkalosis, n (%)	2 (6)	4 (44)	0.010
Aspiration from the nasogastric tube, n (%)	15 (42)	9 (100)	0.002
Number of vomiting, n (%)			
≤ 5	34 (94)	4 (44)	0.002
> 5	2 (6)	5 (56)	
Length of stay in hospital (days), median (range)	10 (4–22)	12 (11–17)	0.023
Atropine sulfate dose, n (%)			
initially high	28 (78)	2 (22)	0.003
progressively increased	8 (22)	7 (78)	

SD – standard deviation.

Successful treatment with atropine was achieved in 36 (80%) patients with very high statistical significance ($p = 0.0008$).

Age, birth weight, body weight on admission, duration of symptoms, pyloric muscle thickness, as well as pyloric muscle length had no statistically significant individual effect on the success of the atropine treatment or the need for converting to surgery (Table 1).

Patients who did not go for surgery had statistically significantly less frequent HCA on admission in 2 out of 36 patients (6%) compared to 4 out of 9 patients (44%) ($p = 0.010$) who were shifted to surgical treatment. Aspiration of gastric content from the nasogastric tube prior to atropine administration was observed in a statistically significantly lower number of patients, in 15 out of 36 (42%) non-operated compared to all 9 (100%) operated patients ($p = 0.002$). Thirty-four (94%) patients who did not

high statistically significant difference ($p = 0.0001$) demonstrating a significant influence of the initially high dose of atropine on the success of conservative treatment. Eight (53%) out of 15 patients from the subgroup Ia were successfully cured by atropine and 7 (47%) underwent surgery, which means that there was no statistically significant difference between the observed patients, indicating lower efficacy of the initially lower dose of atropine (Table 1).

In the multivariate logistic regression model, a statistically significant predictor was the regimen of atropine-dosing patients, who received an initially lower dose of atropine with its progressive increase. They had an 18 times greater risk of surgery [odds ratio (OR) 17.9; $p = 0{,}033$]. The HCA values and the number of vomiting episodes were at the margin of statistical significance – patients who had HCA were at a 15 times greater risk of

surgery (OR 15.06; $p = 0.084$), and ones who experienced vomiting > 5 times within the first 3 days from the onset of atropine administration were at a 9 times greater risk of surgery (OR 9.45; $p = 0.059$) (Table 2).

Table 2

Multivariate logistic regression model of the impact of predictive factors on the atropine treatment outcome of infantile hypertrophic pyloric stenosis

Parameter	OR (95% CI)	*p*
Gender		
male	1	
female	1.30 (0.08–20.2)	0.853
Age	0.96 (0.87–1.06)	0.404
Hypochloremic alkalosis	15.06 (0.69–326.8)	0.084
Number of vomiting		
≤ 5	1	
> 5	9.45 (0.92–97.3)	0.059
Atropine sulfate dose		
initial high	1	
progressively increased	17.9 (1.26–254.0)	0.033

OR – odds ratio; CI – confidence interval.

During the course of the study, no harmful effects of orally administered atropine were observed.

Discussion

Attempts to find an approximate or equally effective conservative method of IHPS treatment have lasted for several decades. The first reason is the generally accepted assumption that prolonged pyloric muscle spasms lead to muscle hypertrophy. However, it is still unclear whether the cause of the spasm is the deficiency of nerve endings in the pyloric muscle, a lower level of NO synthetase, or a reduced number of intestinal Kajal cells [12–15]. The fact that progression of content through the pyloric channel occurs shortly after atropine administration or after pyloromyotomy confirms that muscle spasm, not only hypertrophy, is the main cause of pyloric obstruction, even though ultrasound reveals pyloric muscle hypertrophy continuing to exist for months after healing [16].

Most articles on non-operative treatment of IHPS promote atropine sulfate medical treatment. Medical treatment with atropine sulfate as a non-operative alternative to pyloromyotomy is suggested in 62.5% of these articles [12].

As atropine is parasympatholytic with a strong antimuscarinic effect, which reduces peristaltic contractions and relaxes smooth musculature, in this particular case, its use is completely justified and logical [17].

Although the surgical treatment of IHPS is 100% effective, avoiding the stress for a newborn child that comes with surgery and general anesthesia is also essential. One cannot rule out with certainty the existence of adverse effects of repeated general anesthesia in the neonatal age on the cognitive and motor development of the child [18, 19].

Besides, the complication rate after surgical treatment is about 7.3% ($p < 0.01$) concerning the mucosal perforation, wound infection, or incomplete pyloromyotomy [10].

Since 1991, when the first laparoscopic pyloromyotomy was done, no significant advantage of this approach has been defined in relation to open surgery. However, a slightly higher percentage of incomplete pyloromyotomy with the laparoscopic approach (0.87%) is shown concerning the open surgical method, which is statistically significant ($p = 0.046$) [10]. Atropine treatment of prolonged vomiting due to insufficient pyloromyotomy leads to symptom resolution very quickly and should certainly have an advantage over redo-pyloromyotomy [20–26].

Initial intravenous administration of atropine, with a subsequent transition to the oral route, recommended in most articles, has a higher percentage of efficiency but requires more serious patient monitoring due to the effects on the central nervous system, possible tachycardia, and face blushing [1, 16]. The harmful effects of atropine administered orally were not recorded in the literature nor our patient series. These were the reasons why our patients received atropine orally. The use of a maximum dose of atropine from the very beginning of the treatment was proven to be very safe and with a higher percentage of successful healing [27, 28].

Our study showed that HCA could be considered as a potential predictive factor of the negative outcome of atropine treatment. The literature also reveals the mutual relationship between duration of symptoms and HCA, with the same negative effect on the outcome of atropine treatment of IHPS [29].

Continued vomiting, more than five times in the first three days of the therapy, as well as in the Koike et al. [30] series, was a potential predictive factor of the negative outcome of atropine treatment in our series of patients. The influence of the pyloric muscle thickness is of no statistical significance on the outcome of atropine treatment [31].

There are only a few articles that mention other options of non-operative treatment such as spasmolytic treatment, dating from 1950–1986, balloon catheter dilatation of pyloric stenosis, dating from 1990, and tetrahydrobiopterin for the restoration of nitric oxide synthase activity in pyloric muscle, dating from 1997 [32–34]. Although these few options have been adopted by some researchers, their significance is minor and marginal, with no use in clinical practice.

Conclusion

High success rate and no side effects represent the orally administered atropine treatment as a valid alternative indication for non-operative management of IHPS, allowing discharge from the hospital without an operation. Administrating initially high doses was shown to be more effective in relation to gradually increased oral doses of atropine sulfate. HCA and continued vomiting, more than five times in the first three days of therapy, were considered as potential predictive factors of the negative outcome of atropine treatment.

R E F E R E N C E S

1. *Aspelund G, Langer JC.* Current management of hypertrophic pyloric stenosis. Semin Pediatr Surg 2007; 16(1): 27–33.
2. *Ross A, Johnson P.* Infantile hypertrophic pyloric stenosis. In: *Ameh EA, Bickler SW, Lakhoo K, Nwomeh BC, Poenaru D,* editors. Paediatric surgery: A comprehensive text for Africa. Seattle: Global Help; 2016; p. 12.
3. *Kawahara H, Imura K, Nishikawa M, Yagi M, Kubota A.* Intravenous atropine treatment in infantile hypertrophic pyloric stenosis. Arch Dis Child 2002; 87(1): 71–4.
4. *Kawahara H, Imura K, Yagi M.* Antropyloroduodenal manometry in patients with infantile hypertrophic pyloric stenosis: evidence of pylorospasm. JPN J Pediatr Surg 1997; 29: 1317–23.
5. *Krogh C, Fischer TK, Skotte L, Biggar RJ, Øyen N, Skytthe A.* Familial aggregation and heritability of pyloric stenosis. JAMA 2010; 303(23): 2393–9.
6. *Hernanz-Schulman M.* Infantile Hypertrophic Pyloric Stenosis. Radiology 2003; 227(2): 319–31.
7. *Mohamed A. Eltomey M, Ghareeb HA.* Postoperative ultrasonography changes of the pylorus in infants with hypertrophic pyloric stenosis. Egypt J Radiol Nucl Med 2014; 45: 897–902.
8. *Ramstedt C.* Zur operation der angeborenen pylorus-stenose. Med Klin 1912; 8: 170–5.
9. *Shaw A.* Ramstedt and the centennial of pyloromyotomy. J Pediatr Surg 2012; 47(7): 1433–5.
10. *Hall NJ, Eaton S, Seims A, Leys CM, Densmore JC, Calkins CM,* et al. Risk of incomplete pyloromyotomy and mucosal perforation in open and laparoscopic pyloromyotomy. J Pediatr Surg 2014; 49(7): 1083–6.
11. *Yamataka A, Tsukada K, Yokoyama-Laws Y, Murata M, Lane GJ, Osawa M,* et al. Pyloromyotomy versus atropine sulfate for infantile hypertrophic pyloric stenosis. J Pediatr Surg 2000; 35(2): 338–41; discussion 342.
12. *Lauriti G, Cascini V, Chiesa PL, Pierro A, Zani A.* Atropine Treatment for Hypertrophic Pyloric Stenosis: A Systematic Review and Meta-Analysis. Eur J Pediatr Surg 2018; 28(5): 393–9.
13. *Agata Bizzocchi A, Metz D.* Treating Pyloric Stenosis Medically in a Resource Poor Setting. Ann Pediatr Child Health 2016; 4(1): 1095.
14. *Vanderwinden JM, Mailleux P, Shiffmann SN, Vanderhaeghen JJ, DeLaet MH.* Nitric oxide synthase activity in infantile hypertrophic pyloric stenosis. N Engl J Med 1992; 327(8): 511–5.
15. *Kusafuka T, Puri P.* Altered messenger RNA expression of the neuronal nitric oxide synthase gene in infantile hypertrophic pyloric stenosis. Pediatr Surg Int 1997; 12(8): 576–9.
16. *Nagita A, Yamaguchi J, Amemoto K, Yoden A, Yamazaki T, Mino M.* Management and ultrasonographic appearance of infantile hypertrophic pyloric stenosis with intravenous atropine sulfate. J Pediatr Gastroenterol Nutr 1996; 23(02): 172–7.
17. *Brown JH.* Atropine, scopolamine and related anti-muscarinic drugs. In: *Gilman AG, Rall TW, Nies AS, Taylor P,* editors. Goodman and Gilman's pharmacological bases of therapeutics. Tokyo: Pergamon Press; 1990; p. 150–65.
18. *Montana M, Evers A.* Anesthetic Neurotoxicity: New Findings and Future Directions. J Pediatr 2017; 181: 279–85.
19. *Andropoulos DB.* Effect of Anesthesia on the Developing Brain: Infant and Fetus. Fetal Diagn Ther 2018; 43(1): 1–11.
20. *Alain JL, Grousseau D, Terrier G.* Extramucosal pyloromyotomy by laparoscopy. Surg Endosc 1991; 5(4): 174–5.
21. *Cubas RF, Longshore S, Rodriguez S, Tagge E, Baerg J, Moores D.* Atropine: A Cure for Persistent Post Laparoscopic Pyloromyotomy Emesis. J Neonatal Surg 2017; 6(1): 2.
22. *Owen RP, Almond SL, Humphrey GM.* Atropine sulphate: rescue therapy for pyloric stenosis. BMJ Case Rep 2012; 2012. pii: bcr2012006489.
23. *Chiu SS, Gilbert JC.* Recurrent pyloric stenosis: a form of the incomplete pyloromyotomy. J Pediatr Surg Case Reports 2018; 29: 14–7.
24. *Gumayan RL, Sandoval JA.* Operative Management of Recurrent Hypertrophic Pyloric Stenosis: A Case Report and Review of the Literature. SM Min Inv Surg 2017; 1(1): 1004.
25. *Hukeri* A, *Gupta* A, *Kothari* P, Dikshit V, Kekre G, Patil P, et al. Our experience of laparoscopic pyloromyotomy with ultrasound-guided parameters. J Minim Access Surg 2019; 15(1): 51–5.
26. *Elnaggar IA, Elbatarny AM, Khiralla MG, Mewally MF.* Laparoscopic pyloromyotomy in infantile hypertrophic pyloric stenosis using a myringotomy knife. Ann Pediatr Surg 2018; 14(2): 60–6.
27. *Kasuko O, Yuko Y, Santoshi H, Motoko M, Sigetaka S, Makiko O.* Oral treatment of atropine sulphate for hypertrophic pyloric stenosis. J Jap Fed Soc 2001; 105: 22–8.
28. *Singh UK, Kumar R, Prasad R.* Oral atropine sulfate for infantile hypertrophic pyloric stenosis. Indian Pediatr 2005; 42(5): 473–6.
29. *Wu SF, Lin HY, Huang FK, Chen AC, Su BH, Li CI,* et al. Efficacy of Medical Treatment for Infantile Hypertrophic Pyloric Stenosis: A Meta-analysis. Pediatr Neonatol 2016; 57(6): 515–21.
30. *Koike Y, Uchida K,* Nakazawa M, Inoue M, Kusunoki M, Tsukamoto Y. Predictive factors of negative outcome in initial atropine therapy for infantile hypertrophic pyloric stenosis. Pediatr Int 2013; 55(5): 619–23.
31. *Fan J, Shi Y, Cheng M, Zhu X, Wang D.*Treating idiopathic hypertrophic pyloric stenosis with sequential therapy: A clinical study. J Paediatr Child Health 2016; 52(7): 734–8.
32. *Rasmussen L, Hansen LP, Pedersen SA.* Infantile hypertrophic pyloric stenosis: the changing trend in treatment in a Danish county. J Pediatr Surg 1987; 22(10): 953–5.
33. *Hayashi AH, Giacomantonio JM, Lau HY, Gillis DA.* Balloon catheter dilatation for hypertrophic pyloric stenosis. J Pediatr Surg 1990; 25(11): 1119–21.
34. *Braegger CP, Schwöbel M, Känel J, Werner ER, Thöny B, Blau N.* Tetrahydrobiopterin in the treatment of infantile hypertrophic pyloric stenosis. Biochem Mol Med 1997, 62(1): 101–5.

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Subgingival air-polishing treatment in patients with aggressive periodontitis

Tretman subgingivalnog peskarenja kod obolelih od agresivne parodontopatije

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Abstract

Background/Aim. Periodontal tissue maintenance therapy is an important phase of the overall periodontal disease therapy. This paper aims to determine subgingival air-polishing efficacy with glycine powder in putative paropathogens reduction, plaque index, gingival bleeding index and probing depth. **Methods.** The study included 44 patients with aggressive periodontitis of both sexes, aged between 21 and 50, divided into two groups. Subgingival air-polishing was applied in the first group and sonic scaling in the control (second) group. Biofilm samples were taken from 5 deepest periodontal pockets before the therapy and 3 and 15 months after it. Paropathogens *Aggreggatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsithya*, *Prevotella intermedia*, *Treponema denticola* were detected by PCR analysis. **Results.** Paropathogens values were decreased after applied treatments. There was a statistically significant reduction in the mean value of full-mouth plaque (FMPS), from 43.00 to 14.90 (first group) and from 44.71 to 15.54 (second group), full-mouth bleeding score (FMBS) from 42.55 to 13.85 (first group) and from 43.04 to 15.17 (second group), as well as in probing depth from 3.40 to 2.64 (first group) and from 3.85 to 2.91 (second group), three months after the therapy. **Conclusion.** Subgingival air-polishing successfully reduces putative paropathogens and clinical parameters three months after the treatment.

Key words:
periodontal diseases; periodontal pocket; glycine; therapeutics; bacteria; polymerase chain reaction.

Apstrakt

Uvod/Cilj. Terapija održavanja zdravlja potpornog tkiva zuba predstavlja važnu fazu terapije u celokupnom tretmanu obolelog parodoncijuma. Cilj rada bio je utvrditi efikasnost subgingivalnog peskarenja glicinskim prahom na smanjenje verovatnih paropatogena, kliničkih parametara plak indeksa, indeksa krvarenja gingive kao i dubine parodontalnog džepa. **Metode**. U istraživanju su učestvovala 44 bolesnika sa dijagnostikovanom agresivnom parodontopatijom, oba pola, uzrasta od 21 do 50 godina, podeljena u dve grupe. Prvoj grupi je rađeno subgingivalno peskarenje površine korena zuba glicinskim prahom, a kontrolnoj (druga grupa) je rađena klasična sonična obrada površine korena zuba. Uzorci biofilma uzeti su iz 5 najdubljih parodontalnih džepova pre terapije, tri i 15 meseci nakon terapije. Paropatogeni *Aggreggatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsithya, Prevotella intermedia, Treponema denticola* detektovani su PCR analizom. **Rezultati.** Vrednosti traženih paropatogena su opale posle primenjenih tretmana. Došlo je do statistički značajnog smanjenja srednjih vrednosti FMPS (*full-mouth plaque score*), sa 43.00 na 14.90 (prva grupa) i sa 44.71 na 15.54 (druga grupa), FMBS (*full-mouth bleeding score*) sa 42.55 na 13.85 (prva grupa) i sa 43.04 na 15.17 (druga grupa), kao i dubine sondiranja sa 3.40 na 2.64 (prva grupa) i sa 3.85 na 2.91 (druga grupa), tri meseca posle terapije. **Zaključak.** Subgingivalno peskarenja uspešno dovodi do smanjenja paropatogena i kliničkih parametara tri meseca nakon terapije.

Ključne reči:
periodontalne bolesti; periodontalni džep; glicin; lečenje; bakterije; polimeraza, reakcija stvaranja lanaca.

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Introduction

Periodontal diseases include chronic inflammatory-destructive changes in the periodontal tissue. The disease arises as a result of the response of host defense factors to the presence of bacteria in the dentogingival junction [1]. The periodontal disease, in the form of generalized aggressive periodontitis, is the most severe of all forms of the supporting tissue disease of the teeth. However, it does not happen often. Periodontitis is one of the leading factors of tooth loss in adult population [2]. Numerous factors are involved in the onset of this disease: microorganisms of dental biofilm, poor oral hygiene, bad habits, etc. Familial predisposition is prominent in this case, therefore, this form of periodontal disease is considered genetic [3, 4]. According to some authors, this form of periodontal disease is a consequence of a reduced immune response to dental biofilm antigen. Persons suffering from aggressive periodontitis have disorders of the function of neutrophil granulocytes and monocytes [5]. It is believed that the greatest potential in causing periodontal diseases is found in the following bacteria: *Aggregatibacter actinomycetemcomitans* (*Aa*), *Porphyromonas gingivalis* (*Pg*), *Prevotella intermedia* (*Pi*), *Tannerella forsythia* (*Tf*), *Treponema denticola* (*Td*), as confirmed by numerous studies [6–10]. These bacteria are designated as putative periodontal pathogens [11–13]. Since aggressive periodontitis is characterized by a disrupted function of defense of the organism from pathogenic microorganisms and other factors, this disease is very unpredictable, both by its clinical progression and its therapeutic response [14, 15]. Contemporary trends in dentistry require a quick, efficient and atraumatic approach to treating the disease. Minimally invasive procedures are used in the treatment of periodontal diseases as well. In this regard, in the treatment of periodontal pockets, researchers try to remove dental biofilm with its contents, which is the main cause of periodontal disease [16]. The removal of biofilm consequently leads to the reduction and withdrawal of inflammation from the soft wall of the periodontal pocket without damaging the surrounding periodontal tissue [17]. The minimal invasive procedure in processing periodontal pockets has replaced the previously used procedure of scaling and root planing (SRP) of the surface of the root, as Ower [18] wrote about it. Subgingival air-polishing using glycine powder on periodontal pockets (Full Mouth PerioFlow – FMPF) is one form of a minimally invasive procedure of treating periodontal pockets, as it is effective in the removal of biofilms from natural tooth structure [19], and at the same time, it does not damage both soft and hard periodontal tissue [20–22]. Flemmig et al. [23] also states there is no damage to soft tissue when subgingival air-polishing with glycine powder is used. This non-surgical, therapeutic approach includes treating the whole mouth as quickly as possible, which differs from the previous treatment that entailed working on periodontal pockets over a longer period of time [24]. FMPF involves the removal of biofilm, and other sediments from the root surface using compressed air, water and powder with varying types of abrasivity [19, 21]. Sodium bicarbonate has been used for air-polishing, which has effectively removed the biofilm. However, sodium bicarbonate particles were up to four times the particle size of glycine powder. Particles of sodium bicarbonate, formed in such a manner, left even larger abrasive traces on the root surface and caused greater damage to the soft tissue [25]. While glycine powder particles are 45–60 microns in size, sodium bicarbonate particles are up to 250 microns in size. In addition, the usage of the periodontal pockets polishing (PFLOW) method, including a mixture of glycine powder, causes considerably smaller damage to the soft tissue in comparison to the previous technologies [26, 27]. Furthermore, air-polishing, in comparison to other techniques of treatment of periodontal pockets, has the advantage of preserving the cement of the root of the tooth [20]. The new PFLOW technology uses a specially designed nozzle with three outlets, one for air, one for powder, and one for water. This improves the mixing of water and air with powder, the extraction of the powder from the depth of the periodontal pocket while preventing, at the same time, emphysema of the periodontal pocket soft tissue. Designed in such a manner, the nozzle allows access to the periodontal pocket with the depth of up to 5 mm. The new subgingival air-polishing method increases the efficiency of work and provides better comfort to the patient [28].

The goal of this paper was to determine the effect of subgingival glycine air-polishing on the subgingival periodontal pathogens during maintenance therapy. Moreover, it was intended to estimate the clinical parameters of periodontal tissue, like plaque index, gingival bleeding index and probing depth.

Methods

The maintenance therapy results were recorded 3 and 15 months after applying the first treatment. The study included 44 patients suffering from aggressive periodontitis, both genders of different ages, 27 were non-smokers, and 17 were smokers.

The patients were selected according to the criteria defined for diagnosing aggressive periodontitis [29].

Inclusion criteria were as follows: clinical and radiological features of generalized aggressive periodontitis; patients with good general health; at least 20 teeth present.

Exclusion criteria were as follows: clinical and radiological features of localized aggressive periodontitis; presence of some kind of systemic disease; pregnancy or lactation; scaling and root planing within three months of examination; periodontal surgery and/or antibiotic therapy within six months of examination.

The study was approved by the Research Board of the Medical Faculty, University of Banja Luka. The research has been conducted in full accordance with the ethical principle of the Declaration of Helsinki. All patients involved in the study had signed a letter of approval confirming they would take part in the study of their own will. Upon receiving their consents, thorough medical and dental histories were taken from the subjects. Dental exams and dental radiographs

analysis were done for each patient 15 months after the basic therapy. Two co-authors participated in the patient selection, as well as in the basic therapy, besides the main researcher, the author of the study (SM, TN). All clinical assessments, radiographic analysis, sampling for microbiological and laboratory tests were performed by one person for the uniformity of the results.

The samples of subgingival biofilm, required for microbiological analysis, were taken from the five deepest periodontal pockets with sterile paper points (#30, Absorbent paper points, Taper 0.2 VDW GmbH, Germany) for each patient separately. The selected area was well isolated from the saliva with cotton rolls and dried thoroughly. Samples of subgingival biofilm were taken by using five sterile paper points, with one paper point placed in each of the periodontal pockets. Ninety seconds later, the paper points were gently taken out from the pockets and placed in a sterile 1.5 mL Eppendorf tube and transported the same day to the laboratory that used the micro-IDent test (Hain Lifescience GmbH, Nehren, Germany) for the blind analysis. The necessary DNA extraction from periodontal pathogens was done between 24 and 48 hours from the moment of taking the sample. In case the analysis could not be done within this timeframe, the samples were frozen at -20° C and the analysis was carried out within seven days of the sampling time.

PCR analysis was applied for analyzing DNA from periodontal pockets, according to the manufacturer's instructions. The microbiological assessment of the samples consisted of isolating the DNA from the sample, followed by the PCR process, and concluded by the identification of PCR products (amplicons) by reverse hybridization.

Sampling and monitoring of the results was done at baseline and 3 and 15 months after the initial therapy. The initial division of the patients was followed, which means that during the maintenance therapy, one group underwent the subgingival air-polishing, while the second group had the classic sonic treatment of periodontal pockets. Samples of subgingival biofilm were taken from the same periodontal pockets. Taking samples from periodontal pockets was done before determining clinical parameters in order to avoid biofilm destruction by periodontal probing.

To assess the state of oral hygiene and gingivitis the following indexes were used: Full Mouth Plaque Score (FMPS) and Full Mouth Bleeding Score (FMBS) [30].

The FMPS is determined by examining four surfaces of the tooth (vestibular, oral, mesial and distal) and recording a positive or negative value for each surface of the tooth, depending on whether the dental biofilm was present or absent. All present teeth were examined, except for third molars. The presence (+) or the absence (-) of the dental biofilm is recorded in the dental record, and the total value of dental biofilm is expressed in percentages:

FMPS = Number of dental surfaces with biofilm ×100/number of examined dental surfaces.

Therefore, the FMPS is the percentage of teeth surface with plaque accumulation that was evaluated using a periodontal probe.

The FMBS is determined by probing the gingiva associated with four teeth surfaces (vestibular, oral, mesial and distal) and recording a positive or negative value for each surface of the tooth, depending on whether the gingiva bleeding was present 30 seconds after probing or not. The gingiva is probed with all teeth present, except for third molars. The presence (+) or the absence (-) of bleeding is recorded in the dental record, and the total value of this index is expressed in percentages:

FMBS = Number of dental surfaces where gingiva bleeding is present x100/ number of examined dental surfaces.

The FMBS is the percentage of teeth surface with bleeding upon.

To assess the condition of deeper structures of periodontal tissues, probing depth (PD) was measured with a periodontal probe. These measurements were made using the periodontal probe PCP-UNC 15®, Hu-Friedy, Chicago, IL, USA.

Clinical work in patients' mouths was performed in the following way:

During the first checkup and upon the completion of examination and sample taking, supragingival calculus was removed in all patients, supragingival air-polishing was done and subgingival concrements were removed as well. After this, the patients were divided into two groups.

Subgingival air polishing of root surfaces was performed on the first (test) group of patients, using a glycine amino acid in the form of 45–60 micron powder granules (EMS Air-Flow Master). Each root surface area was air-polished for 4 or 5 seconds. The nozzle for subgingival air-polishing is presented in Figure 1.

Fig.1 – Specially designed nozzle for subgingival air-polishing.

Sonic SRP of the root surfaces was performed on the second (control) group of patients. The procedure was done with a sonic device and periodontal nozzles (Figure 2).

Fig. 2 – Periodontal nozzles for sonic devices.

This systemic treatment of all periodontal pockets was performed in the following four appointments. The patients took care of their oral hygiene in the home environment according to our instructions. An ultra-soft manual toothbrush was used for oral hygiene.

The patients rinsed their oral cavities with 0.12% chlorhexidine digluconate (Curasept ADS 212, Curaden, Kriens, Switzerland) twice a day during the course of the therapy and continued to do so for another 10 days. Upon the completion of the fourth appointment and within 24 hours upon the finished treatment of periodontal pockets, the patients in both groups were prescribed antibiotic therapy, depending on the results of the microbiological analysis. In the tested group, there were no subjects allergic to penicillin or metronidazole. Therefore, the antimicrobial therapy consisted of amoxicillin 500 mg capsules and metronidazole 400 mg tablets or a combination of the two medicines. The subjects took the medication or medications three times a day for 7 days [31–33].

Follow-up checkups were performed 6 to 8 weeks after the first appointment and periodontal tissues were re-assessed (FMPS, FMBS, and PD were measured again). The second stage came three months after the start of the therapy. All the aforementioned measurements were repeated, as was the laboratory PCR analysis, which examined the possible presence of periodontal pathogens. On the basis of the obtained results, it was assessed whether or not a further therapeutic procedure is necessary and what kind.

A re-evaluation of the required parameters was performed 15 months after the first appointment to examine the effect of maintenance therapy. All parameters from the first visit were re-determined, and PCR analysis was also performed. Removal of any solid deposits was carried out using Gracy's curettes.

Statistical analysis

IBM SPSS Statistics 19.0: MS Office Word 2010 was the software used for the statistical analysis and tables.

The Wilcoxon sign test is a statistical comparison of the average of two dependent samples. The Wilcoxon sign test works with metric (interval or ratio) data that is not multivariate normal or with ranked/ordinal data. Generally, it is the non-parametric alternative to the dependent samples *t*-test. The Wilcoxon sign test tests the null hypothesis that the average signed-rank of two dependent samples is zero.

Mann-Whitney *U* test is used to compare two independent groups. This test is equivalent to a *t*-test of independent samples, however, this test does not assume a normal distribution of the analyzed data. This test is used when the analyzed data are not in normal distribution and it is inappropriate to use the *t*-test on independent samples.

Fisher's exact test is used when the sample size is too low to use chi-square (χ^2) test.

The χ^2 test serves to determine whether some of the obtained (observed) frequencies deviate from the frequencies expected under a particular hypothesis. This test also requires the correlation of two variables and it shows the probability of the variables' correlation.

Statistically significant values are assumed values of $p < 0.05$.

Results

Forty-four patients (24 females, 20 males; 27 non-smokers and 17 smokers) suffering from aggressive periodontitis were included in the study. Twenty and 24 of them were treated by the PFLOW and SRP, respectively.

PCR analysis demonstrates the reduction in the presence of the baseline bacteria observed through the monitoring period of 3 and 15 months, in relation to the number and percentage of patients examined (Table 1).

After the PFLOW therapeutic procedure, the percentage of patients with very elevated *Aa* decreased from 30% to 5% (3 months) and 5% (15 months). The percentage of patients in whom this bacteria was not detected increased from 45% to 80% and 80%, respectively.

After the SRP therapeutic procedure, the percentage of patients with very elevated *Aa* dropped from 29.2% to 8.3% and 8.3%, respectively. The percentage of patients in whom this bacteria was not detected increased from 50% to 87.5% and 87.5%, respectively.

The PFLOW treatment, applied at the beginning of the therapy, and 3 and 15 months after the maintenance therapy was performed, led to a reduction of the percentage of highly elevated *Pg* with the initial value of 65% to 5% and 5%, respectively. The percentage of patients who did not have *Pg* bacteria detected increased from the initial 10% to 50% and 45%, respectively. During the SRP therapeutic procedures, at the beginning of the treatment, 58.3% of patients had a very high *Pg* value of bacteria, and after 3 and 15 months of maintenance therapy, none of the patients had a very high value of this bacteria. The percentage of patients without detected bacterium *Pg* from the initial 16.7% increased to 54.2%, 3 months later and to 58.3%, 15 months later.

After the PFLOW therapeutic procedure, the percentage of patients with very high *Pi* bacteria decreased from the initial 15% to 0%, both 3 and 15 months after the performed maintenance therapy. In 45% of patients, *Pi* bacteria were

Table 1

Microbiological data expressed by the number and percentage of patients

Bacteria	Treatment		Checkup					
			T0		T3		T15	
			n	%	n	%	n	%
Aa	PFLOW	Not detected	9	45	16*	80	16*	80
		Slightly elevated	5	25	2	19	2	10
		Elevated	0	0	1	5	1	5
		Highly elevated	6	30	1*	5	1*	5
	SRP	Not detected	12	50	21*	87.5	21*	87.5
		Slightly elevated	5	20.8	1	4.2	1	4.2
		Elevated	0	0	0	0	0	0
		Highly elevated	7	29.2	2	8.3	2	8.3
Pg	PFLOW	Not detected	2	10	10*	50	9*	45
		Slightly elevated	2	10	9*	45	9*	45
		Elevated	3	15	0	0	1	5
		Highly elevated	13	65	1*	5	1*	5
	SRP	Not detected	4	16.7	13*	54.2	14*	58.3
		Slightly elevated	2	8.3	9*	37.5	9*	37.5
		Elevated	4	16.7	2	8.3	1	4.2
		Highly elevated	14	58.3	0*	0	0*	0
Pi	PFLOW	Not detected	9	45	18*	90	16*	80
		Slightly elevated	5	25	1	5	0*	0
		Elevated	3	15	1	5	0	0
		Highly elevated	3	15	0	0	0	0
	SRP	Not detected	10	41.7	20*	83.3	22*	91.7
		Slightly elevated	2	8.3	4	16.7	1	4.2
		Elevated	8	33.3	0*	0	0*	0
		Highly elevated	4	16.7	0*	0	0*	0
Tf	PFLOW	Not detected	0	0	10*	50	12*	60
		Slightly elevated	1	5	4	20	4	20
		Elevated	7	35	5	25	4	20
		Highly elevated	12	60	1*	5	0*	0
	SRP	Not detected	0	0	17*	70.8	15*	62.5
		Slightly elevated	3	12.5	5	20.8	5	20.8
		Elevated	5	20.8	2	8.3	3	12.5
		Highly elevated	16	66.7	0*	0	1*	4.2
Td	PFLOW	Not detected	2	10	10*	50	13*	65
		Slightly elevated	4	20	7	35	5	25
		Elevated	8	40	3	15	2*	10
		Highly elevated	6	30	0*	0	0*	0
	SRP	Not detected	1	4.2	16*	66.7	13*	54.2
		Slightly elevated	7	29.2	6	25	8	33.3
		Elevated	11	45.8	2*	8.3	3*	12.5
		Highly elevated	5	20.8	0*	0	0*	0

***Aa – Aggregaticacter actinomycetemcomitans*; *Pg – Porphyromonas gingivalis*; *Pi-Prevotella intermedia*; *Tf – Tannerella forsithya*; *Td – Treponema denticola*; PFLOW – Subgingival Air-polishing; SRP – Scaling and Root Planing; T0 – baseline (no treatment); T3 – 3 months after tretament; T15 – 15 months after treatment; n (%) – number (percentage) of patients.**

***statistical significance was present 3 and 15 months after PFLOW and SRP therapy compared with the baseline values ($p < 0.05$).**

not detected before therapy, while 3 months after therapy, the percentage of patients without these bacteria increased to 90%, and 15 months after initial therapy, the percentage was 80%.

Very elevated *Pi* bacteria were recorded in 16.7% of patients. After the SRP treatment (3 and 15 months duration), this value was 0%. The percentage of patients that did not have *Pi* bacteria was 41.7%, and after the maintenance therapy, this value was 83.3% (3 months) and 91.7% (15 months).

After the PFLOW treatment, the percentage of patients with highly elevated levels of *Tf* dropped from 60% to 5% after 3 months, and after 15 months, no patients with very high values of these bacteria were recorded. The percentage of patients without detected *Tf* bacteria increased from the initial 0% to 50% after 3 months and to 60% after 15 months.

After the SRP therapeutic procedure, the percentage of patients with very high *Tf* bacteria values from the initial 66.7% decreased to 0% after 3 months and to 4.2% after 15 months. The percentage of patients without detected *Tf* bacteria increased from the initial 0% to 70.8% after 3 months and to 62.5% after 15 months.

After the PFLOW treatment, the percentage of patients with a very elevated value of *Td* bacteria value from the initial 30% dropped to 0% after 3 months and to 0% after 15 months. The percentage of patients without detected *Td* bacteria from the initial 10% increased to 50% after 3 months and to 65% after 15 months.

The SRP treatment led to a reduction in the highly elevated *Td* bacteria from the initial 20.8% to 0% after 3 months and to 0% after 15 months. The percentage of patients without *Td* bacteria at the beginning of the treatment was 4.2%, 3 months after SRP treatment, this value was 66.7% and after 15 months 54.2%.

Full mouth plaque score

In the group of patients that underwent the PFLOW therapy, there was a statistically significant ($p < 0.05$) enhancement of the FMPS index value 3 months after the performed therapy.

However, 15 months after PFLOW therapy, FMPS index values were not statistically nor significantly different ($p > 0.05$) than the initial state (Table 2).

In the group of patients that underwent the SRP therapy, there was a statistically significant ($p < 0.05$) improvement in the FMPS index value 3 months after the therapy compared to the initial value. However, 15 months after the initial therapy, the FMPS index value was not statistically different ($p > 0.05$) compared to the initial value (Table 2).

Full mouth bleeding score

In the group of patients that underwent the PFLOW therapy, there was statistically significant ($p < 0.05$) improvement in the FMBS index value 3 months after the performed therapy. However, 15 months after PFLOW therapy, the values of the FMBS index were not significantly different ($p > 0.05$) compared to the initial state (Table 3).

In the group of patients that underwent the SRP therapeutic method, there was a statistically significant ($p < 0.05$) improvement in the FMBS index value 3 months

Table 2

Clinical data for FMPS

Parameters	PFLOW				SRP			
	n	mean ± SD	min.	max.	n	mean ± SD	min.	max.
FMPS T0	20	43.00 ± 24.66	12.00	81.00	24	44.71 ± 25.03	11.00	88.00
FMPS T3	20	14.90 ± 7.50	4.00	30.00	24	15.54 ± 7.33	2.00	30.00
FMPS T15	20	42.35 ± 24.68	12.00	88.00	24	45.25 ± 24.96	11.00	87.00

FMPS – Full Mouth Plaque Score; PFLOW – Subgingival Air-polishing; SRP – Scaling and Root Planing; T0 – baseline (no treatment); T3 – 3 months after treatment; T15 – 15 months after treatment; n – number of patients; min – minimum; max – maximum; SD – standard deviation.
***p* < 0.05, 3 months after PFLOW and SRP therapy.**

Table 3

Clinical data for FMBS

Parameters	PFLOW				SRP			
	n	mean ± SD	min.	max.	n	mean ± SD	min.	max.
FMBS T0	20	43.00 ± 24.66	12.00	81.00	24	44.71 ± 25.03	11.00	88.00
FMBS T3	20	13.85 ± 8.20	1.00	29.00	24	15.17 ± 9.28	2.00	37.00
FMBS T15	20	42.55 ± 22.14	9.00	84.00	24	43.04±19.36	9.00	75.00

FMBS – Full Mouth Bleeding Score; PFLOW - Subgingival Air-polishing; SRP – Scaling and Root Planing; T0 – baseline (no treatment); T3 – three months after treatment; T15 – 15 months after treatment; n – number of patients; min – minimum; max – maximum; SD – standard deviation.
***p* < 0.05, 3 months after PFLOW and SRP therapy.**

after the therapy compared to the initial value. However, 15 months after the initial therapy, the FMBS index value was not statistically different ($p > 0.05$) compared to the initial value (Table 3).

Probing depth

In the group of patients that underwent the PFLOW therapy, there was a statistically significant ($p < 0.05$) reduction in the probing depth 3 months after the initial value. However, 15 months after PFLOW therapy, the probing depth was not statistically significantly different ($p > 0.05$) compared to the initial state (Table 4).

In the group of patients that underwent the SRP therapeutic method, there was a statistically significant ($p < 0.05$) decrease in the depth of the pocket 3 months after the therapy compared to the initial value. However, 15 months after initial therapy, the probing depth value was not significantly different ($p > 0.05$) compared to the initial values (Table 4).

Correlation in the PFLOW therapy group was found between *Aa* bacteria and some clinical parameters. In the group of patients undergoing PFLOW therapy, there was a statistically significant ($p < 0.05$) medium-strong positive correlation, before the therapy itself (T0), between the bacteria *Aa* and the probing depth. A higher percentage of *Aa* bacteria was present in deeper periodontal pockets. Three months after PFLOW therapy (T3), there was a mean strong positive statistically significant correlation ($p < 0.05$) between the bacteria *Aa* and the PD. The correlation positivity shows that a higher percentage of *Aa* bacteria is present in deeper periodontal pockets. Statistically significant ($p < 0.05$) medium-strong positive correlation existed between *Aa* and FMBS parameter after 15 months (T15). The correlation positivity indicates that higher FMBS parameters increase the frequency of *Aa* bacteria (Table 5).

Discussion

When certain types of bacteria were tested, subgingival air-polishing of periodontal pockets accompanied by antimicrobial therapy showed equally efficient results as the sonic scaling of periodontal pockets. *Aggregatibacter actinomycetemcomitans* is frequently present in subgingival biofilm in patients suffering from generalized aggressive periodontitis, which is also confirmed by studies carried out globally [6, 10, 34]. *Aa* was found in slightly more than half (52.27%) of the patients in the tested group. The data from other studies state a similar prevalence of *Aa* in subjects suffering from aggressive periodontitis [7, 13, 35]. Taking into account the results received in this but also in some other studies, it can be noted that patients suffering from aggressive periodontitis have a lower percentage of *Aa*. A positive effect of the applied method is also proved by data that *Aa* was found in only 15.9% of the patients at the end of the study.

A high percentage of *Porphyromonas gingivalis* was detected during the study (86.36%). Among the tested periodontal pathogens in the studies of aggressive periodontitis, *Pg* was present in the very high percentage [13], which indicates a significant role of this periodontal pathogen in the pathogenesis of aggressive periodontitis. In some studies conducted in India, *Pg* was detected only in persons with advanced periodontitis, unlike the persons with healthy periodontal tissue, indicating a link between *Pg* and a disease of periodontal tissue [36]. High prevalence of this bacterium in subgingival biofilm in persons with generalized aggressive periodontitis indicates its connection with this

Table 4

Clinical data for PD

Parameters	PFLOW				SRP			
	n	mean ± SD	min.	max.	n	mean ± SD	min.	max.
PD T0	20	3.40±0.73	2.4	5.03	24	3.85±0.77	2.85	5.87
PD T3	20	2.64±0.41	2.09	3.44	24	2.91±0.61	2.19	4.79
PD T15	20	3.57±0.81	2.4	5.87	24	3.71±0.76	2.44	5.44

PD – periodontal depth; PFLOW – Subgingival Air-polishing; SRP – Scaling and Root Planing; T0 – baseline (no treatment); T3 – 3 months after treatment; T15 – 15 months after treatment; n – number of patients; min – minimum; max –maximum; SD – standard deviation.
***p* < 0.05, 3 months after PFLOW and SRP therapy.**

Table 5

Influence of PFLOW treatment on correlation between *Aggregatibacter actinomycetemcomitans* and clinical parameters in patients (n = 20) with aggressive periodontitis

Clinical parameters	T0		T3		T15	
	r	*p*	r	*p*	r	*p*
FMPS	0.170	0.475	0.127	0.593	0.180	0.448
FMBS	0.145	0.542	0.024	0.920	0.451	0.046
PD	0.471	0.036	0.474	0.035	0.085	0.721

PFLOW – Subgingival Air-polishing; T0 – baseline (no treatment); T3 – 3 months after treatment; T15 – 15 months after treatment; FMPS – Full Mouth Plaque Score; FMBS – Full Mouth Bleeding Score; PD – Periodontal Depth; r – coefficient of correlation; *p* – significance.

disease. The data from a study that used PCR methodology confirmed that *Pg* was present only in the regions with the disease of periodontal tissue, unlike the healthy regions where *Pg* was not identified [13, 37]. This bacterium was found in over 60% of subjects with aggressive periodontitis with pocket depth up to 5 mm and over 90% of subjects with pocket depth more than 5 mm [38]. This indicates that bacteria *Pg* is significantly correlated with aggressive periodontitis. The grater the destruction of periodontal tissue, the greater the percentage of bacteria *Pg*. Other studies, too, record a high prevalence of this putative periodontal pathogen among the subjects suffering from aggressive periodontitis, bringing it in connection with periodontal disease [7, 9]. A positive effect of applied therapies is also confirmed by the fact that this periodontal pathogen was found in 47.72% of the patients at the end of the therapy.

The prevalence of *Prevotella intermedia* was 56.81% in this study. In comparison with healthy regions of periodontal tissue, the microbiological profile of subgingival biofilm of the regions with aggressive periodontitis contained a significant amount of *Pi*, which confirms that *Pi* is a significant etiological factor in the occurrence of aggressive periodontitis [37]. Studies conducted in Japan point out that the prevalence of *Pi* was similar to the one registered in this study [39]. PCR analysis of subgingival biofilm in Chinese patients confirmed that *Pi* was a dominant periodontal pathogen in persons suffering from aggressive periodontitis [40]. A study conducted in Lebanon also confirmed that this was one of the possible causes of periodontal tissue disease. In the patients suffering from periodontitis, *Pi* was also present in subgingival biofilm samples, making it an integral part of the microbiological profile of patients suffering from aggressive periodontitis [11]. Studies conducted in Marocco indicated a higher prevalence of this periodontal pathogen in the subgingival biofilm of a diseased periodontium [7]. The data shows that the percentage of *Pi* detected at the end of therapy was 13.63%, which proves that the applied method had a positive effect.

The findings of this study confirm the role of *Tannerella forsythia* in the pathogenesis of periodontitis. Of all the five putative periodontal pathogens tested during this study, this was the only bacterium that was present in all patients of the study. Identical results were obtained in a study carried out among the Bulgarian patients suffering from aggressive periodontitis [13]. The periodontal pathogens were found to exist in high percentages in Maroccan patients as well, where the bacterium was dominant [7]. Persistent bleeding on probing, an indicator of an infection, encouraged numerous researchers to repeat their studies, which then confirmed the presence of *Tf* in those regions [41]. Studies conducted worldwide point out that *Tf*, as a resident of subgingival biofilm in persons suffering from aggressive periodontitis, was present in high percentages [6–8]. After therapy, this periodontal pathogen was present in only 38.63% of the patients, indicating a positive effect of the applied therapy.

Treponema denticola is also considered one of the possible causes of periodontal tissue destruction occurring in aggressive periodontitis, detected in 93.18% of the patients. Recent studies have shown the prevalence of *Td* in more than 80% of the patients and that is significantly associated with the severity of periodontal tissue destruction. High percentages of *Td* were found especially in the regions where periodontal pockets were deeper than 4 mm [36]. High percentages of *Td* in the subgingival biofilm were registered among the residents of different geographical areas, which was confirmed by numerous studies [6, 8, 9, 13]. The efficacy of the applied therapy is evident from the *Td* finding, the percentage being 40.90% at the end of therapy.

The efficacy of individual therapeutic procedures applied in this study was examined with the FMPS and FMBS indexes and monitoring of PD. Both therapeutic procedures, the PFLOW and the SRP, applied in the patients, produce an equally statistically significant reduction in the FMPS in both groups, from 43.00 to 14.90, (first group) and from 44.71 to 15.54 (second group) and FMBS, also in both groups, from 42.55 to 13.85 (first group) and from 43.04 to 15.17 (second group), three months after the treatment. These results match the results obtained in the studies carried out in China and some other parts of the world [9, 21, 31]. There are various mechanical control techniques of biofilm accumulation that consequently lead to a reduction in the signs of inflammation in periodontal soft tissue [41].

The results obtained in this study indicate that the new therapeutic procedure PFLOW successfully stopped further destruction of periodontal tissue by monitoring PD (probing depth), with the initial value of 3.40 dropping to 2.64 (first group) and from 3.85 to 2.91 in the second group, three months after therapy. Other studies also indicate that all of the applied techniques successfully reduce the levels of inflammation and stop further damage to the periodontal tissue [9, 42]. Monitoring the parameters of gingival inflammation and signs of periodontal destruction through PD, some other studies also find that PFLOW can be considered successful in removing the subgingival biofilm and therefore stop the further destruction of periodontal tissue [8, 27, 31]. Since both the SRP and PFLOW have similar clinical achievements, especially in maintaining healthy periodontal tissue, precedence can be given to the PFLOW as the patient accepts it more easily and the dentist spends less time working on it.

Fifteen months after therapy, the FMPS, FMBS and PD parameters showed statistical equality compared to the values recorded at the beginning of the study, indicating a drop in patient motivation and irregular oral hygiene maintenance. It is very important to emphasize to patients the role of biofilm in the occurrence and development of periodontal tissue destruction during every control checkup. Regular removal of biofilm reduces and eliminates the agglomeration of probable periodontal bacteria that have a large share in the occurrence of periodontitis. By constant motivation and education in

maintaining oral hygiene, patients must become aware of their own roles in maintaining the health of the periodontal tissue.

Conclusion

During the maintenance therapy of the health of the tooth-supporting tissue, there was no growth and proliferation of the required periodontal pathogens, after both 3 and 15 months. The new therapeutic procedure in the scope of minimally invasive treatment of periodontal tissue proved to be equally successful in the eradication of the studied putative periodontal pathogens, as well as in the reduction of the monitored clinical parameters such as plaque index, gingival bleeding index and PD, as the previous sonic SRP therapy.

The regular subgingival air-polishing and the regular re-motivation of patients for maintaining oral hygiene in home conditions in the first three months did not lead to an increase in the value of the clinical parameters monitored. However, a control examination after 15 months showed that there was no improvement in clinical parameters indicating that the motivation of patients undergoing the maintenance therapy of the health of the teeth supporting tissue had dropped. Based on this, it can be seen that the subgingival air-polishing of the surface of the root of the tooth has successfully led to the reduction of the tracked microbiological parameters, and it can be recommended to be used during the maintenance therapy. It is especially acceptable to be used in patients with a compromised periodontal status who have fixed prosthetics in order to prolong the duration of such prosthetic work. It is necessary to carry out more frequent controls with these patients so that the lack of motivation in maintaining oral hygiene is avoided.

The dentist is supposed to choose a mode of therapy concerning the feasibility of certain procedures, following the patients' needs.

R E F E R E N C E S

1. *Cekici A, Kantarci A, Hasturk H, Van Dyke TE.* Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontol 2000 2014; 64(1): 57–80.
2. *Tonetti MS, Jepsen S, Jin L, Otomo-Corgel J.* Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call forglobal action. J Clin Periodontol 2017; 44(5): 456–62.
3. *Vieira AR, Albandar JM.* Role of genetic factors in the pathogenesis of aggressive periodontitis. Periodontol 2000 2014; 65(1): 92–106.
4. *Rusyanti Y, Widyaputra S, Maskoen AA.* Periodontal tissue destruction in aggressive periodontitis: Determination of gene or environmental factors. Saudi Dent J 2019; 31(2): 290–9.
5. *Tapashetti RP, Sharma S, Patil SR, Guwa S.* Potential effect of neutrophil functional disorders on pathogenesis of aggressive periodontitis. J Contemp Dent Pract 2013; 14(3): 387–93.
6. *Könönen E, Müller HP.* Microbiology of aggressive periodontitis. Periodontol 2000 2014; 65(1): 46–78.
7. *Chahboun H, Arnau MM, Herrera D, Sanz M, Ennibi OK.* Bacterial profile of aggressive periodontitis in Morocco: a cross-sectional study. BMC Oral Health. 2015; 15: 25.
8. *Kargas K, Tsalikis L, Sakellari D, Menexes G, Konstantinidis A.* Pilot study on the clinical and microbiological effect of subgingival glycine powder air polishing using a cannula-like jet. Int J Dent Hyg 2015; 13(3): 161–9.
9. *Lu H, He L, Zhao Y, Meng H.* The effect of supragingival glycine air polishing on periodontitis during maintenece therapy: a randomized controlled trial. PeerJ 2018; 6: e4371.
10. *Lourenço TG1, Heller D, Silva-Boghossian CM, Cotton SL, Paster BJ, Colombo AP.* Microbial Signature Profiles of Periodontally Healthy and Diseased Patients. J Clin Periodontol 2014; 41(11): 1027–36.
11. *Al Yahfoufi Z.* Prevalence of Periodontal Destruction and Putative Periodontal Pathogens in the Same Lebanese Family. J Contemp Dent Pract 2017; 18(10): 970–6.
12. *Mombelli A, Almaghlouth A, Cionca N, Cancela J, Courvoisier DS, Giannopoulou C.* Microbiologic Response to Periodontal Therapy and Multivariable Prediction of Clinical Outcome. J Periodontol 2017; 88(12): 1253–62.
13. *Kotsilkov K, Popova C, Boyanova L, Setchanova L, Mitov I.* Comparison of culture method and real-time PCR for detection of putative periodontopathogenic bacteria in deep periodontal pockets. Biotechnol Biotechnol Equip 2015; 29(5): 996–1002.
14. *Teughels W, Dhondt R, Dekeyser C, Quirynen M.* Treatment of aggressive periodontitis. Periodontol 2000 2014; 65(1): 10733.
15. *Nibali V.* Aggressive Periodontitis: microbes and host response, who to blame? Virulence 2015; 6(3): 223–8.
16. *Sans M, Beighton D, Curtis MA, Cury JA, Dige I, Dommisch H,* et al. Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease. J Clin Periodontol 2017; 44(Suppl18): S5–S11.
17. *Larsen T, Fiehn NE.* Dental biofilm infections – an update. APMIS 2017; 125(4): 376–84.
18. *Ower P.* Minimally-invasive non-surgical periodontal therapy. Dent Update 2013; 40(4): 289–90, 293–5.
19. *Lu H, He L, Zhao Y, Meng H.* The effect of supragingival glycine air polishing on periodontitis during maintenance therapy: a randomized controlled trial. PeerJ 2018; 6: e4371.
20. *Bozbay E, Dominici F, Gokbuget AY, Cintan S, Guida L, Aydin MS,* et.al. Preservation of root cementum: a comparative evaluation of power-driven versus hand instruments. Int J Dent Hyg 2018; 16(2): 202–9.
21. *Simon CJ, Munivenkatappa Lakshmaiah Venkatesh P, Chickanna R.* Efficacy of glycine powder air polishing in comparison with sodium bicarbonate air polishing and ultrasonic scaling - a double-blind clinico-histopathologic study. Int J Dent Hyg 2015; 13(3): 177–83.
22. *Bühler J, Amato M, Weiger R, Walter C.* A systematic review on the effects of air polishing devices on oral tissues. Int J Dent Hyg 2016; 14(1): 15–28.
23. *Cobb CM, Daubert DM, Davis K, Deming J, Flemmig TF, Pattison A,* et al. Consensus Conference Findings on Supragingival and Subgingival Air Polishing. Compend Contin Educ Dent 2017; 38(2): e1–e4.
24. *Flemmig TF, Arushanov D, Daubert D, Rothen M, Mueller G, Leroux BG.* Randomized controlled trial assessing efficacy and safety of glycine powder air polishing in moderate-to-deep periodontal pockets. J Periodontol 2012; 83(4): 444–52.

25. *Bühler J, Schmidli F, Weiger R, Walter C.* Analysis of the effects of air polishing powders containing sodium bicarbonate and glycine on human teeth. Clin Oral Investig 2015; 19(4): 877–85.
26. *Graumann SJ, Sensat ML, Stoltenberg JL.* Air Polishing: A Review of Current Literature. J Dent Hyg 2013; 87(4): 173–80.
27. *Caygur A, Albaba MR, Berberoglu A, Yilmaz HG.* Efficacy of glycine powder air-polishing combined with scaling and root planing in the treatment of periodontitis and halitosis: A randomised clinical study. J Int Med Res 2017; 45(3): 1168–74.
28. *Sultan DA, Hill RG, Gillam DG.* Air-Polishing in Subgingival Root Debridement: A Critical Literature Review. J Dent Oral Biol 2017; 2(10): 1065.
29. *Albandar JM.* Aggressive periodontitis: case definition and diagnostic criteria. Periodontol 2000 2014; 65(1): 13–26.
30. *The British Society of Periodontology.* Periodontology in General Dental Practice in the United Kingdom. A policy statement. Liverpool: The British Society of Periodontology; 2001.
31. *Usin MM, Tabares SM, Menso J, deAlbera ER, Sembaj A.* Generalized aggressive periodontitis: microbiological composition and clinical parameters in non-surgical therapy. Acta Odontol Latinoam 2016; 29(3): 255–61.
32. *Pretzl B, Sälzer S, Ehmke B, Schlagenhauf U, Dannewitz B, Dommisch H,* et al. Administration of systemic antibiotics during non-surgical periodontal therapy-a consensus report. Clin Oral Investig 2019; 23(7): 3073–85.
33. *Keestra JA, Grosjean I, Coucke W, Quirynen M, Teughels W.* Non-surgical periodontal therapy with systemic antibiotics in patients with untreated aggressive periodontitis: a systematic review and meta-analysis. J Periodontal Res 2015; 50(6): 689–706.
34. *Liu J, Zhao J, Li C, Yu N, Zhang D, Pan Y.* Clinical and microbiological effect of nonsurgical periodontal therapy on patients with chronic and aggressive periodontitis. Quintessence Int 2013; 44(8): 575–83.
35. *Belibasakis GN, Schmidlin PR, Sahrmann P.* Molecular microbiological evaluation of subgingival biofilm sampling by paper points and curette. Acta Pathol Microbiol Immunol Scand 2014; 122(4): 347–52.
36. *Kumawat RM, Ganvir SM, Hazarey VK, Qureshi A, Purohit HJ.* Detection of *Porphyromonas gingivalis* and *Treponema denticola* in chronic and aggressive periodontitis patients: A comparative polymerase chain reaction study. Contemp Clin Dent 2016; 7(4): 481–6.
37. *Stingu CS, Jentsch H, Eick S, Schaumann R, Knöfler G, Rodloff A.* Microbial profile of patients with periodontitis compared with healthy subjects. Quintessence Int 2012; 43: 23–31.
38. *Kulkarni PG, Gosavi S, Haricharan PB, Malgikar S, Mudrakola DP, Turagam N,* et al. Molecular Detection of Porphyromonas gingivalis in Chronic Periodontitis Patients. J Contemp Dent Pract 2018; 19(8): 992–6.
39. *Tomita S, Kasai S, Ihara Y, Imamura K, Kita D, Ota K,* et al. Effects of systemic administration of sitafloxacin on subgingival microflora and antimicrobial susceptibility profile in acute periodontal lesions. Microb Pathog 2014; 71–72: 1–7.
40. *Feng X, Zhang L, Xu L, Meng H, Lu R, Chen Z,* et al. Detection of eight periodontal microorganisms and distribution of Porphyromonas gingivalis fimA genotypes in Chinese patients with aggressive periodontitis. J Periodontol. 2014; 85(1): 150–9.
41. *Tomita S, Komiya-Ito A, Imamura K, Kita D, Ota K, Takayama S,* et al. Prevalence of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Tannerella forsythia in Japanese patients with generalized chronic and aggressive periodontitis. Microb Pathog 2013; 61-62: 11–5.
42. *Jiao J, Zhang L, Meng HX, Shi D, Lu RF, Xu L,* et al. Clinical performance of non-surgical periodontal therapy in a large Chinese population with generalized aggressive periodontitis. J Clin Periodontol. 2018; 45(10): 1184–97.

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PRELIMINARY REPORT

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Reference charts of birth weight and birth length by gestational age in the southeast Serbian newborns – preliminary results

Neonatalne antropometrijske karte novorođenčadi u jugoistočnoj Srbiji – preliminarni rezultati

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Abstract

Background/Aim. To date, there has been no population-based neonatal anthropometric chart published in Serbia. Charts based on infants born in a single hospital (hospital-based) in the 1990s are still widely used in our country, as well as the Alexander chart. The aim of this study was to construct population-based centile, gender-specific charts for birth weight and length for singleton infants born in Southeast Serbia from 24 to 42 weeks of gestation and to compare them with other previously published charts. **Methods.** Data on 39,842 singleton live infants, delivered from 2006 to 2015 in three maternity wards in Southeast Serbia (Niš, Prokuplje, and Aleksinac), were analyzed. **Results.** The inclusion criteria met 37,169 newborns. Preterm births were relatively uncommon (5.25%). Estimated centile charts for male and female birth weights and lengths were constructed showing the 3rd, 10th, 25th, 50th, 75th, 90th, and 97th centiles. **Conclusion.** Our birth weight percentiles provide a population norm for singleton infants adjusted for gender, born in Southeast Serbia. These references are both of epidemiological and clinical use. There is a need for large-scale research that will include a larger number of preterm newborns which were represented in limited numbers in our study. There is also a need for setting up the gold standard method for the precise determination of the gestational age, i.e. the use of the early fetal ultrasound.

Key words:
infant; serbia; anthropometry; fetal development; reference values.

Apstrakt

Uvod/Cilj. U Srbiji do sada nije objavljena populaciona neonatalna antropometrijska karta. U svakodnevnom radu se još uvek široko koriste antropometrijske karte napravljene na osnovu merenja u jednom porodilištu u našoj zemlji ili karte stranih autora kao što je Alexander-ova karta. Cilj ove studije bio je izrada populacionih antropometrijskih karti za dužinu i masu na rođenju za novorođenčad rođenu od 24. do 42. nedelje gestacije iz jednoplodnih trudnoća u jugoistočnoj Srbiji i poređenje sa drugim ranije objavljenim kartama. **Metode.** Analizirani su podaci za 39 842 novorođenčadi rođenih iz jednoplodnih trudnoća u periodu od 2006. to 2016. godine u tri porodilišta u jugoistočnoj Srbiji (Niš, Prokuplje i Aleksinac). **Rezultati.** Kriterijume za ulazak u studiju je ispunilo 37 169 novorođenčadi. Prevremeno rođene dece bilo je 5,25%. Napravljeni su percentili za dužinu i masu na rođenju za mušku i žensku novorođenčad pokazujući 3., 10., 25., 50., 75., 90. i 97. percentil. **Zaključak.** Ovim istraživanjem dobijene su prve populacione antropometrijske, polno-specifične neonatalne karte za novorođenčad iz jugositočne Srbije. Dobijeni rezultati imaju klinički i epidemiološki značaj. Zbog ograničenog broja prevremeno rođenih beba postoji potreba za dodatnim istraživanjem na većem uzorku kako bi se preciznije odredili percentili za ovu grupu novorođenčadi. Takođe, potrebno je uspostaviti zlatni standard za precizno određivanje gestacijske dobi, tj. ranu upotrebu fetalnog ultrazvuka.

Ključne reči:
novorođenče; srbija; antropometrija; trudnoća, razvoj fetusa; referentne vrednosti.

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Introduction

Birth weight and length are quite sensitive indicators of children's health. Small-for-gestational-age (SGA) neonates have a long-term risk of short stature [1], neurocognitive impairment [2], metabolic disorders [3] , and cardiovascular diseases [4, 5]. Similarly, the large-for-gestational-age (LGA) are also at increased risk of short and long-term health problems [6, 7]. The values that identify infants at high and low risk cannot be clinically defined. Therefore, the adoption of statistical definitions instead of using clinical ones is advised [8]. By this, a neonate is defined as SGA when his or her weight and/or length is below the 10th, 5th, or 3rd centile of the neonatal chart, and LGA when his or her anthropometric values are above the 90th centile [9, 10].

These observations justify the use of neonatal charts. For more than fifty years, clinicians and investigators have proposed reference data for assessing birth weight and length for gestational age. Currently used neonatal charts are different regarding exclusion and inclusion criteria, instruments of measurement, methods of assessing gestational age and calculating centiles. There are several proposed characteristics that a reliable neonatal chart should have [8].

To date, there has been no population-based neonatal anthropometric chart published in Serbia. Charts based on infants born in a single hospital (hospital-based) in the 1990s [11] are still widely used in our country, as well as the Alexander chart [12].

Previously, comparative anthropometric data of Roma and non-Roma newborns, born between the 36th and 42nd gestational week, were published [13]. The aim of this study was to construct gender-specific charts for birth weight and length for singleton infants born in Southeast Serbia from 24 to 42 weeks of gestation and compare them with other previously published charts.

Methods

Data on 39,842 infants were analyzed. The study included all live singleton newborns delivered from 2006 to 2015 in three maternity wards in Southeast Serbia (Niš, Prokuplje, and Aleksinac).

Data were obtained from the computerized birth files of the National Institute of Health. The gestational age had been calculated in completed weeks based on the last menstrual period, and/or early date ultrasound, and/or neonatal examination. The weight was measured by a mechanical scale with 10 g precision. The length was measured using a non-stretch plastic tape from crown to heel.

Infants with major congenital anomalies and those with uncertain gestational age were excluded.

The LMS method was used to estimate the birth weight centimes. The L (Box-Cox power), M (median), and S (coefficient of variation) parameters were estimated [13]. This method uses smoothed values of L, M, and S to transform the observed distribution of birth weights and lengths to a standard normal distribution. This allows the calculation of centiles by using the appropriate SD score [14]. The scatter data plots and *Z* scores obtained from the LMS method were used to identify the outliers. Observations lying beyond ± 3 *Z* score were deleted.

Centiles were calculated using the LMS Chart Maker Light 2.54 version software, and the other analysis was carried out using SPSS, version 16.

Ethical approval to proceed without individual consent was given based upon the fact that this was retrospective anonymous clinical research.

Results

The inclusion criteria met 37,169 newborns. Tables 1 and 2 summarize the number of infants born in each gestational week and estimated values of L, M, and S by gender and gestational week. Preterm births were relatively uncommon (5.25%). Estimated centile charts for male and female birth weights (Figure 1) and lengths (Figure 2) were constructed showing the 3rd, 10th, 25th, 50th, 75th, 90th and 97th centiles. Female infants were lighter and shorter than the male infants, especially from 36 weeks onwards.

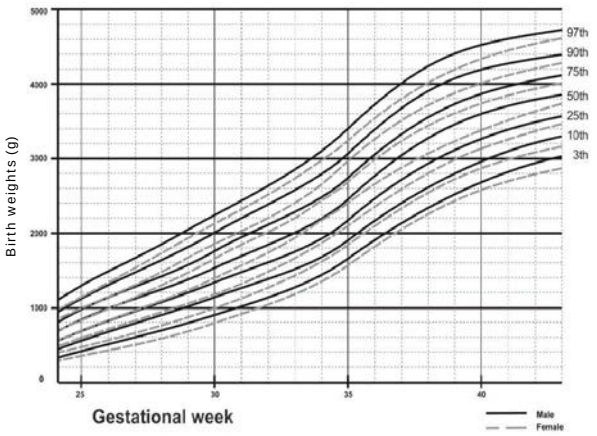

Fig. 1 – Estimated 3rd, 5th, 10th, 50th, 75th, 90th and 97th centiles for male and female birth weights.

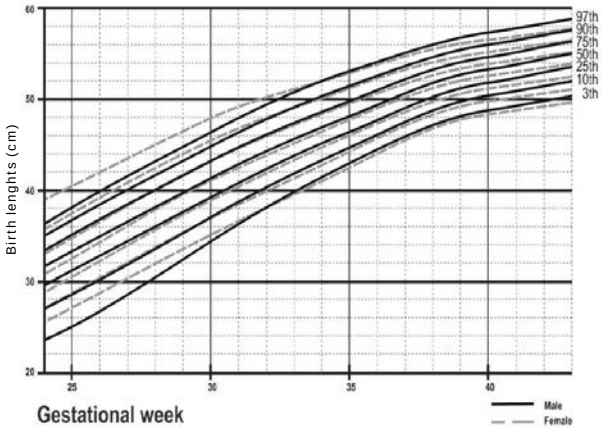

Fig. 2 – Estimated 3rd, 5th, 10th, 50th, 75th, 90th, and 97th centiles for male and female birth lengths.

Table 1

Number of infants and estimated values for L, M, and S for the birth weight*

Gestation (week)	Male				Female			
	n	L	M	S	n	L	M	S
24	7	1.78	627.32	0.16	10	1.56	615.12	0.20
25	12	1.76	833.74	0.16	10	1.54	730.82	0.19
26	7	1.76	965.92	0.16	12	1.53	852.41	0.19
27	15	1.75	1,083,85	0.16	15	1.51	993.17	0.19
28	20	1.73	1,230.88	0.16	10	1.50	1,134.35	0.19
29	23	1.71	1,379.96	0.16	9	1.51	1,314.08	0.18
30	38	1.68	1,553.15	0.16	24	1.52	1,514.76	0.18
31	21	1.66	1,753.11	0.16	24	1.54	1,690.01	0.17
32	63	1.62	1,947.16	0.16	51	1.56	1,882.12	0.17
33	73	1.59	2,068.48	0.16	45	1.56	2,049.68	0.16
34	109	1.52	2,270.82	0.16	98	1.49	2,302.78	0.16
35	189	1.33	2,540.79	0.15	171	1.28	2,539.93	0.15
36	472	1.02	2,804.59	0.15	425	0.96	2,779.62	0.14
37	1073	0.71	3,107.31	0.14	948	0.67	3,007.74	0.14
38	2618	0.57	3,297.06	0.13	2340	0.53	3,153.91	0.13
39	4838	0.49	3,463.48	0.13	4369	0.42	3,322.90	0.12
40	8508	0.46	3,602.50	0.12	8147	0.36	3,443.87	0.12
41	2226	0.45	3,725,22	0.12	2239	0.32	3,532.73	0.12
42	294	0.44	3,802.69	0.12	270	0.29	3,602.39	0.12

***LMS – method for the birth weight centimes [L (Box-Cox power), M (median), S (coefficient of variation)]**

Table 2

Number of infants and estimated values for L, M, and S for the birth length*

Gestation (week)	Male				Female			
	n	L	M	S	n	L	M	S
24	8	0.10	31.69	0.08	9	-1.04	30.68	0.08
25	13	0.54	33.55	0.08	11	-0.64	32.17	0.08
26	7	0.96	35.31	0.07	13	-0.24	33.77	0.08
27	15	1.34	37.01	0.07	15	0.15	35.54	0.07
28	20	1.69	38.71	0.07	10	0.54	37.43	0.07
29	22	1.99	40.34	0.06	9	0.91	39.32	0.07
30	42	2.24	41.84	0.06	32	1.29	41.01	0.06
31	19	2.43	43.12	0.06	24	1.65	42.35	0.06
32	54	2.57	44.23	0.05	39	1.98	43.52	0.06
33	67	2.67	45.27	0.05	42	2.25	44.72	0.05
34	88	2.72	46.47	0.05	76	2.44	46.08	0.05
35	190	2.71	47.93	0.05	169	2.52	47.62	0.05
36	521	2.64	49.52	0.05	497	2.50	49.22	0.05
37	1,158	2.55	51.01	0.04	1,027	2.41	50.53	0.04
38	2,653	2.47	52.01	0.04	2,332	2.30	51.31	0.04
39	4,873	2.41	52.72	0.04	4,393	2.19	52.02	0.04
40	8,434	2.36	53.31	0.04	8,107	2.09	52.55	0.04
41	2,192	2.32	53.95	0.04	2,231	1.97	53.05	0.04
42	290	2.28	54.52	0.04	267	1.83	53.38	0.04

***For abbreviations see under Table 1.**

Discussion

Our birth weight centiles provide a population norm for singleton infants adjusted for gender, born in Southeast Serbia. These references are both of epidemiological and clinical use, and they may have applicability as a tool for epidemiological comparisons between geographic locations and cultures. Data from an entire population were used and they provided a more valid standard than those based on hospital data. Hospital-based studies are often prescriptive, mostly based on a small number of infants without known risk factors for intrauterine growth retardation, and thus, may have limited usage in populations with mixed low and high-risk pregnancies. Population-based studies are more descriptive. In the absence of criteria regarding risk factors for fetal growth, these studies describe "what growth is actually like" in examined population [8].

The study cohort was stratified for gender. The known larger birth weight and length for gestational age in male versus female infants were shown.

Our measurements were quite similar to those of Abrahamowicz et al. [15], Fenton and Kim [16] , and Roberts and Lancaster [17]. On the other hand, clear differences between our measurements and those made in Brasil [18] and Israel [19] justify the fact that each specific population group should have its own neonatal anthropometric charts developed.

There are several limitations to our study. Our data were provided from the routine care, hence measurements were not standardized. The measurements were done by different members of staff, and this may have contributed to the inter-observer difference. Infants were not adjusted for parity. The secular trend has not been taken into account, having in mind a long period of data acquisition, even though there are plenty of studies with a similar disadvantage. The study was limited by the small size of the sample of 24 to 33 weeks gestation (50 male, 32 female). Therefore, using results from significant international experience in the large-scale population-based studies in the developed countries [15, 20] might be the best way to estimate the fetal growth and centiles of preterm infants in our population.

The other problem is the calculation of the exact gestational age, which is very important considering the fact that the fetus describes the fastest human growth. In our country, the gestational age is calculated by the last menstrual period, or by the neonatal examination more frequently than by the early fetal ultrasound, which is considered the gold standard [19]. Although menstrual dating is generally accurate for term neonates (± 7 days of the ultrasound estimate), the error rises with prematurity and postmaturity.

Conclusion

Thorough neonatal anthropometric data obtained in this study and centile charts of the Serbian population were constructed and made available for the first time. Consequently, it will improve assessing the growth and nutritional status of newborn infants during the perinatal period, classifying them as small, appropriate, or large for gestational age.

There is a need for large-scale research that will include a larger number of preterm newborns which were represented in limited numbers in our study. There is also a need for setting up the gold standard method for the precise determination of the gestational age, i.e. the use of the early fetal ultrasound.

R E F E R E N C E S

1. *Paz I, Seidman DS, Danon YL, Laor A, Stevenson DK, Gale R.* Are children born small for gestational age at increased risk of short stature? Am J Dis Child 1993; 147(3): 337–9.
2. *Puga B, Gil P, de Arriba A, Labarta JI, Romo A, Mayayo E*, et al. Neurocognitive development of children born small for gestational age (SGA). An update. Pediatr Endocrinol Rev 2012; 9(4): 716–26.
3. *Jaquet D, Gaboriau A, Czernichow P, Levy-Marchal C.* Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. J Clin Endocrinol Meta 2000; 85(4): 1401–6.
4. *Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ.* Weight in infancy and death from ischaemic heart disease. Lancet 1989; 2(8663): 577–80.
5. *Baker J, Olsen L, Sørensen T.* Weight at birth and all-cause mortality in adulthood. Epidemiology 2008; 19(2): 197–203.
6. *Chiavaroli V, Giannini C, D'Adamo E, de Giorgis T, Chiarelli F, Mohn A.* Insulin resistance and oxidative stress in children born small and large for gestational age. Pediatrics 2009; 124(2): 695–702.
7. *Das UG, Sysyn GD.* Abnormal fetal growth: intrauterine growth retardation, small for gestational age, large for gestational age. Pediatr Clin N Am 2004; 51(3): 639–54, viii.
8. *Bertino E, Milani S, Fabris C, Curtis MD.* Neonatal anthropometric charts: what are they and what are they not. Arch Dis Child Fetal Neonatal Ed 2007; 92(1): F7–F10.
9. *Kramer MS.* Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organ 1987; 65(5): 663–737.
10. *Bakketteig LA.* Current growth standards, definitions, diagnosis and classification of fetal growth retardation. Eur J Clin Nutrition 1998; 52(1): S1–S4.
11. *Durutović-Gligorović S.* Anthropometric newborn norms. 2nd ed. Belgrade, Medical Faculty, 2000.
12. *Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M.* A United States national reference for fetal growth. Obstet Gynecol 1996; 87(2): 163–8.
13. *Stankovic S, Zivic S, Ignjatovic A, Stojanovic M, Bogdanovic D, Novak S*, et al. Comparison of weight and length at birth of non-Roma and Roma newborn in Serbia. Int J Pub Health 2016; 60(6): 69–73.
14. *Cole TJ.* Fitting smoothed centile curves to reference data. J R Stat Soc 1988; 151(2): 385–418.
15. *Abrahamowicz M, Blondel B, Bréart g, Kramer MS, Platt RW, Wen SW*, et al. A New and Improved Population-Based Canadian Reference for Birth Weight for Gestational Age.Pediatrics 2001; 108(2): e35.
16. *Fenton TR, Kim JH.* A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. BMC Pediatr 2013; 13: 59.

17. *Roberts CL, Lancaster PA*. Australian national birthweight percentiles by gestational age. Med J Aust 1999; 170(3): 114–8.
18. *Pedreira CE, Pinto FA, Pereira SP, Costa E*. Birth weight patterns by gestational age in Brazil. An Acad Bras Cienc 2011; 83(2): 619–22.
19. *Davidson S, Sokolover N, Erlich A, Litwin A, Linder N, Sirota L*. New and Improved Israeli Reference of Birth Weight, Birth Length, and Head Circumference by Gestational Age: A Hospital-Based Study. Isr Med Assoc J 2008; 10(2): 130–4.
20. *Sherry B, Mei Z, Grummer-Strawn L, Dietz WH*. Evaluation of and recommendations for growth references for very low birth weight (<or = 1500 grams) infants in the United States. Pediatrics 2003; 111(4): 750–8.

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Anti-epidemic measures for protection against COVID-19 in institutions for execution of criminal sanctions

Antiepidemijske mere za zaštitu od COVID-19 u ustanovama za izvršenje krivičnih sankcija

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Ključne reči:
covid-19; zatvorenici; pandemija; zdravstvena zaštita, obezbeđenje kvaliteta; rizik, procena.

Introduction

On the last day of 2019, the health authorities of the People's Republic of China informed the World Health Organization (WHO) about the occurrence of several cases of respiratory infections in people in the city of Wuhan. An unknown coronavirus [severe acute respiratory syndrome coronaviris 2 (SARS-Cov-2)] was identified as the cause of the disease on January 7, 2020, and the disease was named COVID-19. In different variations, six types of coronavirus have been identified in the human population: HCoV-229E, HCoV-OC43, SARS-CoV, HCoV-NL63, HCoV-HKU1, and MERS-CoV [1]. The first cases are related to the fish market, where locals sell animals and poultry [2]. COVID-19, besides health implications, has produced many sociological, political, and security implications in many countries worldwide. The security challenges, risks, and threats posed by COVID-19 are multidimensional and complex. The multiplication of these challenges, risks, and threats has influenced prisons to become risky institutions in the emergence of COVID-19. The reasons for this are multiple: the very security nature of these institutions in which people with security risk to society are housed, the complexity and dynamism of functioning of these institutions (which represent micro-social environments for themselves), and the difficulty of providing medical care for convicts in primary medical institutions in cases of illness.

The appearance of COVID-19 and the introduction of the special functioning regime in Italian prisons on March 6, 2020, resulted in many riots in prisons across this country. Disturbing pictures from Italian prisons were seen all over the world on March 9, 2020. The head of the Italian prison administration, Francesco Basentini, stated that three prisoners died during the riot in prison in Modena, while three died after being transferred from the prison. Basentini stated: "There have been several serious riots in prisons across the country. In Pavia, two guards were taken hostage but released after police intervention" [2]. The Italian Ministry of Justice said a fire broke out in several prisons. The Italian government has introduced quarantine in most of the northern regions to curb the coronavirus epidemic [3].

In the Republic of Serbia, the first case of COVID-19 in the general population was registered on March 6, 2020 [4]. The appearance of COVID-19 caused a special legal functioning regime and introduced a state of emergency in the Republic of Serbia, and persons who violated anti-epidemic measures were sanctioned. For that purpose, the Ministry of Justice of the Republic of Serbia, the Directorate for Execution of Criminal Sanctions, opened three special facilities: 1. Penitentiary in Pančevo (a facility in Vršac), 2. Penitentiary Požarevac-Zabela, and 3. Penitentiary in Niš, Department in Pirot for detaining people deprived of their liberty during the state of emergency declared due to the COVID-19 pandemic, caused by SARS-Cov-2. The research is based on the analysis of anti-epidemic measures for protection against

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COVID-19 in institutions for execution of criminal sanctions and the proposal of the optimal model of protection.

The research was also based on analyzing comparative practice in applying the anti-epidemic protection measures and the results in the Republic of Serbia and European countries selected according to the territory, the Unite states of America (USA), and the United Kingdom (UK). The timing of the research subject covered the period March-April 2020. The reference framework was the complex application of health and organizational protection measures.

Protective measures against COVID-19 in some European countries, the USA and the UK

The European Prison Observatory's publication "COVID-19 – what is happening in European prisons?" shows numerous complaints about the functioning of prisons and the measures that have been implemented in many European countries. The measures to protect prisoners from COVID-19 in some countries (except Serbia) are shown in Table 1 [5–10]. Having in mind the above, anti-epidemic protection measures arise as first-class for the system of execution of criminal sanctions both in the Republic of Serbia and in other countries of the world.

Table 1

Measures to protect prisoners from Covid-19 in some European countries, the United States of America (USA) and the United Kingdom (UK) [6]

Country	Measures
Austria	Telephone time limited to 10 minutes per day; Video calls are enabled; Prisoners in open wards were instructed to stay in their cells all day; Prison staff members do not wear masks and do not keep a 1-meter distance; Staying in the open air was reduced from 1 hour to 15 minutes.
Denmark	The Danish Prison Administration has closed its doors to new prisoners and limited family visits. Daily perm and all activity groups have been suspended. Convicted prisoners will serve their sentences alone in their cells.
Estonia	The Ministry of Justice has introduced an extra measure to avoid the spread of coronavirus: prison guards in Estonia are now switching to a 24/5 work schedule, which means they will work five days in a row and spend their free time after work in prison. Family visits, group work, and walks have been suspended, and prisoners must remain in their living rooms. Prisoners have access to more printed material and TV channels, more food choices, and more phone calls.
France	According to the French Prison Administration, 63 prisoners and 145 staff members were tested positive for coronavirus, and on April 3, 2020, one prisoner died of Covid-19. Trial postponement measures have been applied, proceedings are being conducted by remote video calls and many prisoners have been released on parole, 6,266 prisoners, but overcrowding remains a major problem in French prisons.
Greece	"Coridallos Prison Hospital" and the wing of the local public hospital "Diavata" in Thessaloniki are planned to be used to house prisoners in case they are infected with the Covid-19 virus.
Hungary	Lawyers may enter penitentiaries for consultations after temperature measurements; communication takes place with plexiglass barriers between lawyers and convicts and by telephone; the premises used for consultations are disinfected; lawyers are prohibited from handing over documents to defendants; lawyers are directed to consult with their clients by phone or Skype. The Hungarian prison service called on priests and members of the convict's relatives to "minimize the number of visits." Visits are still allowed if family members are separated from the convicts by plexiglass barriers. No physical contact, kisses, or hugs are allowed. Also, the number of visitors was reduced to two per visit; new detainees were isolated for two weeks; new detainees with any Covid-19 symptoms were transferred to hospitals; leaving the institution is not allowed. All activities in prisons were suspended, except for one-hour outdoor walks, free use of the gym and television that was allowed for each cell, and early release of elderly and sick criminals who are vulnerable to Covid-19 was introduced.
Italy	In March 2020, the Criminal Administration announced that the virus had spread in Italian prisons: 94 detainees tested positive for the virus, 19 recovered and 2 died. The first detainee to die because of Covid was a 76-year-old man; He was detained in the Bologna prison and died in the Bologna hospital. The second death was confirmed on April 10. A 58-year-old man was imprisoned in Voghera Prison. Quarantine and isolation wards have been set up in prisons and emergency departments. By April 15, 2020, 6,000 convicts had been released in the prison system.
Norway	By an internal regulation, the Prison and Probation Service (KDI) has sent an order to regions across the country that over 200 prisoners be released as a measure to minimize the risk of infection in prisons. The medical staff has tested prisoners, and there are about 200 prisoners over the age of 60 who are part of the risk group.
Poland	Since March 12, 2020, everyone has had their body temperature measured upon entering the prisons, access to telephone communication has been increased. Prisoners also have greater access to television and the press, additional therapeutic and educational activities have been carried out, visits to all detention centers and prisons have been suspended since March 19, officers who come into contact with prisoners must wear protective masks, and prisoners are banned from doing outside jobs. All prisons and detention centers are provided with disinfectants, sanitary masks, hygiene and medical supplies, protective suits, and gloves, and all cells are disinfected several times a day.

Portugal	Visits to all prisons have been suspended since March 16. To overcome the isolation of prisoners, the state increased the number of allowed telephone calls, giving the right to 3 calls a day of five minutes. Extended video calls are also allowed in some prisons. Educational, recreational, and religious activities have also been suspended. The work performed by the prisoners was also suspended except for some services such as cooking, cleaning, agricultural work (in some prisons). The Santa Cruz do Bispo women's prison has produced protective materials, masks, uniforms for health workers, and other products.
Slovenia	The measures include: suspension of prison sentences, early release for prisoners who have less than six months in prison left, interrogation of a person by videoconference is possible except for the public from all proceedings. Those measures should be in force until July 1, 2020.
Ukraine	Due to the Covid-19 pandemic, the Ministry of Justice drafted the Amnesty Law to release 900 prisoners, who do not pose a threat to society.
USA	By June 23, at least 48,764 people in prison had tested positive for Covid-19. Much of the tremendous increase in coronavirus cases has been due to some states – including Michigan, Ohio, Tennessee, Texas – that have begun testing almost everyone in prisons where people have become ill. This scope of testing would suggest that the coronavirus circulated in prisons in much greater numbers than was known in the past weeks of the pandemic [6].
UK	Due to the Covid-19 pandemic, the British model of protection of persons deprived of their liberty has included many restrictions in the institutions for executing criminal sanctions. The restrictions remain in force to this day. Social visits to prisons have been abolished, alternative ways of contacting persons deprived of their liberty have been introduced, for example, by leaving a voice message using the prison voicemail service or sending e-mails using e-mail for prison services, unlimited writing at the expense of the prison has been approved. As a temporary measure, secure telephone handsets were provided to inmates at 55 prisons, allowing high-risk inmates to talk to a few approved contacts. As support for persons deprived of their liberty and their families, secure video calling lines have been introduced in some prisons. Video calls are free for both prisoners and their families [7]. Handwashing facilities are available to prisoners, staff, and visitors. Work with suppliers is managed in order to ensure sufficient supplies of soap and cleaning products. Prisoners who have a low assessed risk and whose sentence expires will be temporarily released from prison, as part of a National Health Service (NHS) protection and life-saving plan [8]. The usual regime in prisons has been suspended to apply social distance. This is crucial to protect the prisoners and staff and prevent the spread of the virus. This means that prisoners can no longer participate in normal recreational activities such as using the gym, going to church, or visiting the library. Only basic workers such as kitchen staff or wing cleaners will continue with their jobs, and all of those who are hired will still get paid. Support was provided to prisoners, such as life advice and advice on how to manage life problems. Informative videos about Covid-19 were made for friends and relatives of the persons deprived of their liberty [9]. All hearings of the Probation Committee and new trials were suspended. The Probation Committee will analyze cases through a combination of remote hearings and a paper review process. Contact between prisoners and their legal teams has been facilitated by increasing video conferencing capacity in prisons [10].

COVID-19 protection measures in institutions for execution of criminal sanctions in Serbia

In the Republic of Serbia, measures were implemented under the Law on Protection of Population from Infectious Diseases and the International Health Regulations, which included surveillance of passengers coming from hotspots of the new coronavirus and isolation of patients and health surveillance of contacts [11]. This was regulated by the Law on Execution of Criminal Sanctions, which has been in force since 01/01/2006, with later changes and additions from 2009, significant systemic changes from 2014, and current changes and additions from May 2019. According to this law, the execution of a prison sentence is carried out, organized, and supervised by a single central institution called the Directorate for Execution of Criminal Sanctions, which is part of the Ministry of Justice of the Republic of Serbia. Besides the Administration for Execution of Criminal Sanctions and its organizational units at the headquarters, the system of execution of criminal sanctions comprises 29 institutions. The structure goes as follows: the Training Center, then nine Penitentiary Institutions in Pančevo, Belgrade, Belgrade-Padinska Skela, Požarevac-Zabela, Sremska Mitrovica, Niš, Ćuprija, Šabac, Sombor, then the Penitentiary for Juveniles in Valjevo, the Penitentiary for Women in Požarevac, the Correctional Home in Kruševac, the Special Prison Hospital in Belgrade, and 16 District Prisons in Belgrade, Novi Sad, Leskovac, Čačak, Zrenjanin, Subotica, Vranje, Kragujevac, Kraljevo, Kruševac, Prokuplje, Užice, Zaječar, Novi Pazar, Negotin and Smederevo [12]. Within these institutions for execution of criminal sanctions, besides the Special Prison Hospital in Belgrade, there is a special organizational unit, the Health Care Service, at each of these institutions.

The Health Care Service performs health prevention, treats convicts and detainees, monitors hygiene and the quality of food and water, and takes part in determining and implementing the program of treatment of convicts. The institution has at least one doctor and two medical technicians and must provide the services of one psychiatrist. When hospital treatment is organized in an institution, the institution must have an educated doctor and hospital staff, and it also must possess hospital facilities, medical materials, accessories, devices, and medicines. The institution must have a special room for separating sick

convicts, depending on the disease. The Women's Institute must have special equipment for taking care of pregnant women, mothers, and women. A health professional who examines and treats a convict is guaranteed and so is the full professional independence under the law and the code of ethics [13].

The territorial distribution of these institutions meant that some of these institutions were in the epicenter of urban areas where the emergence of COVID-19 escalated. This made the security situation much more complicated since there is only one specialized institution for treating persons deprived of freedom, and that is the Prison Hospital in Belgrade. Here, in the case of COVID-19 in prisons, the pressure on the capacity of this institution would be maximum, and the treatment in regular medical centers would be complex due to the provision of persons deprived of liberty and the risk of spreading the infection to employees in the security service. The complex structure of COVID-19 has shown the following clinical picture in previous cases: pneumonia and respiratory problems, a body temperature of ≥ 38°C, radiographic identification of pneumonia, and changes in lymphocytes [14]. During a pandemic, the health system of criminal institutions would not be capable of responding to these challenges. With COVID-19, which causes additional restrictions, such as restriction or cancellation of visits by family and friends, the ban on receiving packages, and additional social distancing, the increase on social deprivation of convicts can cause various security implications in penitentiary institutions.

Extraordinary events that may occur due to appearance of COVID-19 in prisons are shown in Table 2.

Table 2

Extraordinary events that may result from the appearance of COVID-19 in prisons

Events
Attempt to escape/rebellion
escape from the closed part of the institution
removal from the semi-open part of the institution
escape during apprehension
Self-harm
hunger strike
by cutting, swallowing objects, poisoning
attempted suicide by cutting, poisoning, hanging
suicide by all typologies
Attacks
on officials
on convicts
on other persons
destruction of property
Offenses
possession of a mobile phone
theft
possession of money
indiscipline

Preventive measures have been taken to protect persons deprived of their liberty and employees in these institutions (Table 3). First of the measures was adopted on March 11,

Table 3

Number of infected with SARS-Cov-2 in the Republic of Serbia until 11/04/2020

Rank	City	Infected [16]	Location of prison	Infected
1	Belgrade	1,495	✓	0
2	Niš	536	✓	0
3	Ćuprija	180	✓	0
4	Novi Sad	169	✓	0
5	Kruševac	84	✓	0
6	Valjevo	82	✓	0
7	Leskovac	76	✓	0
8	Zaječar	59	✓	0
9	Vranje	57	✓	0
10	Kragujevac	52	✓	0
11	Čačak	42	✓	0
12	Subotica	36	✓	0
13	Pančevo	35	✓	0
14	Požarevac	34	✓	0
15	Užice	33	✓	0
16	Paraćin	32	x	0
17	Kraljevo	30	✓	0
17	Novi Pazar	30	✓	0
19	Jagodina	25	x	0
19	Šabac	25	✓	0
21	Pirot	24	✓	0
22	Surdulica	20	x	0
22	Sremska Mitrovica	20	✓	0
24	Aleksinac	19	x	0
25	Zrenjanin	16	✓	0
26	Kikinda	13	x	0
27	Sombor	12	✓	0
28	Varvarin	8	x	0
29	Smederevo	7	✓	0

SARS-Cov – severe acute respiratory syndrome coronavirus; x – no prison.

2020. Under the recommendations of the Institute of Public Health of Serbia "Dr Milan Jovanović Batut" and "Infectious Diseases Clinic" of the Clinical Center of Serbia, the following measures were introduced: the procurement of contactless thermometers for all institutions for execution of criminal sanctions; all employees who suffer from acute respiratory infections, show symptoms of dry cough or have a fever, must contact competent health institutions in order to go on sick leave and not come to work for two weeks; all employees who came from risk areas or were in contact with people whose infection was confirmed or suspected, had to use annual leave or open sick leave for two weeks; travel of employees to countries that are hotbeds of the epidemic was prohibited; measuring body temperature at the entrance points in the institutions was obliged; all admitted people in the institutes were separated in smaller rooms and under increased health supervision; noninstitutional rights, benefits, and work outside the institution were postponed; the ban on receiving packages was considered as a last resort; all group visits to prisons were postponed; it was recommended that people deprived of their liberty leave the institution as little as possible to visit courts, post offices, banks and health care institutions, except for emergency interventions and recommended measures of enhanced disinfection. Considering the epidemiological situation from as early as March 16, 2020, the following categories of employees were allowed to work from home: older than 60 years, chronically ill, under the request of the employee, one parent of a minor child up to the fourth grade of primary school, given that there is no one to take care of the child because of the closing of schools and kindergartens. All visits to convicted persons are prohibited, except for lawyer visits to their clients in custody, for 30 minutes, only if they have a trial scheduled in the next seven days, so that they can prepare the defense, by respecting all protection measures (a barrier between interlocutors, protective masks and gloves). The system of execution of criminal sanctions had to be adjusted to the environment and specific living and working conditions in institutions for execution of criminal sanctions, which resulted in the non-entry of COVID-19 into prison institutions of Serbia until April 11, 2020 (Table 3) [15].

On 13/04/2020, following the epidemiological situation, upon the previously available data (Table 4), the measure for banning visits to persons deprived of their liberty was extended. All persons who would show symptoms of acute respiratory infection had to be isolated, including the people deprived of liberty who were in contact with them and further action was to be coordinated with competent epidemiologists. The possibility of a larger number of persons deprived of liberty showing symptoms of COVID-19 was analyzed. In that case, those with a milder clinical picture would be referred for inpatient treatment to an improvised COVID Hospital, in one of the sports halls in Belgrade, which were guarded by security services and members of the Serbian Army. People deprived of liberty with a more severe clinical picture would be referred to the competent territorial clinical centers, and depending on the assessed risk of people deprived of liberty (low or medium), they would be provided with members of the Security Service and the Serbian Army. There was also a special protocol for treating persons deprived of their liberty with a high-security risk. Then on 27/04/2020 extended measure on prohibiting visits to persons deprived of liberty was introduced.

Table 4

Preventive measures for protecting persons deprived of their liberty and employees in prisons against COVID-19

Measures toward an individual

1. Limitation of the duration of visits and subsequent total suspension of visits;
2. Increased definition of premises in which persons deprived of liberty live, of their personal hygiene, and of premises in which employees live;
3. Increased duration of staying in fresh air;
4. Ventilation of all premises where persons deprived of liberty and employees live;
5. Wearing protective masks and gloves in the institution and during the funeral of persons deprived of liberty outside the institution, and measuring the temperature after leaving the institution (for all persons in institutions).
6. Increased laundry and laundry of belongings of persons deprived of liberty;
7. Zoning of movements within institutions and reduced social contacts between persons deprived of liberty, and between employees;
8. Enhanced protein and vitamin nutrition of persons deprived of their liberty;
9. Isolation of all arrived persons deprived of liberty and enhanced health supervision;
10. Permitted telephone communication for all persons deprived of their liberty to the extent necessary to reduce the deprivation caused by Covid-19;
11. Prohibition of many visits to persons deprived of their liberty and subsequent relaxation of this prohibition regarding all health care measures.

General organizational and security measures

1. Non-contact temperature measurement of employees and all persons entering the facilities of institutions for execution of criminal sanctions and displayed articles on protection measures;
2. Use of barriers for all vehicles entering the facilities of institutions for execution of criminal sanctions;
3. Disinfection of hands and shoes for all persons entering the facilities of institutions for execution of criminal sanctions;
4. Dressing of civilian clothes of members of the Security Service in the facilities of institutions for execution of criminal sanctions;
5. Disinfection of all areas within the institutions for execution of criminal sanctions;
6. Zoning of movements within institutions for execution of criminal sanctions;
7. Giving guidelines on safety and health culture to all employees of institutions for execution of criminal sanctions;
8. Wearing protective masks, gloves, and face shields for all persons entering the facilities of institutions for execution of criminal sanctions;
9. Installation of barriers at the entrances to all facilities of institutions for execution of criminal sanctions;
10. Mandatory Covid-19 test for all employees coming from sick leave and vacation;
11. Prohibition of using the gym for all employees in institutions for execution of criminal sanctions.
12. Restaurant catering is organized in such a way that employees can order meals and pick them up in a way that they do not concentrate and stay on the restaurant premises.
13. Prohibition of many group visits to institutions for execution of criminal sanctions.

At the beginning of June, instructions were given for actions in the institutions for execution of criminal sanctions, taking into account the current epidemiological situation. Guidelines were given to organize family visits with all protection measures, with continuing the disinfection measures and all other precautions. With the worsening of the epidemiological situation on 03/07/2020, additional instructions were given. It was forbidden to gather in the same room to consume food and drinks (to sit in restaurants), all meetings are held online, and working from the office was to be organized in such a way that only one employee was in the office, ventilation was recommended in all rooms and using central air conditioning was discouraged, disinfecting all work surfaces was necessary. In the case of COVID-19 in employees, the employee was sent for testing in the COVID clinic of the competent Health Center, where a protocol for treatment in the case of a positive test was provided. All employees that were in contact with the infected were sent to home isolation for seven days, and were kept as COVID contacts in the records of attendance at work. In case of a larger absence of employees, the work process should be organized in such a way as to continue uninterrupted. Table 4 lists the measures that have been permanently used in institutions for execution of criminal sanctions since the outbreak of the epidemic and which in these specific socio-environments have acted to prevent the spread of COVID-19.

Discussion of research results

The emergence of COVID-19 has caused various social, political, and security implications globally. The unpredictability of this health threat has affected the change of organizational and business policies of all decision-makers in the changed security context.

Persons deprived of their liberty, such as people in prisons, are more susceptible to the epidemic (COVID-19). People in prison live in and around the environment and can thus be exposed to the infection, be a source of the infection and spread infectious diseases, inside and outside the prison. A significant risk with this type of virus is the huge potential for infection and virulence [15, 16]. As the pandemic spreads, the response to COVID-19 in prisons is becoming more challenging and requires access from the entire society. Efforts to control COVID-19 in the prison community are unlikely to succeed unless measures are taken to prevent and control the infection, testing, treatment, and care in prisons. Prison health is part of public health so it should not lag behind. As part of the public health system, the WHO has collaborated with partners to develop a set of new materials on COVID-19 preparedness, prevention, and control in prisons [17]. Since the beginning of the pandemic, the Directorate for Execution of Criminal Sanctions in Serbia has been following the recommendations of the Institute of Public Health of Serbia "Dr Milan Jovanović Batut". In cooperation with the Ministry of Health and competent health institutions, the Institute of Public Health monitors the epidemiological situation of diseases caused by the new coronavirus in the world (COVID-19), records new findings and recommendations of the WHO, and issues public notices and instructions to health institutions and other competent authorities and institutions [18].

In many countries, the responsibility for providing health care in prisons lies with the Ministry of Justice/ Interior, not the Ministry of Health. Coordination and cooperation between the health and justice sectors are of the utmost importance if people's health in prisons are to be protected [19]. People in prisons and other places of detention are already deprived of their liberty, and they could react to further restrictive measures imposed on them [20].

Conclusion

Institutions for execution of criminal sanctions as dynamic and specific organizational units are at multiple risks of the COVID-19 pandemic. The complex organizational and security infrastructure of these institutions, where the perpetrators of various criminal acts live, who at the same time have their obligations and rights, has caused a change in the concept of business. The new business concept is based on the strategy of reducing the risk of COVID-19 and simulating the regular functioning of institutions. The goal was to spare the persons deprived of their liberty of feeling in their treatment the application of restrictive health and anti-epidemiological protection measures, imposed on the whole society, and that they perform their activities regularly, which reduces psychological and social deprivation caused by COVID-19 in the convicted population. The widespread transmission of an infectious pathogen affecting the community poses a great threat to introducing infectious agents into prisons; the risk of rapid disease transmission within prisons is likely to have an intensifying effect on the epidemic, rapidly multiplying the number of patients. Efforts to control COVID-19 in the prison community are unlikely to yield adequate results unless strong infection prevention and control measures, adequate testing, treatment, and care in prisons are carried out.

R E F E R E N C E S

1. *Adhikari SP, Meng S, Wu YJ, Mao YP, Ye RX, Wang QZ,* et al. Epidemiology, causes, clinical manifestation and diagnosis, prevention and control of coronavirus disease (COVID-19) during the early outbreak period: a scoping review. Infect Dis Poverty 2020; 9(1): 29.
2. *City Institute for Public Health Belgrade*. Corona virus. [cited 2020 July 15]; Available from: https://www.zdravlje.org.rs/index.php/aktuelnevesti/855-korona-virus (Serbian)
3. Six prisoners were killed in a riot at a prison in Italy. Tanjug.

[cited 2020 July 13]. Available from: https://www.021.rs/story/Info/Region-i-svet/236194/ (Serbian)

4. *Ministry of Health of the Republic of Serbia, Institute of Public Health of Serbia "Dr Milan Jovanović Batut"*. Covid 19. Belgrade: Institute of Public Health of Serbia "Dr Milan Jovanović Batut"; 2020. [cited 2020 July 17]; Available from: https://covid19.rs/ (Serbian)
5. *European prison observatory*. Covid – 19: What is happening in European prisons. [cited 2020 April 17]; Available from: http://www.prisonobservatory.org/upload/17042020European_prisons_during_covid19%233.pdf
6. *The Marshall project*. A State-by-State Look at Coronavirus in Prisons. [cited 2020 July 14]. Available from: https://www.themarshallproject.org/2020/05/01/a-state-by-state-look-at-coronavirus-in-prisons
7. *Governament United of Kindom*. Coronavirus (Covid-19) in Prisons; [cited 2020 July 19]; Available from: https://www.gov.uk/guidance/coronavirus-covid-19-and-prisons#prison-social-visits-in-england-and-wales
8. *Governament United of Kindom*. Coronavirus (Covid-19) in Prisons; [cited 2020 July 20]; Available from: https://www.gov.uk/guidance/coronavirus-covid-19-and-prisons#prison-social-visits-in-england-and-wales
9. *Governament United of Kindom*. Coronavirus (COVID-19) in Prisons; [cited 2020 July 23]; Available from: https://www.gov.uk/guidance/coronavirus-covid-19-and-prisons#prison-social-visits-in-england-and-wales
10. *City Institute for Public Health Belgrade*. Corona virus. [cited 2020 July 11]; Available from: https://www.zdravlje.org.rs/index.php/aktuelne-vesti/855-korona-virus (Serbian)
11. *City Institute for Public Health Belgrade*. Corona virus. [cited 2020 July 21]; Available from: https://www.zdravlje.org.rs/index.php/aktuelne-vesti/855-korona-virus (Serbian)
12. Strategy for the Development of the System of Execution of Criminal Sanctions in the Republic of Serbia until 2020. [cited 2020 July 03]. Available from: https://www.pravno-informacioni-sistem.rs/SlGlasnikPortal/eli/rep/sgrs/vlada/strategija/2013/114/1/reg (Serbian)
13. Article 24 of the Law on Execution of Criminal Sanctions, no 35/19; [cited 2020 July 04]; Available from: https://www.pravno-informacioni-sistem.rs/SlGlasnikPortal/eli/rep/sgrs/skupstina/zakon/2014/55/2/reg (Serbian)
14. *Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y*, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. N Engl J Med 20206; 382(13): 1199–207.
15. *Ministry of Health of the Republic of Serbia, Institute of Public Health of Serbia "Dr Milan Jovanović Batut"*. Number of people infected with the Covid-19 virus in the Republic of Serbia until April 11, 2020. (general population). Belgrade: Institute of Public Health of Serbia "Dr Milan Jovanović Batut"; 2020. [cited 2020 April 27]; Available from: https://covid19.rs/ (Serbian)
16. *Corman V, Muth D, Niemeyer D, Drosten C*. Hosts and Sources of Endemic Coronaviruses. Advances in Virus Research, Vol. 100. Bethseda: Elsevier Public Health Emergency Collection; 2018. p. 163–88.
17. *World Health Organization*. Prevention and control of COVID-19 in prisons and other places of detention. [cited 2020 Juny 20]. Available from: https://www.euro.who.int/en/health-topics/health-determinants/prisons-and-health/focus-areas/prevention-and-control-of-covid-19-in-prisons-and-other-places-of-detention
18. *City Institute for Public Health Belgrade*. Corona virus. [cited 2020 July 17]; Available from: https://www.zdravlje.org.rs/index.php/aktuelne-vesti/855-korona-virus (Serbian)
19. 2019 Novel Coronavirus (2019-nCOV): Strategic Preparedness and Response Plan. Geneva: World Health Organization; 2020; [cited 2020 July 17]; Available from: https://www.who.int/docs/default-source/coronaviruse/srp-04022020.pdf?sfvrsn=7ff55ec0_4&download=true).
20. Good governance for prison health in the 21st century: a policy brief on the organization of prison health. Copenhagen: WHO Regional Office for Europe/Vienna: United Nations Office on Drugs and Crime; 2013. [cited 2020 July 17]; Available from: http://www.euro.who.int/__data/assets/pdf_file/0017/231506/Good-governance-for-prison-health-in-the-21st-century.pdf.

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CASE REPORTS

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The first case of surgical myocardial revascularization and endarterectomy of the right carotid artery in the same procedure in a patient with haemophilia A

Prvi slučaj hirurške revaskularizacije miokarda i endarterektomije desne karotidne arterije u istom aktu kod bolesnika sa hemofilijom A

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Abstract

Introduction. Haemophilia A is the most common hereditary coagulation disturbance occurring due to the lack of coagulation factor VIII. It is widely accepted that people with haemophilia have a reduced incidence of coronary artery disease, potentially because of the protective effect of the impaired coagulation against the pathogenic mechanisms of the acute coronary syndrome. **Case report.** A 53-year-old man with mild haemophilia [FVIII 22% (mild form: more than 5%–40% of normal)] was hospitalized because of frequent anginal pain at rest. Selective coronary angiography revealed a severe three-vessel coronary disease. A need for urgent surgical revascularization was indicated. The color duplex scan showed the existence of hemodynamically significant stenosis on the right internal carotid artery. After consulting a haematologist, a cardiac surgeon, and a vascular surgeon, it was concluded that due to high bleeding risk, the patient should undergo an endarterectomy of the right carotid artery and a triple aortocoronary bypass in the same procedure. Procedures were performed with a substitution of FVIII concentrate. The patient firstly underwent the endarterectomy of the right carotid artery. Then, the left mammary artery graft was implanted to the left anterior descending artery as well as the venous grafts to the first obtuse marginal artery and posterior descending branch. There were no complications. During the revascularization, there was no need for blood transfusion, nor was there excessive bleeding in the postoperative period. The patient was discharged with antithrombotic therapy (aspirin, 50 mg). **Conclusion**. Patients with haemophilia are not protected against the development of atherosclerosis. Cardiac surgery in these patients presents a unique challenge for medical teams in securing haemostasis. Adequate substitution with factor VIII concentrate provides adequate haemostasis and the possibility for treatment with antiplatelet therapy.

Key words:
hemophilia a; coronary disease; coronary artery bypass; endarterectomy, carotid; cardiovascular surgical procedures; treatment outcome.

Apstrakt

Uvod. Hemofilija A je najčešći nasledni poremećaj koagulacije koji nastaje zbog deficita faktora VIII. Opšte je prihvaćeno da ljudi sa hemofilijom imaju smanjenu incidencu koronarne bolesti, uglavnom zbog protektivnog efekta narušenog sistema koagulacije nasuprot patogenetskim mehanizama za razvoj akutnog koronarnog sindroma. **Prikaz bolesnika.** Muškarac star 53 godine sa blagom hemofilijom A [FVIII 22% (blaga forma – više od 5%–40% od normale)] hospitalizovan je zbog učestalih anginoznih bolova u miru. Selektivna koronarografija je pokazala tešku trosudovnu koronarnu bolest koja je zahtevala hitnu hiruršku revaskularizaciju miokarda. Kolor dopler krvnih sudova vrata je pokazao hemodinamski značajnu stenozu na desnoj karotidnoj arteriji. Posle konsultacije sa kardiohirurgom, hematologom i vaskularnim hirurgom, zbog povećanog rizika od krvarenja odlučeno je da se hirurška revaskularizacija miokarda i endarterektomija desne karotidne arterije rade u istom aktu. Operacije su urađene uz supstituciju koncentratom faktora VIII. Najpre je urađena endarterektomija desne karotidne arterije, a zatim

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je iskorišćena leva arterija mamarija kao graft na levu prednju descendentnu arteriju, kao i dva venska grafta na prvu optuzu u marginalnu granu i zadnje descendentne grane. Tokom intervencije nije bilo hemoragijskih komplikacija, niti potrebe za transfuzijom krvi. Bolesnik je otpušten kući sa antitrombocitnom terapijom (acetilsalicilna kiselina, 50 mg). **Zaključak.** Bolesnici sa hemofilijom nisu zaštićeni od razvoja ateroskleroze. U cilju obezbeđivanja zadovoljavajuće hemostaze, kardiohirurške operacije kod ovih bolesnika, predstavljaju pravi izazov za ceo medicinski tim. Adekvatna supstitucija koncentratom FVIII osigurava adekvatnu hemostazu i daje mogućnost za primenu antitrombocitne terapije.

Ključne reči:
hemofilija; koronarna bolest; aortokoronarno premošćavanje; endarterektomija a. carotis; hirurgija, kardiovaskularna, procedure; lečenje, ishod.

Introduction

Haemophilia A is the most common hereditary coagulation disorder occurring due to the lack of coagulation factor VIII (FVIII). The life expectancy of persons born with haemophilia, who have access to adequate treatment, should approach the average with the currently available treatment [1].

It is widely accepted that people with haemophilia have a reduced incidence of coronary artery disease, potentially because of the protective effect of the impaired coagulation against the pathogenic mechanisms of acute coronary syndrome [2, 3]. Also, data have demonstrated that mortality due to ischaemic heart disease is lower in haemophilia patients than in the general male population [4]. Advances in managing haemophilia increase the life expectancy and the development of age-related and lifestyle-associated disorders, such as atherosclerosis and ischaemic heart disease [2].

Cardiac surgery in these patients presents a unique challenge to medical teams in securing haemostasis.

Case report

A 53-year-old male patient was hospitalized because of frequent chest pain at rest. The patient had a history of hypertension, hyperlipidemia, and long-term smoking experience. The personal history revealed haemophilia A with FVIII activity around 20%.

Laboratory examination verified decreased activity of factor FVIII [FVIII 22% (normal haemostasis requires at least a quarter (25%) of factor VIII activity)], normal activity of von Willebrand factor (vWF 112%), and prolonged activated partial thromboplastin time (aPTT) 37.5 s (reference range 29.1–41.9 s). He did not have FVIII inhibitors. The patient has been treated with cryoprecipitate in case of joint haemorrhages or dental interventions since childhood. Since 1980, the patients has been treated with FVIII concentrate from human plasma in preparation for tonsillectomy and cholecystectomy.

Following the advice of a haematologist, the patient received 3,000 IU of human FVIII concentrate prior to coronary angiography (Figure 1). After substitution, the level of FVIII was 104%. Catheterization was performed via the right radial artery and revealed a severe three-vessel coronary disease with high degree left main stenosis (Figure 2). It indicated a need for urgent surgical revascularization. There were no haemorrhagic complications after the procedure.

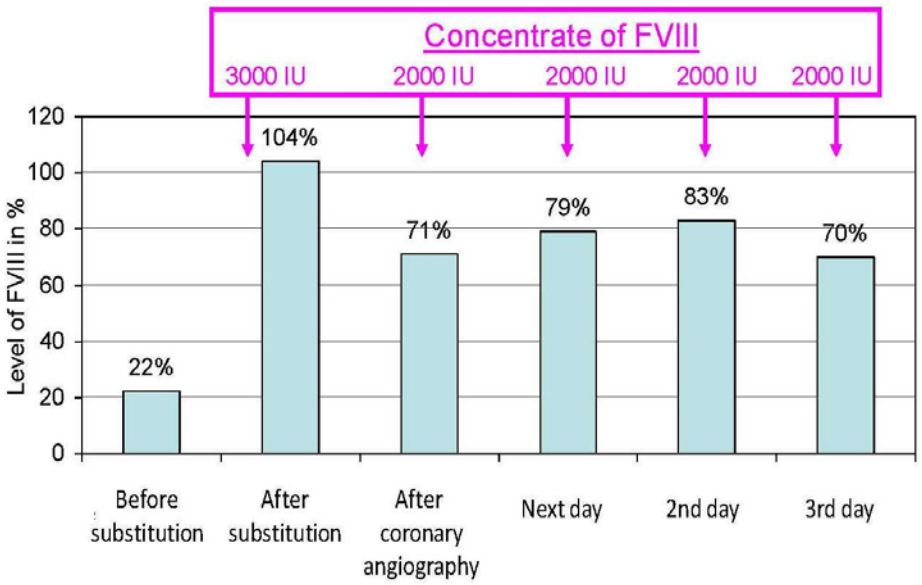

Fig. 1 – Substitution with FVIII concentrate before and after coronary angiography.

Fig. 2 – Coronary angiography (severe three-vessel coronary disease: LM stenosis 50%, LAD mid 70–90%, Cx prox 90–99%, Cx mid 90–99%, Cx dist 90–99%, OM2 70–90%, RCA prox 70–90%, RCA mid 100%).
LM – left main coronary artery; LAD – left anterior descending artery; Cx – circumflex artery; prox – proximal segment; mid – middle segment; dist – distal segment; OM2 – obtuse marginal 2; RCA – right coronary artery.

In preparation for surgery, according to the existing guidelines and the history of transient ischemic attack less than six months prior to surgery, a color duplex scan of carotid arteries was performed [5]. It showed the existence of ulcerated plaque on the right internal carotid artery that gives haemodynamically significant stenosis of 85%. The computed tomography (CT) scan of carotid arteries and aorta was performed, and the ultrasound findings were confirmed. There were no significant plaques or calcifications in the ascending aorta. Endarterectomy was indicated due to the medical record of the transient ischaemic attack, the characteristic of the plaque, and the degree of stenosis [6].

In patients with haemophilia, the surgical treatment raises costs due to the need for FVIII administration. A more complex perioperative period and a multidisciplinary approach are required, and there is also an increased bleeding risk. Because of that, simultaneous interventions are advisable when possible. In addition to this, it is known that haemophilic patients can develop anti-FVIII antibodies after 20–50 repeated FVIII administrations, which speaks in favour of a joint operation. After consulting a haematologist, cardiologist, cardiac surgeon, and vascular surgeon, it was concluded that the patient should undergo an endarterectomy and a triple aortocoronary bypass in the same procedure.

Due to an unsuitable radial artery diameter, the quality of preoperative ultrasound screening, and the fact that the patient was obese (higher risk of deep sternal wound infection is present if both mammary arteries were harvested), a surgeon decided to choose the left mammary artery in addition to the great saphenous vein as the grafts of choice for this procedure. The on-pump approach was selected over the off-pump after analyzing the coronary anatomy and the fact that there were no significant calcifications in the ascending aorta (with no significant risk of cross-clamp injury).

Before, during, and after surgery, the patient was constantly monitored by a haematologist. Every 30 min during surgery, the level of FVIII and aPTT were measured. FVIII was given according to given values. During the total heparinisation, activated clotting time was used to measure the heparinisation level. For the entire perioperative period, FVIII level was kept in a normal range.

Just before the surgery, the patient received a bolus of 3,500 IU of FVIII. After fifteen minutes, the level of FVIII was 101%, and after one hour, 90%. During that time, an endarterectomy was performed on the right carotid artery and the great saphenous vein from the right leg was harvested for the use as a venous graft. After that, a median sternotomy was done and the left mammary artery was harvested. Systemic heparin was given in 30,000 units and the extracorporeal circulation (ECC) was started. The anticoagulation activity of heparin was monitored by

activated clotting time (ACT) and protamine sulphate was given twice to maintain ACT around 400 s. During the procedure, FVIII was added several times (Figure 3). The patient underwent implantation of the left mammary artery to the left anterior descending artery (LAD) and two vein grafts to the first obtuse marginal (OM) artery and posterior descending (PD). There was no need for blood transfusion and antifibrinolytics were not prescribed. ECC lasted for 91 min, and the total revascularization time was 4 h. At the end of the procedure, the level of FVIII was 60%.

On the first postoperative day, aspirin in low-dose of 50 mg and low-molecular-weight heparin were introduced to the therapy. The level of FVIII was between 83% and 104% (Figure 4).

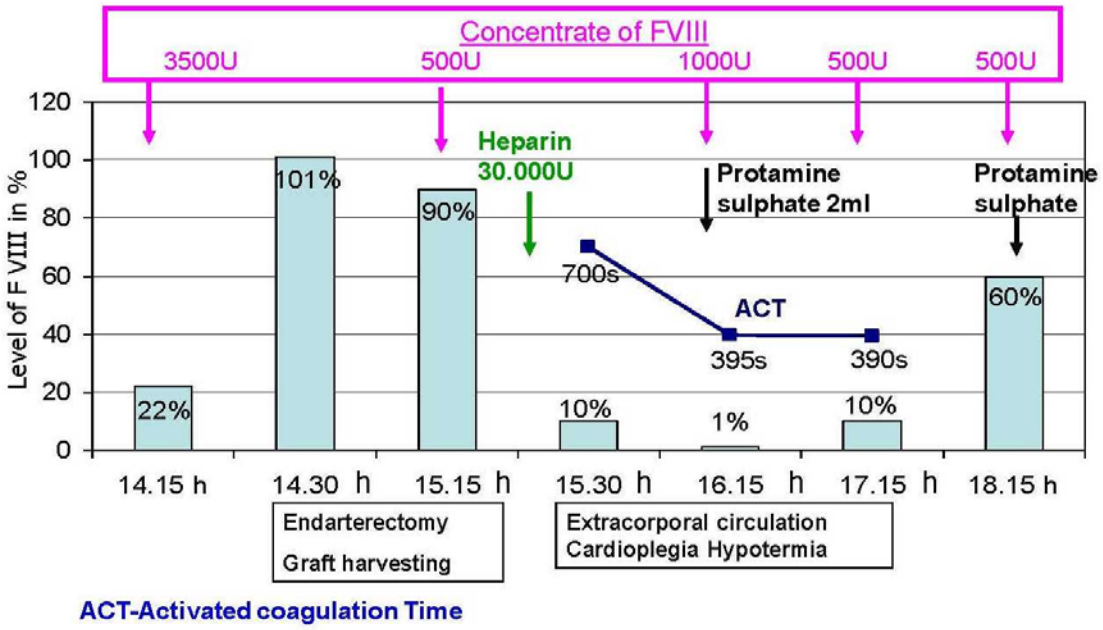

Fig. 3 – Substitution with FVIII concentrate before and during the surgery.

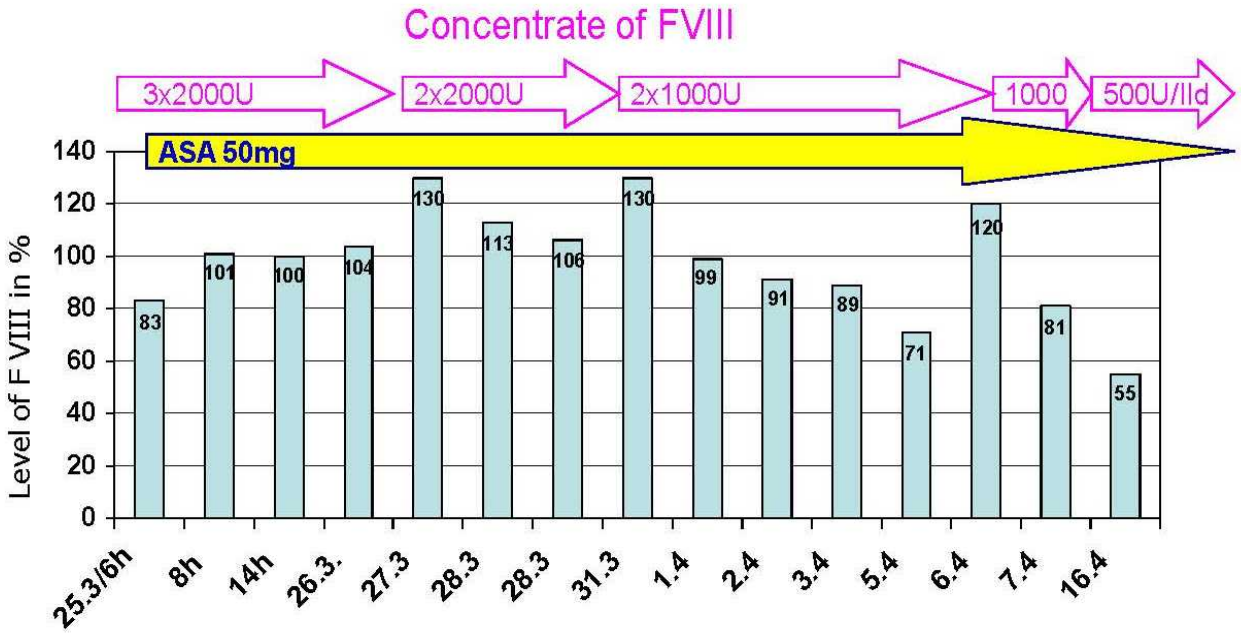

Fig. 4 – Substitution with FVIII concentrate after surgery.
ASA – acetylsalicylic acid.

From the second to the sixth postoperative day, 2 × 2,000 units of FVIII were prescribed. In the coming days, the dose was reduced (Figure 4). There was no excessive bleeding in the postoperative period.

At discharge, the level of FVIII was 55%. The patient was discharged with aspirin 50 mg, a statin, and a beta-blocker. The haematologist decided that the patient should not receive FVIII as long as its levels stay above 30%.

Fourteen months after the surgery, the level of FVII was 37% and during that period there were no haemorrhagic complications.

Discussion

As the haemophilia population is getting older, studies have established that cardiovascular mortality is three times more common as a cause of death [7], but that mortality is 60% lower than in the general population [8–11]. Several potential reasons may explain the low incidence of coronary artery disease in patients with haemophilia. These patients have hypocoagulable status and are significantly less likely to form a thrombotic mass. They also have a less established atheroma in blood vessels [12]. However, the patients with haemophilia might not be protected from atherosclerosis, as demonstrated by clinical studies [13] and autopsy reports on haemophiliacs with fatal myocardial infarction showing extensive atherosclerotic lesions and only rarely fresh thrombi [14].

Patients with haemophilia have an increased tendency to bleed, and, therefore, any invasive procedure is associated with an increased risk of hemorrhagic complications. Artery incision is confined with a high risk of local complications, but it can be effectively reduced by substituting a coagulation factor or by choosing access via radial artery for catheterization [15], as was the case with our patient. Considering that different complications are possible during invasive procedures, and sometimes urgent surgery is necessary, it was decided that higher doses of FVIII be given.

However, applying the missing coagulation growth factor may increase the risk of acute thrombosis in patients with unstable atherosclerotic plaques. Girolami et al. [15] studied 36 cases of acute coronary syndrome in patients with haemophilia A. In most cases, the event occurred during or after the infusion of recombinant FVIII, desmopressin, and prothrombin complex concentrates [14].

In addition, one of the treatment-related complications occurring mainly in haemophilia A patients is the development of an inhibitor, usually an IgG antibody, which is directed against the specific deficient factor and may occur shortly after the replacement therapy has been initiated [16].

Cardiac surgery constitutes a major haemostatic challenge because of sternotomy, the need for total heparinization, ECC, mild hypothermia, and cardiac arrest. However, there is no uniform protocol for the substitution of FVIII that can be applied in a bolus or an infusion [17]. According to the World Federation of Haemophilia recommendations, patients with haemophilia A undergoing major surgery should be supplemented with FVIII before the procedure to achieve the level of 80%–100% of FVIII activity [1].

Procedures using cardiopulmonary bypass incorporated standard heparinization protocols in many patients with haemophilia described in the literature, after 100% correction of factor levels by a bolus or continuous administration of factor concentrates [25–31].

During surgery, the level of FVIII was monitored and the concentrate was added according to values gathered. On heparin induction, the level of FVIII decreased to 10% and after that to 1%. Then, the patient received protamine-sulphate and 500 IU more of FVIII. After that, the level of FVIII increased to 60%. The level of FVIII increased due to the substitution and neutralization of heparin with protamine-sulphate.

Antiplatelet therapies are important for preventing thrombosis after cardiac surgery [30]. Haemophilia is not associated with abnormalities of platelet number or platelet function. However, antiplatelet therapy can increase the haemorrhagic tendency [19]. To minimize these risks, the clotting factor deficiency has to be corrected. As coronary artery bypass grafting and patients with coronary disease are required to be on single or dual antiplatelet therapy, for haemophilic patients, the use of aspirin in 50 mg dosage is recommended as long as FVIII is above 30% with regular measurements of FVIII level. Dual antiplatelet therapy is discouraged in these patients [31].

Conclusion

Patients with haemophilia are not protected from the development of atherosclerosis. Cardiac surgery in these patients presents a unique challenge to medical teams in securing haemostasis. Adequate substitution with FVIII concentrate provides adequate haemostasis and the possibility for treatment with antiplatelet therapy.

REFERENCES

1. *Srivastava A, Brewer AK, Mauser-Bunschoten EP, Key NS, Kitchen S, Llinas A,* et al. Treatment Guidelines Working Group on Behalf of The World Federation Of Hemophilia. Guidelines for the management of hemophilia. Haemophilia 2013; 19(1): e1–47.
2. *Mafrici A, Baudo F.* Hemophilia and percutaneous coronary interventions. Ital Heart J 2003; 4(10): 731–3.
3. *Bilora F, Dei Rossi C, Girolami B, Casonato A, Zanon E, Bertomoro A,* et al. Do hemophilia A and von Willebrand disease protect against carotid atherosclerosis? A comparative study between coagulopathics and normal subjects by means of carotid echo-color Doppler scan. Clin Appl Thromb Hemost 1999; 5(4): 232–5.
4. *Rosendaal FR, Briët E, Stibbe J, van Herpen G, Leuven JA, Hofman A,* et al. Haemophilia protects against ischaemic heart disease: a study of risk factors. Br J Haematol 1990; 75(4): 525–30.

5. *Aboyans V, Ricco JB, Bartelink MEL, Björck M, Brodmann M, Cohnert T,* et al. ESC Scientific Document Group. 2017 ESC Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in collaboration with the European Society for Vascular Surgery (ESVS): Document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteries. Endorsed by: the European Stroke Organization (ESO)The Task Force for the Diagnosis and Treatment of Peripheral Arterial Diseases of the European Society of Cardiology (ESC) and of the European Society for Vascular Surgery (ESVS). Eur Heart J 2018; 39(9): 763–816.
6. *Neumann FJ, Sousa-Uva M, Ahlsson A, Alfonso F, Banning AP, Benedetto U,* et al. ESC Scientific Document Group. 2018 ESC/EACTS Guidelines on myocardial revascularization. Eur Heart J 2019; 40(2): 87–165.
7. *Mannucci PM, Schutgens RE, Santagostino E, MauserBunschoten EP.* How I treat age-related morbidities in elderly patients with hemophilia. Blood 2009; 114(26): 5256–63.
8. *Soucie JM, Nuss R, Evatt BG, Abdelhak A, Cowan L, Hill H,* et al. Mortality among males with hemophilia: relations with source of medical care. The Hemophilia Surveillance system Project Investigators. Blood 2000; 96(2): 437–42.
9. *Darby SC, Kan SW, Spooner RJ, Giangrande PL, Hill FG, Hay CR,* et al. Mortality rates, life expectancy, and causes of death in people with hemophilia A or B in the United Kingdom who were not infected with HIV. Blood 2007; 110(3): 815–25.
10. *Plug I, Van Der Bom JG, Peters M, Mauser-Bunschoten EP, de Goede-Bolder A, Heijnen L,* et al. Mortality and causes of death in patients with hemophilia, 1992-2001: a prospective cohort study. J Thromb Haemost 2006; 4: 510–6.
11. Triemstra M, Rosendaal FR, Smit C, Van der Ploeg HM, Briët E. Mortality in patients with hemophilia. Changes in a Dutch population from 1986 to 1992 and 1973 to 1986. Ann Intern Med 1995; 123(11): 823–7.
12. *Berger K, Schopohl D, Lowe G, Holme PA, Tait RC, Combescure C,* et al. How to compare cardiovascular disease and risk factors in elderly patients with haemophilia with general population. Haemophilia 2016; 22(5): e406–16.
13. *Biere-Rafi S, Zwiers M, Peters M, van der Meer J, Rosendaal FR, Büller HR,* et al. The effect of haemophilia and von Willebrand disease on arterial thrombosis: a systematic review. Neth J Med 2010; 68(5): 207–14.
14. *Srámek A, Reiber JH, Gerrits WB, Rosendaal FR.* Decreased coagulability has no clinically relevant effect on atherogenesis: observations in individuals with a hereditary bleeding tendency. Circulation 2001; 104(7): 762–7.
15. *Girolami A, Ruzzon E, Fabris F, Varvarikis C, Sartori R, Girolami B.* Myocardial infarction and other arterial occlusions in hemophilia a patients. A cardiological evaluation of all 42 cases reported in the literature. Acta Haematol 2006; 116(2): 120–5.
16. *Smolka G, Kulach A, Dabek J, Szulc A, Gasior Z.* Percutaneous coronary intervention with stent implantation in haemophilic A patient with unstable angina. Haemophilia 2007; 13(4): 428–31.
17. *Bhave P, McGiffin D, Shaw J, Walsh M, McCarthy P, Tran H,* et al. Guide to performing cardiac surgery in patients with hereditary bleeding disorders. J Card Surg 2015; 30(1): 61–9.
18. *Kim DK, Kim DI, Kim MS, Lee EJ, Kim YB, Cho HJ,* et al. Successful percutaneous coronary intervention for acute coronary syndrome in a patient with severe hemophilia a. Korean Circ J 2010; 40(10): 527–9.
19. *Tang M, Wierup P, Terp K, Ingerslev J, Sørensen B.* Cardiac surgery in patients with haemophilia. Haemophilia 2009; 15(1): 101–7.
20. *MacKinlay N, Taper J, Renisson F, Rickard K.* Cardiac surgery and catheterization in patients with haemophilia. Haemophilia 2000; 6(2): 84–8.
21. *Rodriguez V, Burkhart HM, Schmidt KA, Pruthi RK.* Hemostatic management of an infant with severe hemophilia A and tetralogy of Fallot for cardiac bypass surgery. Pediatr Blood Cancer 2010; 55(7): 1399–401.
22. *Gasparović H, Zupancic-Salek S, Brida V, Dulić G, Jelić I.* Aortic valve replacement in a patient with severe hemophilia. Coll Antropol 2007; 31(1): 355–7.
23. *Kaminishi Y, Aizawa K, Saito T, Misawa Y, Madoiwa S, Sakata Y.* Modified Bentall operation in a patient with hemophilia A. Jpn J Thorac Cardiovasc Surg 2003; 51(2): 68–70.
24. *Donahue BS, Emerson CW, Slaughter TF.* Case 1--1999. Elective and emergency cardiac surgery on a patient with hemophilia B. J Cardiothorac Vasc Anesth 1999; 13(1): 92–7.
25. *Meagher PD, Rickard KA, Richards JG, Baird DK.* Aortic and mitral valve replacement in a patient with severe haemophilia A. Aust N Z J Med 1981; 11(1): 76–9.
26. *Murugan SJ, Viswanathan S, Thomson J, Parsons JM, Richards M.* Heart surgery in infants with hemophilia. Ann Thorac Surg 2006; 81(1): 336–9.
27. *Eren A, Friedl R, Hannekum A, Gulbins H.* Cardiac surgery in a patient with haemophilia A. Thorac Cardiovasc Surg 2006; 54(3): 212–4.
28. *Stine KC, Becton DL.* Use of factor VIII replacement during open heart surgery in a patient with haemophilia A. Haemophilia 2006; 12(4): 435–6.
29. *De Bels D, Demeere JL, Dugauquier J, Louis P, Van der Vorst E.* Continuous infusion of factor VIIIc during heart surgery in a patient with haemophilia A. Eur J Anaesthesiol 2004; 21(12): 984–6.
30. *Scharfman WB, Rauch AE, Ferraris V, Burkart PT.* Treatment of a patient with factor IX deficiency (hemophilia B) with coronary bypass surgery. J Thorac Cardiovasc Surg 1993; 105(4): 765–6.
31. *Kanellopoulou T, Nomikou E.* Replacement therapy for coronary artery bypass surgery in patients with hemophilia A nnd B. J Card Surg 2018; 33(2): 76–82.

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CASE REPORT

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Nonsteroidal anti-inflammatory drug-induced colopathy: an uncommon cause of positive immunochemical faecal occult blood test in the program for colorectal cancer screening

Kolopatija izazvana nesteroidnim antiinflamatornim lekovima: redak uzrok pozitivnog imunohistohemijskog fekalnog testa na okultno krvarenje u programu skrininga kolorektalnog karcinoma

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Abstract

Introduction. Nonsteroidal anti-inflammatory drug-induced colopathy is an uncommon condition associated with the long-term use of enteric-coated and slow-release nonsteroidal anti-inflammatory drugs. This paper presents a case of colopathy showing no symptoms or signs, discovered by a positive immunochemical faecal occult blood test. **Case report.** Performed within the framework of the National Program for Screening of Colorectal Cancer, the immunochemical faecal occult blood test was positive in a 56-year-old female patient. The colonoscopy revealed three lesions in the right colon: one erosion-ulceration and two concentric "diaphragm-like" strictures passable by the endoscope. The patient reported that she had been taking diclofenac 100 mg twice a day for the past seven years. After withdrawing the offending drug, the second colonoscopy indicated a marked improvement in the colonic mucosa while the "diaphragm-like" strictures persisted. **Conclusion.** The cases of nonsteroidal anti-inflammatory drug-induced colopathy are likely to become more frequent. It would be, therefore, advisable to consider the long-term use of such drugs as being a possible factor that leads to mucosal injury, particularly in the right colon, as well as being a rare reason for a positive immunochemical faecal occult blood test.

Key words:
colorectal neoplasms; diagnosis; feces; occult blood; colonic diseases; anti-inflammatory agents; treatment outcome.

Apstrakt

Uvod. Kolopatija izazvana nesteroidnim antiinflamatornim lekovima je retko stanje uslovljeno dugotrajnim uzimanjem entero-rezistentnih i sporo-oslobađajućih nesteroidnih antiinflamatornih lekova. U radu je prikazana kolopatija izazvana nesteroidnim antiinflamatornim lekovima, bez ispoljenih simptoma ili znakova, otkrivena pozitivnim imunohistohemijskim fekalnim testom na okultno krvarenje. **Prikaz bolesnika.** U okviru Nacionalnog programa skrininga kolorektalnog karcinoma, imunohistohemijski fekalni test na okultno krvarenje je bio pozitivan kod 56-godišnje bolesnice. Kolonoskopski su nađene tri lezije desnog kolona: erozija-ulceracija i dve koncentrične „dijafragmi-slične" strikture prolazne za endoskop. Detaljnom anamnezom utvrđeno je da je bolesnica uzimala diklofenak od 100 mg, dva puta dnevno, tokom poslednjih sedam godina. Nakon isključivanja diklofenaka, kontrolnom kolonoskopijom je ustanovljeno značajno poboljšanje sluznice kolona, uz zaostale „dijafragmi-slične" strikture. **Zaključak.** Učestalost kolopatije izazvane nesteroidnim antiinflamatornim lekovima će vrlo verovatno biti sve veća. Zbog toga bi bilo preporučljivo uzeti u obzir dugotrajnu upotrebu ovih lekova kao mogući faktor koji dovodi do oštećenja sluznice, posebno u desnom kolonu, ali i kao retki uzrok pozitivnog imunohemijskog fekalnog testa na okultno krvarenje.

Ključne reči:
kolorektalne neoplazme; dijagnoza; stolica; okultna krv; kolon, bolesti; antiinflamatorici; lečenje ishod.

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Introduction

To avoid possible upper gastrointestinal side effects of non-steroidal anti-inflammatory drugs (NSAIDs), 'slow' or 'modified-release' preparations have been used increasingly, thus resulting in a higher incidence of colopathy, even though it remained rare [1]. Since NSAIDs have become so widely prescribed and some even available as over-the-counter drugs, increased awareness of this largely underestimated clinical condition is necessary in order to reduce morbidity by prevention, promote early recognition [2] and, thereby, mitigate further complications. The patient reported here was present with NSAID-induced colopathy, showing no symptoms or signs, with only a positive immunochemical faecal occult blood test (iFOBT).

Case report

A 56-year-old female was referred to a gastroenterologist due to a positive iFOBT: 477.173 ng/mL (reference value 0–99) performed in the National Colorectal Cancer screening program for the average-risk population. Physical examination and laboratory findings were unremarkable. The leukocyte count was 8.15×10^9/L, the erythrocyte count was 5.04×10^{12}/L, the hemoglobin level was 137 g/L, and the mean corpuscular volume (MCV) was 82.3 fL. The colonoscopy revealed three lesions in the right colon: one erosion-ulceration and two concentric 'diaphragm-like' strictures with a mucosal lesion passable by the endoscope (Figure 1). The histopathological examination showed erosions of the mucosa, reactive changes in the epithelial cells, irregularity of the crypts, and mixed inflammatory infiltrates of lymphocytes, plasma cells, and eosinophilia in the lamina propria (Figure 2). The patient then reported that she had been taking diclofenac 100 mg twice a day for the past seven years because she had chronic lower backache. This statement on her long-term diclofenac use, along with the endoscopic and histological findings, led to the diagnosis of NSAID-induced colopathy. The NSAID was discontinued, and a colonoscopy was repeated eight weeks later. While the 'diaphragm-like' strctures persisted, the second colonoscopy showed a marked improvement in the colonic mucosa, with tissue restitution in segments containing mucosal lesions (erosions-ulcerations) (Figure 3). It was decided not to perform the balloon dilatation as the diaphragm strictures were passable by the endoscope.

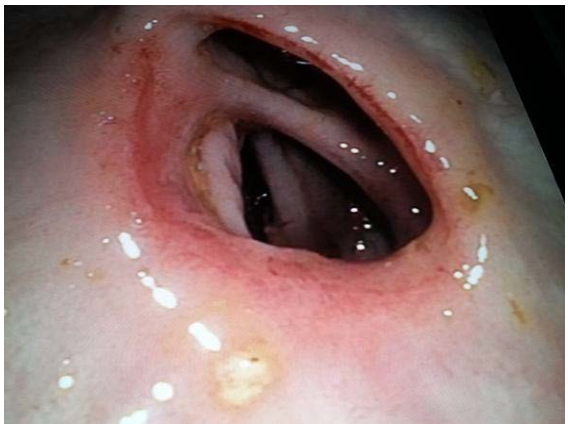

Fig. 1 – Diaphragm disease on the first colonoscopy.

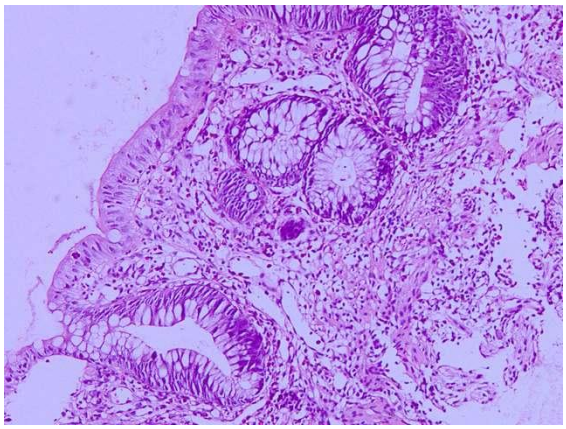

Fig. 2 – Mixed inflammatory infiltrates in the lamina propria, irregularity of the crypts (hematohylin & eosin staining, 100×).

Fig. 3 – Discontinuation of the non-steroidal antiinflammantory drug: a marked improvement in the colonic mucosa on the second colonoscopy.

Discussion

Despite the extensive use of NSAIDs in the general population, NSAIDs-induced colopathy is a condition that often goes unrecognized or even misdiagnosed [3]. Symptomatic patients are usually present with chronic (median 3 months) and multiple symptoms [1], such as anaemia, rectal bleeding, abdominal pain, diarrhoea, obstruction, perforation, and peritonitis [4, 5].

In the case reported here, the patient had no symptoms or signs; she only had a positive iFOBT, accompanied by a history of taking diclofenac for seven years. In support of this finding, the literature data does indicate the majority of cases to be asymptomatic, with a diagnosis made incidentally during the investigations of bowel symptoms or upon endoscopic examination [4, 5].

NSAID-induced colopathy usually involves the right colon due to a higher concentration of the enteric-coated and slow-release preparations of NSAIDs on this site [6]. The mechanism of NSAID-induced colopathy is still unclear. Since most NSAIDs undergo enterohepatic circulation, the proximal colon is directly exposed to the intact drug following a bacterial breakdown in the distal ileum, and herein the cecum acts as a reservoir [2, 7].

However, the duration of treatment is highly associated with the spectrum of endoscopic findings in NSAID-induced colopathy [8]: 51% of patients are reported to have either one or two lesions, while multiple diaphragms are seen in 33%. In our patient, the colonoscopy revealed two concentric "diaphragm-like" strictures passable by the endoscope, accompanied by mucosal lesions.

A histological diagnosis of NSAID colopathy can prove to be difficult. Consequently, a multidisciplinary approach is important, and correlations with the anamnesis, clinical and endoscopic data are crucial. There are no specific histopathological features of NSAID colopathy; most report non-specific inflammation with mixed inflammatory infiltrates (in some cases with lymphocyte dominance), erosions of the mucosa, and fibrosis of the lamina propria [7]. However, eosinophilia in the lamina propria, apoptosis of the epithelial cells, and penetration of lymphocytes into the superficial epithelium may indicate an NSAID injury.

Primary management of an NSAID colopathy is simple and includes the withdrawal of the offending NSAID. All 13 patients who had uncomplicated ulceration and had no strictures in the cohort described by Kurahara et al. [5] showed marked improvement on a repeated colonoscopy 3–10 weeks after withdrawing the NSAIDs. These results are similar to the case reported here. While the ulcers are likely to resolve, the strictures - already formed diaphragms - may sometimes persist despite the cessation of the NSAID use. Balloon dilatation proved to be effective in treating colonic and ileocolonic strictures, and surgery is reserved for multiple strictures or complications [8, 9]. In the case presented here, balloon dilatation was not performed since the diaphragm strictures were passable by the endoscope.

In 1965, Ser Austin Bradford Hill published nine viewpoints for determining causality: the strength of association, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment, and analogy. These viewpoints are regarded as the criteria for identifying causality in clinical practice even beyond epidemiological studies [10]. Although NSAID-induced colopathy is a rare event (yielding a small number of reports in literature), the systematic review by Munipalle et al. [1] provided an optimistic foundation for future research on the strength of association between the NSAID use and colonic lesions. The existence of several case reports and case series (which have been referenced in this paper) accounts for the consistency of this association. Still, the plausibility and coherence can be estimated with the knowledge of NSAID metabolism, even though the pathophysiology of colopathy is not yet fully understood. In the case presented here, the essential criterion of temporality is satisfied through the NSAID exposure occurring before the positive iFOBT. The lack of other causes of colonic lesions (such as inflammatory bowel disease, ischemic colitis, or vasculitis – assessed with endoscopy and histopathological examination), as well as other causes of positive iFOBT, result in the specificity of this adverse effect as the cause of colopathy. Küttner Magalhães et al. [11] consider the presence of colonic inflammation, ulcers, and diaphragm-like strictures to be pathognomonic for this disease. The amount of diclofenac that the patient had used, as well as the duration of the drug use, account for the biological gradient (the presence of a dose-response relationship) and the temporality, as previously stated by Aftab et al. [7]. Finally, the recovery of the eroded colonic mucosa after cessation of diclofenac acts as experimental evidence, under the suggestion that trial cessation of the NSAIDs should be implemented in the presence of colonic diaphragms [1].

One of the most frequent tools used in adverse drug reaction assessment is the Naranjo probability scale. The scale features a list of weighted questions related to the following: the presence of previous reports on a specific reaction, the temporal relationship between the drug and the effect, alternative causes for the event, previous reactions to the same drug, and the drug dosage. The score ranges in four levels, from doubtful to definite adverse reaction. The Naranjo scale is frequently used for its simplicity and the fact that it is less time-consuming than other assessment tools [12]. The case presented herein scored positive on the questions related to the previous reports (1 point), the temporal sequence between the drug and the effect (2 points), the improvement of the symptoms after discontinuation of the drug (1 point), the lack of other alternative causes of this effect (2 points; a decision was made for this answer to be positive since the endoscopic and histological examination failed to identify any other causes of colonic lesions), and the presence of objective evidence of the effect (1 point, with a detailed endoscopic and histological description of the disease). There were no points on questions regarding the repeated use of the drug, the use of a placebo, the toxic concentration of the drug, the influence of different drug dosages, and the presence of previous exposure and effects – because these criteria were not applicable in this case. The

total score was 7, amounting to a probable adverse reaction to the drug.

Given these facts, it was concluded that diclofenac was the causative agent of colopathy in this patient. Future research should aim at understanding the pathophysiology of this adverse effect, as well as to describe its true prevalence.

Conclusion

Due to the increased use of enteric-coated and slow-release NSAIDs, cases of NSAIDs-induced colopathy are likely to become more frequent. Therefore, it would be advisable to consider this entity as a rare reason for a positive iFOBT.

R E F E R E N C E S

1. *Munipalle PC, Garud T, Light D.* Diaphragmatic disease of the colon: systematic review. Colorectal Dis 2013; 15(9): 1063–9.
2. *Tonolini M.* Acute nonsteroidal anti-inflammatory drug-induced colitis. J Emerg Trauma Shock 2013; 6(4): 301–3.
3. *Margolius DM, Cataldo TE.* Nonsteroidal anti-inflammatory drug colopathy mimicking malignant masses of the colon: a report of three cases and review of the literature. Am Surg 2010; 76(11): 1282–6.
4. *Masannat AY, Harron M, Harinath G.* Nonsteroidal anti-inflammatory drugs-associated colopathy. ANZ J Surg 2010;80(1–2): 96–9.
5. *Kurahara K, Matsumoto T, Iida M, Honda K, Yao T, Fujishima M.* Clinical and endoscopic features of nonsteroidal anti-inflammatory drug-induced colonic ulcerations. Am J Gastroenterol 2001; 96(2): 473–80.
6. *Mokhtare M, Valizadeh SM, Emadian O.* Lower gastrointestinal bleeding due to Non-steroid Anti-inflammatory Drug-induced Colopathy Case Report and Literature Review. Middle East J Dig Dis 2013; 5(2): 107–11.
7. *Aftab AR, Donnellan F, Zeb F, Kevans D, Cullen G, Courtney G.* NSAID-induced colopathy. A case series. J Gastrointestin Liver Dis 2010; 19(1): 89–91.
8. *Püspök A, Kiener HP, Oberhuber G.* Clinical, endoscopic, and histologic spectrum of nonsteroidal anti-inflammatory drug-induced lesions in the colon. Dis Colon Rectum 2000; 43(5): 685–91.
9. *El Hajj I, Hawchar M, Sharara A.* NSAID-induced colopathy: case report and review of the literature. J Med Liban 2009; 57(4): 274–6.
10. *Fedak KM, Bernal A, Capshaw ZA, Gross S.* Applying the Bradford Hill criteria in the 21st century: how data integration has changed causal inference in molecular epidemiology. Emerg Themes Epidemiol 2015; 12: 14.
11. *Küttner Magalhães R, Ferreira JM, Pedroto I.* Nonsteroidal anti-inflammatory drug (NSAID)-induced colopathy with diaphragm-like strictures. J Gastrointestin Liver Dis 2014; 23(1): 9.
12. *Belhekar MN, Taur SR, Munshi RP.* A study of agreement between the Naranjo algorithm and WHO-UMC criteria for causality assessment of adverse drug reactions. Indian J Pharmacol 2014; 46(1): 117–20.

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CASE REPORT

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Cataract surgery in a patient with bilateral necrotising scleritis and peripheral ulcerative keratitis associated with granulomatosis with polyangiitis (Wegener's granulomatosis)

Operacija katarakte kod bolesnika sa obostranim nekrotizujućim skleritisom i perifernim ulceroznim keratitisom u sklopu granulomatoze sa poliangiitisom (Wegener-ova granulomatoza)

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Abstract

Introduction. We report a rare case of cataract surgery in a patient with an extreme, widespread anterior staphyloma following severe bilateral necrotising anterior scleritis associated with granulomatosis with polyangiitis (GPA). **Case report.** A 61-year-old man with a history of GPA developed bilateral, rapidly progressive necrotising scleritis and peripheral ulcerative keratitis (PUK). Inflammation compromised the entire anterior globe and peripheral cornea in both eyes. More than 90% of the surface area healed within 8 weeks, following the treatment with 3 pulsed doses of methylprednisolone in addition to the cyclophosphamide treatment. Systemic steroid therapy was slowly tapered over a period of 6 months. Extraordinary scleral loss with a uveal bulge developed, following severe necrotising anterior scleritis associated with PUK. Once the full remission had been achieved after 6 months, uncomplicated phacoemulsification was performed in his left eye, the only functional one. **Conclusion.** Preoperative and postoperative control of inflammation, careful surgical planning, and meticulous surgical techniques are critically important for optimal surgical outcome in such patients. To our knowledge, phacoemulsification in a patient with coexisting uveitic cataract and severe anterior staphyloma has not been previously reported.

Key words:
scleritis; corneal ulcer; granulomatosis with polyangiitis; cataract extraction; treatment outcome.

Apstrakt

Uvod. U radu je prikazan veoma redak slučaj operacije katarakte kod bolesnika sa ekstremnim, opsežnim prednjim stafilomom sklere koji je nastao nakon bilateralnog nekrotizujućeg skleritisa u sklopu granulomatoze sa poliangiitisom (GPA). **Prikaz bolesnika.** Muškarac, starosti od 61 godine, razvio je obostrani, progresivni nekrotizujući skleritis i periferni ulcerozni keratitis (PUK). Inflamacija je obuhvatila celu prednju polovinu bulbusa i periferiju rožnjače na oba oka. Nakon primene 3 pulsne doze metilprednizolona uz povećanje doze održavanja ciklofosfamida, inflamacija je sanirana u više od 90% površine nekrotične sklere u periodu od 8 nedelja. Sistemska steroidna terapija postepeno je redukovana tokom 6 meseci. Kao posledica teškog oblika nekrotizujućeg skleritisa i PUK-a došlo je do ekstremnog istanjenja sklere sa prolabiranjem uvealnog tkiva. Šest meseci nakon potpune remisije bolesti, urađena je nekomplikovana operacija katarakte na levom, jedinom funkcionalnom oku. **Zaključak.** Preoperativna i postoperativna kontrola inflamacije, pažljivo planiranje operacije i izbor hirurške tehnike od kritičnog su značaja za optimalan ishod operacije kod ovakvih bolesnika. Prema našem saznanju, fakoemulzifikacija kod bolesnika sa uvetičnom kataraktom i ekstremnim prednjim stafilomom do sada nije publikovana.

Ključne reči:
Skleritis; rožnjača, ulceracije; granulomatoza sa poliangiitisom; katarakta, ekstrakcija; lečenje, ishod.

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Introduction

Granulomatosis with polyangiitis (GPA), formerly known as Wegener's granulomatosis, is proteinase-3-ANCA-associated vasculitis with a presumed autoimmune aetiology. Necrotising scleritis is an uncommon inflammatory disorder of the sclera. This severe form of scleritis is almost always extremely painful and can lead to vision-threatening complications and visual loss. The presence of necrotising changes and inflammation of the adjacent cornea is highly suggestive of underlying systemic vasculitis, and GPA is the most common form [1]. Reported here was a rare case of severe necrotising scleritis associated with peripheral ulcerative keratitis (PUK) that simultaneously occurred in both eyes of the patient with GPA. Recently, uncomplicated cataract surgery was reported in a patient with refractory GPA present with scleral thinning; however, this was without any signs of associated inflammation or active necrosis [2]. To our knowledge, the case presented here is the first report of uneventful cataract surgery in a patient with extreme, widespread staphyloma following inflammation that compromised the entire anterior globe and peripheral cornea.

Case report

A 61-year-old man with a one-year history of GPA developed bilateral, rapidly progressive necrotising scleritis and PUK. Acute exacerbation of ocular inflammation occurred during the maintenance treatment with oral cyclophosphamide (CYP) (100 mg per day), and 3 months after the induction, a regimen with 6 CYP pulsed was given (1,000 mg per month). The patient was present with extreme discomfort and visual loss. Upon admission, visual acuity was light perception with projection (L+P+) in the right eye and 20/200 (Snellen) in the left eye. An examination revealed white, thinned avascular areas of the sclera and conjunctiva. The inflamed area involved the entire anterior globe and peripheral cornea of both eyes (Figures 1A and 1B). However, PUK was slightly less severe on his left eye, and a small part of the limbus was uninflamed in the upper temporal quadrant (Figure 1B). A slit-lamp biomicroscopy finding also included anterior chamber inflammation. There were no visible lental opacities in the left eye; however, dense vitreous opacification was observed (vitritis). Fundus examination revealed no clinically significant abnormalities at the posterior pole. Progressive ocular inflammation was associated with a significant increase in serum levels of anti-proteinase-3 (anti-PR3) antibody titre, as well as inflammatory markers. Nonetheless, pulmonary and renal diseases were clinically stable.

More than 90% of the surface area healed within 8 weeks, following the treatment with 3 pulsed doses of methylprednisolone (1,000 mg per day) in addition to oral CYP. Systemic steroid therapy was slowly tapered over a period of 6 months. During this time, the patient's visual acuity further declined to L+P- in the right eye and L+P+ in the left eye. Although inflammation was halted in both eyes, advanced prolonged scleral necrosis associated with PUK led to vision loss in his right eye (no light perception) over a period of 12 months after the disease onset. B-scan ultrasonography of the right eye revealed a large optic disc cup. The flat anterior chamber in this eye (Figure 2) was caused by both extensive posterior synechiae that involved the entire lens surface and

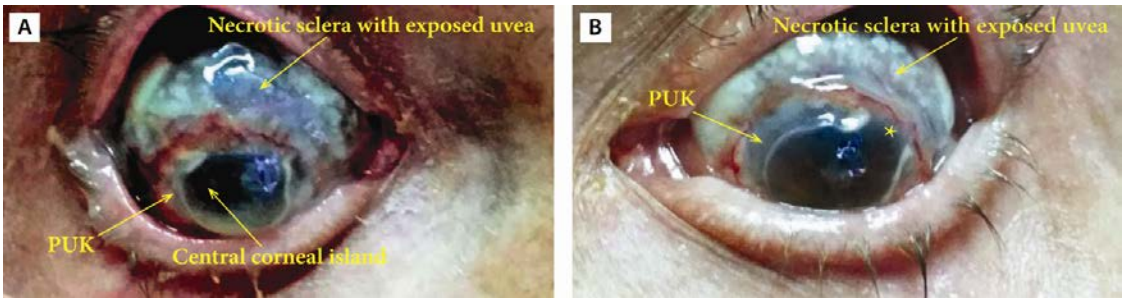

Fig. 1 – Clinical pictures of the right (A) and left eye (B) showing severe bilateral necrotising scleritis associated with peripheral ulcerative keratitis (PUK). Inflammation affected the entire anterior hemisphere of the sclera and peripheral cornea, leaving a central corneal island uninvolved in both eyes.

Fig. 2 – Slit-lamp examination of the patient's right eye showing flat anterior chamber (arrow) associated with corneal scarring following resolution of a severe anterior segment inflammation.

anterior peripheral ring-shaped iris synechiae. Pupillary block glaucoma and secondary angle closure may coexist in the eye as a consequence of severe anterior segment inflammation with uveitis.

In the left eye, following the resolution of PUK, the area of contiguous scleral necrosis developed into furrow-like corneal thinning with adjacent widespread anterior staphyloma (Figure 3). Corneal guttering extended circumferentially, leaving central corneal tissue unaffected. Mature cataract with extensive posterior iris synechiae precluded fundus examination (Figure 3D). Visual acuity in the left eye was L+P+. B-scan ultrasound showed a relatively smaller optic disc cup in the left eye than in the fellow eye. Intraocular pressure was within the normal range in both eyes (up to 21 mmHg) during the follow-up period of the patient. Active inflammation may suppress ciliary body function, whereas scleral necrosis and consequent scleral thinning may lead to increased aqueous outflow and decreased pressure.

Once the full remission had been achieved after 6 months, cataract surgery was performed in his left eye, the only functional one. A perioperative immunomodulatory drug regimen from Foster et al. [3] was adopted. The drug regimen has been proposed to control inflammation when cataract surgery is performed in uveitic eyes. Oral steroid prophylaxis (0.5 mg/kg/day) was commenced 1 day before the surgery and continued with tapering to the preoperative level over the following month (10 mg/day) while maintaining the dose of concurrent immunosuppressive therapy (CYP, 50 mg per day). In addition, topical dexamethasone 0.1% drops were frequently administered 1 day prior to surgery. Topical steroids were continued with tapering for 2 months postoperatively.

Surgery was performed under topical anaesthesia using the Infiniti Vision Phaco System (Alcon, Inc.). A nearly square clear corneal incision of 2.4 mm width was made at the 10 o'clock meridian with a stainless steel keratome. Corneal incision entry was placed at the inner edge of the peripheral corneal gutter in the nasal eye quadrant

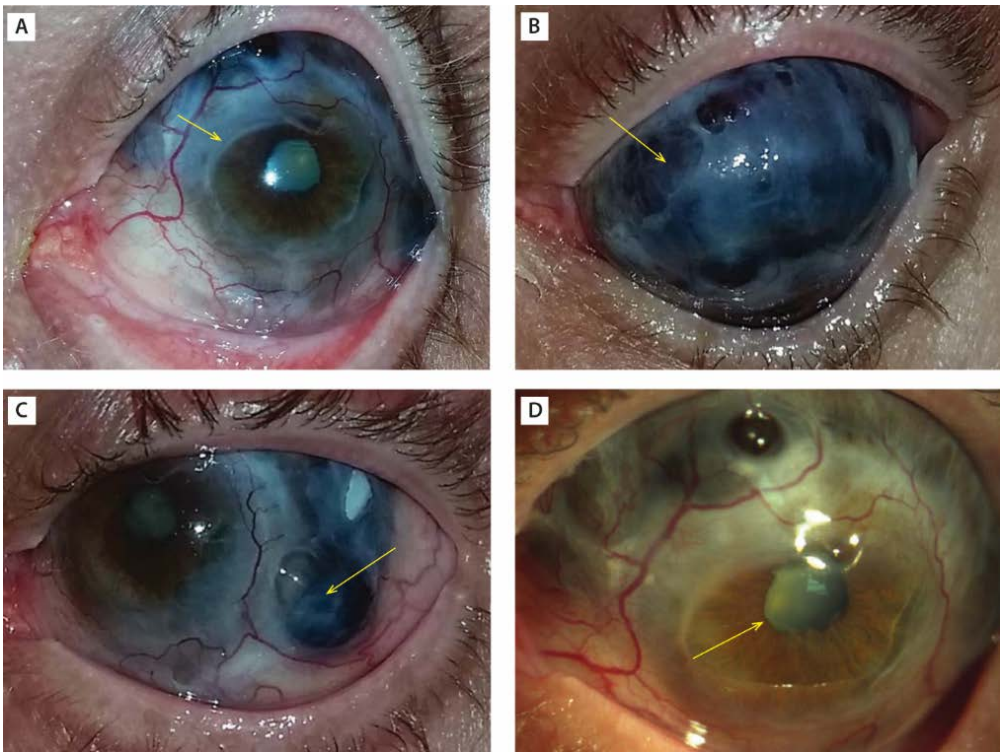

Fig. 3 – Clinical photograph of the patient's left eye, examined in daylight, showing the extensive area of anterior staphyloma and an inactive corneal gutter (arrow) following resolution of a severe sclerokeratitis episode (A). Clinical pictures of the upper scleral hemisphere of the left eye (B) and temporal scleral region in the same eye (C). Of note is the extraordinary degree of scleral loss with a uveal bulge (arrows). This uvea is covered by remaining scleral fibres and a thin layer of conjunctival epithelium only (B and C). Slit-lamp examination showing uveitic cataract with extensive posterior synechiae (D).

corresponding to the area of less severe adjacent anterior staphyloma (Figures 3A and 4). Another 0.6 mm side incision was created in the clear cornea, nearly 90 degrees from the main incision, and the anterior chamber was expanded with a viscoelastic substance comprising sodium hyaluronate 1% (Healon, AMO, Santa Ana, California, USA). Massive posterior iris synechiae were gently loosened by a conventional iris spatula. Next, an additional viscoelastic substance was injected to achieve adequate mydriasis. Trypan blue was used to enhance the visualization of the anterior lens capsule. Continuous curvilinear capsulorhexis measuring approximately 5.5 mm in diameter was performed with microforceps. After hydrodissection, phacoemulsification of the nucleus was performed

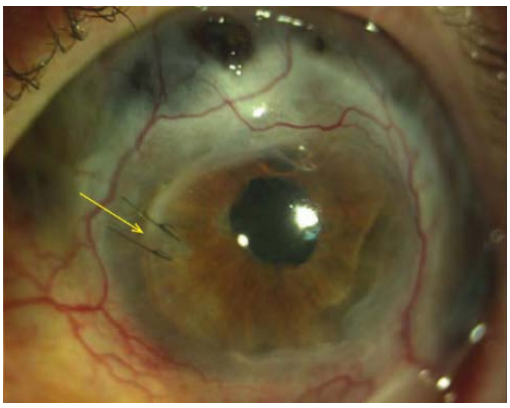

Fig. 4 – Slit-lamp examination of the patient's left eye one week after uneventful cataract surgery. A clear corneal incision was made in the nasal eye quadrant at the edge of the remaining central corneal island (arrow).

intorsional mode using a 0.9 mm mini/flared aspiration by-pass system (ABS; Alcon, Inc.) with a 45° Kelman tip with an ultra sleeve on an Infiniti Vision System (Alcon, Inc.). Torsional ultrasound amplitude was set at a maximum of 100% with linear control. Longitudinal phaco was off, and Intelligent Phaco was on. Surgery was performed with low fluidic parameters (aspiration flow rate 25 cc/min, bottle height 60 cm, and vacuum 350 mm Hg). The same parameters were used for direct chopping and quadrant removal. Following unimanual aspiration of the residual cortex, a foldable hydrophilic intraocular lens (IOL) (LEDAphil IOL, Optix) was implanted in the capsular bag with an injector through the main incision. The method for IOL implantation was strictly consistent with product manuals. Additional 10-0 nylon interrupted sutures were placed along the corneal guttering to provide adequate wound closure (Figure 4). Following cataract surgery, visual acuity slightly improved from L+P+ to 20/400 (Snellen). Fundus examination revealed untreatable findings, including a pale, atrophic optic disc and retinal gliosis.

The patient underwent regular clinical, serological, and immunologic examinations for disease activity and extent, as well as for treatment-related side effects. Serum anti-PR3 antibody titres and inflammatory markers were within the normal range before cataract surgery and during the 1-year follow-up period after the cataract surgery. Two years after the initial presentation and one year following the cataract surgery, the patient remained in remission with an uncorrected visual acuity of 20/400. The mechanisms contributing to vision loss in the right eye and restricted vision in his left eye most likely included vascular occlusion and inflammatory destruction of the retina and optic nerve, as well as retinal findings related to the underlying GPA disease associated with prolonged inflammation, loss of structural tissue and secondary glaucoma.

Discussion

The management of GPA-associated PUK is challenging and lacks definitive guidelines. Rituximab and CYP, either alone or combined with other agents, are the most successful agents in controlling inflammation [1]. In our patient, the introduction of high-dose pulsed methylprednisolone, in addition to maintenance doses of prednisone and increased oral CYP treatment, arrested the bilateral necrotising scleritis and PUK with generalized GPA associated with ophthalmic inflammation refractory to CYP induction regimen.

The presence of long-standing anterior uveitis associated with severe scleral tissue necrosis, as well as chronic corticosteroid usage, may lead to the formation of a cataract. Uneventful cataract extraction or any other surgical procedure can precipitate necrotising scleritis in patients with an underlying autoimmune vasculitic systemic disease [4, 5]. Therefore, surgery should be attempted only in the absence of scleral inflammation and during remission of systemic disease.

Although the diagnostic value of a positive PR-3 ANCA (c-ANCA) for GPA is well established, the usefulness of measuring ANCA titres in assessing disease activity and guiding therapy is somewhat controversial. In one study of 20 patients with refractory ophthalmic GPA, disease relapse seemed to be predicted by rising anti-PR3 titres [6]. However, this finding was not confirmed in another similar study on ocular GPA [7]. Nevertheless, since increases in ANCA occur in some patients prior to relapse, serial measurement of the c-ANCA titre was performed in our patient. Serum anti-PR3 antibody titres were within the normal range before cataract surgery and during the follow-up period after cataract surgery, which lasted 12 months.

Successful surgery generally requires a quiet eye devoid of active inflammation for at least 3 months [1]. In our patient, phacoemulsification was performed 6 months after the full remission of ocular disease activity had been achieved. Preoperative addition or increase in systemic therapy, mainly corticosteroids, seems to be mandatory in eyes at risk of developing disease recurrences, such as necrotising scleritis or PUK. In a study by O'Donoghue [8], patients who had recovered from SINS and required further ocular surgical procedures were given perioperative pulsed methylprednisolone for protection against the recurrence of the necrotising disease. It was demonstrated here that standard perioperative oral steroid prophylaxis, which is currently proposed to control inflammation for cataract surgery in uveitic eyes, was also sufficient to prevent SINS.

Phacoemulsification using a clear corneal approach is generally preferred in patients in remission from PUK [1]. O'Donoghue et al. [8] showed that surgically induced necrotising scleritis usually occurred after cataract surgery and that the disease site was closely related to the wound site; 80% of these sites were limbal. This finding suggests that greater relative vascular disruption associated with the limbal approach may be a contributing factor in scleral disease development [8]. Dick et al. [9] also demonstrated that compared to surgery through a sclerocorneal incision, cataract extraction through a clear corneal incision results in reduced inflammation in the immediate postoperative period. Generally, a clear corneal

incision is made temporally. Here, a clear corneal incision was rotated to the nasal eye quadrant. In this area, the adjacent anterior staphyloma was slightly less severe than the extreme scleral thinning in both superior and temporal eye quadrants (Figures 3B and 3C). A corneal incision was made on the corneal guttering; thus, single corneal sutures with 10-0 nylon were required to ensure adequate wound closure (Figure 4). Interestingly, although necrotising scleritis after ocular surgery has been described in patients after a cataract surgery via a corneal incision, O'Donoghue et al. [8] found that sutures used to close the wound had entered the sclera.

Conclusion

Phacoemulsification can be successfully performed in a patient with coexisting uveitic cataract and staphyloma of the entire anterior globe following necrotising scleritis with PUK. Preoperative and postoperative control of inflammation, careful surgical planning, and meticulous surgical techniques are critically important for optimal surgical outcomes. The final visual outcome depends on the posterior segment complications of necrotising scleritis associated with GPA.

R E F E R E N C E S

1. *Ebrahimiadib N, Modjtahedi BS, Roohipoor R, Anesi SD, Foster CS.* Successful treatment strategies in granulomatosis with polyangiitis-associated peripheral ulcerative keratitis. Cornea 2016; 35(11): 1459–65.
2. *Alfawaz AM.* Successful cataract surgery in a patient with refractory Wegener's granulomatosis effectively treated with rituximab: A case report. Saudi J Ophthalmol 2016; 30(3): 194–7.
3. *Foster CS, Kothari S, Anesi SD, Vitale AT, Chu D, Metzinger JL,* et al. The Ocular Immunology and Uveitis Foundation preferred practice patterns of uveitis management. Surv Ophthalmol 2016; 61(1): 1–17.
4. *Solebo AL, Ahmadi-Lari S, Petrou P, Westcott M.* Bilateral surgically induced scleritis following phacoemulsification. J Cataract Refract Surg 2007; 33(8): 1485–7.
5. *Stokes J, Wright M, Ramaesh K, Smith C, Dhillon B.* Necrotizing scleritis after intraocular surgery associated with the use of polyester nonabsorbable sutures. J Cataract Refract Surg 2003; 29(9): 1827–30.
6. *Joshi L, Lightman SL, Salama AD, Shirodkar AL, Pusey CD, Taylor SR.* Rituximab in refractory ophthalmic Wegener's granulomatosis: PR3 titers may predict relapse, but repeat treatment can be effective. Ophthalmology 2011; 118(12): 2498–503.
7. *Joshi L, Tanna A, McAdoo SP, Medjeral-Thomas N, Taylor SR, Sandhu G,* et al. Long-term outcomes of rituximab therapy in ocular granulomatosis with polyangiitis: Impact on localized and nonlocalized disease. Ophthalmology 2015; 122(6): 1262–8.
8. *O'Donoghue E, Lightman S, Tuft S, Watson P.* Surgically induced necrotising sclerokeratitis (SINS)-precipitating factors and response to treatment. Br J Ophthalmol 1992; 76(1): 17–21.
9. *Dick HB, Schwenn O, Krummenauer F, Krist R, Pfeiffer N.* Inflammation after sclerocorneal versus clear corneal tunnel phacoemulsification. Ophthalmology 2000; 107(2): 241–7.
10. *Vasavada AR, Praveen MR, Vasavada VA, Raj SM, Asnani PK, Garg VS.* Impact of high and low aspiration parameters on postoperative outcomes of phacoemulsification: randomized clinical trial. J Cataract Refract Surg 2010; 36(4): 588–93.
11. *Vasavada V, Raj SM, Praveen MR, Vasavada AR, Henderson BA, Asnani PK.* Real-time dynamic intraocular pressure fluctuations during microcoaxial phacoemulsification using different aspiration flow rates and their impact on early postoperative outcomes: a randomized clinical trial. J Refract Surg 2014; 30(8): 534–40.

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CASE REPORT

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Several different cytogenetic clones arising during treatment of Philadelphia positive chronic myeloid leukemia with tyrosine kinase inhibitors lead to the progression into Philadelphia negative acute myeloid leukemia

Različiti citogenetski klonovi nastali tokom lečenja Filadelfija pozitivne hronične mijelodne leukemije inhibitorima tirozin kinaze sa progresijom u Filadelfija negativnu akutnu mijeloidnu leukemiju

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Abstract

Introduction. Additional karyotype abnormalities in the Philadelphia-positive (Ph+) clone can emerge during the progression of chronic myeloid leukemia (CML) and are often associated with the resistance to treatment with tyrosine kinase inhibitors (TKI). Sometimes, during the TKI treatment, karyotype abnormalities can appear in the Philadelphia-negative (Ph-) cells as well but do not seem to adversely affect the outcome except for chromosome 7 abnormalities. **Case report.** The patient presented was in the chronic phase of Ph+ CML with highly diverse karyotype abnormalities. The abnormalities appeared in three unrelated clones during the TKIs treatment, followed by the evolution of the disease into acute myeloid leukemia (AML). The primary Ph+ clone was revealed during the chronic phase of CML, and therapy with imatinib mesylate was commenced. After a three-year hematologic and cytogenetic remission period, the evolution of the primary clone was noticed. Nilotinib was introduced, leading to a good molecular response and the disappearance/loss of the Ph+ clone with additional abnormalities but with the appearance of the Ph- clone with trisomy 8. Finally, after 5.5 years of nilotinib therapy, the Ph- clone with monosomy 7 occurred during the deep molecular response for BCR-ABL. At that time, the FISH analysis for trisomy 8 was negative, but the rise in blast count was noticed in the bone marrow, and the diagnosis of the secondary AML was established soon after. **Conclusion.** The achievement of the deep molecular response in CML patients does not rule out regular cytogenetic testing of their bone marrow. This is of crucial importance for detecting adverse karyotype abnormalities leading to the development of the myelodysplastic syndrome and AML.

Key words:
leukemia, myelogenous, chronic, bcr-abl positive; leukemia, myeloid, acute; karyotyping; enzyme inhibitors; cytogenetics.

Apstrakt

Uvod. Dodatne kariotipske abnormalnosti u Filadelfija-pozitivnom (Ph+) klonu mogu se javiti tokom progresije hronične mijeloidne leukemije (CML) i često su povezane sa rezistencijom na terapiju tirozin kinaznim inhibitorima (TKI). Ponekad se tokom terapije TKI kariotipske abnormalnosti javljaju i u Filadelfija-negativnim (Ph-) ćelijama, ali ne utiču na progresiju bolesti, izuzev abnormalnosti hromozoma 7. **Prikaz bolesnika.** Kod bolesnice u hroničnoj fazi CML, tokom lečenja TKI uočene su kariotipske abnormalnosti prisutne u tri nezavisna klona sa evolucijom bolesti u akutnu mijeloidnu leukemiju (AML). Primarni Ph+ klon je otkriven tokom hronične faze CML i započeta je terapija imatinib mesilatom. Nakon tri godine hematološke i citogenetske remisije, uočena je evolucija primarnog klona. Započeta je terapija nilotinibom koja je dovela do molekularnog odgovora i povlačenja Ph+ klona sa dodatnim aberacijama, ali i pojavljivanja novog Ph- klona sa trizomijom 8. Nakon 5,5 godina lečenja nilotinibom i postizanja kompletnog molekularnog odgovora, uočen je Ph- klon sa monozomijom 7. Fluorescentna *in situ* hibridizacija (FISH) pokazala je

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odsustvo trizomije 8 i prisustvo monozomije 7. Istovremeno, registrovan je porast broja blasta u koštanoj srži i ubrzo je postavljena dijagnoza sekundarne AML. **Zaključak.** Postizanje kompletnog molekularnog odgovora primenom TKI terapije ne treba da isključi redovno citogenetsko testiranje koštane srži bolesnika sa CML. Otkrivanje kariotipskih abnormalnosti sa lošom prognozom je od velikog značaja zbog mogućnosti razvoja sekundarnih maligniteta – mijelodisplastičnog sindroma i AML.

Ključne reči:
leukemija, mijeloidna, hronična, bcr-abl pozitivna; leukemija, mijelocitna akutna; kariotip, određivanje; enzimi, inhibitori; citogenetika.

Introduction

The BCR-ABL fusion gene, generating the Philadelphia chromosome (Ph), is a sole genetic abnormality in 90% of chronic myeloid leukemia (CML) patients in the chronic phase [1]. With the progression of the disease, additional karyotype changes in the Philadelphia positive (Ph+) clone emerge and are often associated with resistance to imatinib mesylate and/or nilotinib. The resistance can be a consequence of one of the numerous mutations in the tyrosine kinase domain or some other underlying mechanism and is usually overwhelmed with some of the novel tyrosine kinase inhibitor (TKI) drugs. Sometimes, during the TKI treatment, karyotype changes in the Philadelphia negative (Ph-) cells can appear [2]. These aberrations, similar to those frequently seen in the myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), include trisomy 8 (+8), the deletion or monosomy of chromosome 7 (del(7q)/-7), and nullisomy of Y (-Y). However, chromosome abnormalities in the Ph- clone do not seem to adversely affect the outcome with the exception of the chromosome 7 abnormalities [1]. Monosomy 7 and del (7q) require frequent bone marrow follow-up as several case reports indicate the development of the MDS and subsequent AML [3–7].

A CML patient with highly diverse karyotype abnormalities was presented in this paper. The abnormalities appeared in three unrelated clones during the treatment with imatinib mesylate and nilotinib. The patient mentioned developed AML in the end.

Case report

A 44-year-old female was diagnosed with CML in the chronic phase in April 2002. The cytogenetic analysis revealed the translocation t(9;22) (q34;q11) (Ph chromosome) in 20/20 mitoses (Figure 1A). The prognostic Sokal and Hasford scores implied a low-risk patient at presentation, without comorbidities or any other additional treatment. The antileukemic therapy was commenced with hydroxyurea and interferon with a subsequent increase in interferon dosage. Regardless of achieving hematological remission, no cytogenetic response was obtained after 6 months of the treatment. As the patient had no available stem cell donor, imatinib mesylate in a standard dose (400 mg a day) was started in September 2002. Six months later, the patient achieved partial cytogenetic response (PCgR) with 53% Ph-negative metaphases. After 12 months, a complete cytogenetic response (CCgR) was achieved, although a b2a2 transcript of the BCR-ABL fusion was detected by "nested" RT-PCR. During the next three years, the patient remained in a stable chronic phase with CCgR, on 400 mg of imatinib. However, in August 2005, the follow-up cytogenetics revealed complex translocation t(5;6;12) with t(9;22) (Figure 1B) in all analyzed cells, without any clinical or laboratory sign of disease progression. The tyrosine-kinase domain mutations were negative by direct sequencing.

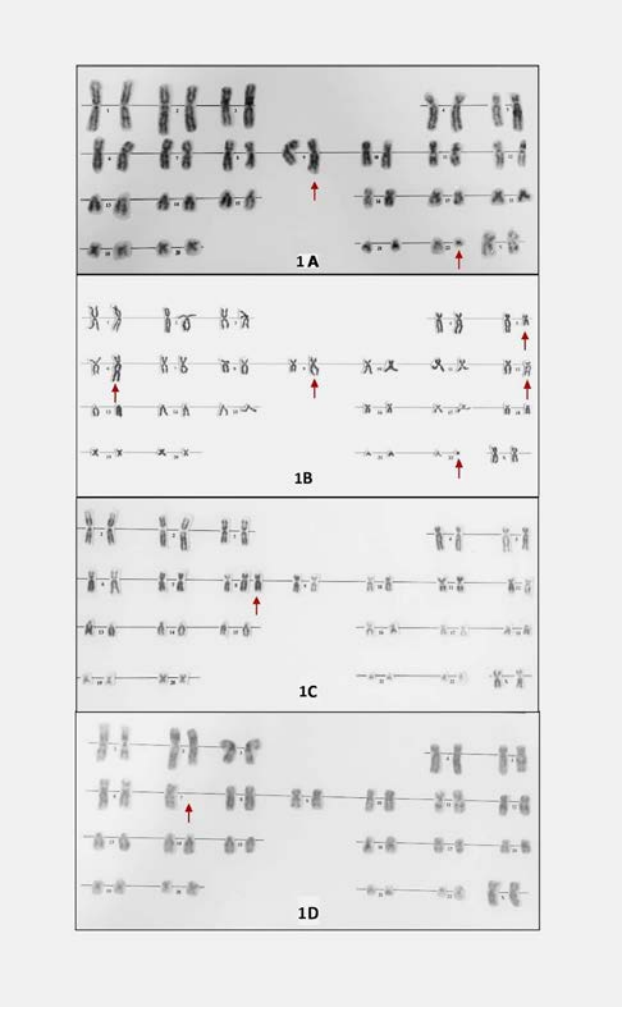

Fig. 1(A–D): A) (46,XX,t(9;22) (q34;q11) at presentation; B) [46,XX,t(5;6;12)(q14?;q21?;q23?),t(9;22)(q34;q11) imatinib (400 mg), TT 36 m]; C) 47,XX,+8 nilotinib (800 mg), TT 6 m; D) 45,XX,-7 nilotinib (800 mg), TT 66 m.

The patient was treated with an escalated dosage of imatinib (800 mg a day) since October 2006 without success. The patient was switched to nilotinib, 800 mg a day, from July 2008 through the "compassionate use program".

However, due to hematologic and hepatic toxicity, after only a month, nilotinib was reduced to 400 mg. After 6 months of 400 mg nilotinib, the patient achieved CCgR again, but at 12 months, the reappearance of Ph+ clone with t(5;6;12) was noticed in 45% of metaphases, suggesting PCgR. Along with the Ph-positive cells, the Ph-negative clone with +8 was seen in 10% of mitoses. After the recovery of blood counts and the hepatic function 6 months later, the patient was escalated to the full dosage of nilotinib (800 mg) again. She achieved a major cytogenetic response [MajCgR, 10% of Ph+ with t(5;6;12) clone] at 24 months of nilotinib treatment, and finally, after 30 months on nilotinib, the patient achieved CCgR. During the next 3 years on 800 mg of nilotinib, her follow-up showed CCgR and a stable molecular response (MR3), though the Ph-negative clone with +8 was constantly present in 10–30% of metaphases (Figure 1C).

However, during the regular follow-up in February 2014 (5.5 years of nilotinib therapy), profound neutropenia without anemia and thrombocytopenia was noticed (hemoglobin 120 g/L, white blood cells 2.5×10^9/L, 76% lymphocytes, 4% blasts, 10% monocytes, platelets 258 × 10^9/L) together with elevated transaminases (alanine aminotransferase − ALT 86 U/L). Immediate bone marrow evaluation revealed dysplastic changes in erythroid and megakaryocyte lineages together with 6% of blasts. The karyotype revealed the poor quality of chromosomes, but the clonal change with a loss of one chromosome from the C group was evident in 60% of mitoses. The fluorescence *in situ* hybridization (FISH) analysis with the BCR-ABL probe was negative both for the Ph chromosome and trisomy 8, but the CEP7 probe revealed the monosomy of chromosome 7 in 80% of interphase nuclei.

The administration of nilotinib was stopped. Two months later, the patient's bone marrow was hypocellular with less dysplasia than at the previous examination but with the rise in blast count (12%). The cytogenetic examination confirmed -7 in all analyzed cells (20/20) and the absence of trisomy 8 (Figure 1D). Real-time qualitative polymerase chain reaction (RQ-PCR) for BCR-ABL revealed a deep molecular response, MR4.

After one month, in April 2014, further evaluation revealed leukemic progression and development of AML (30% of blasts) confirmed by the flow cytometry immunophenotype (HLA-DR^{med}, CD34^{high}, CD117^{med}, CD13^{high}, CD33^{med}, CD7^{+}). The patient was treated with the antileukemic treatment (3 + 7 regimen) without success, followed by the "salvage" protocol FLAG-Ida without achieving any morphological or cytogenetic response. Unfortunately, the patient died of aplasia during the treatment.

Cytogenetic study and response criteria

The cytogenetic study was performed on unstimulated bone marrow cells using a standard technique. The Giemsa-banded metaphases were analyzed, and the result was reported by the International System for Human Cytogenetic Nomenclature standards, 2013. The cytogenetic response was classified according to the standard of the UK Medical Research Council practice as complete (0% Ph+ metaphases), major (1–34% Ph+), partial (35–65% Ph+ metaphases), minor (66–95% Ph+) and no response (95–100% Ph+). The cytogenetic clonal evolution was defined as the presence of any abnormality other than a single Ph chromosome.

Reverse transcription-polymerase chain reaction (RT-PCR) and "nested"

The total RNA was extracted from peripheral blood cells according to the guanidine thiocyanate-phenol-chloroform extraction method [8]. Reverse transcription was performed on 1 μg of total RNA after heating at 65 ºC for 15 minutes. Reverse transcription was performed with the 1st Strand cDNA Synthesis Kit for RT-PCR (AMV) (Roche Diagnostics Corporation, Indianapolis, IN, USA) according to the manufacturer's manual. The amplification was done with slight modifications as described by Moravcová et al. [9].

The FISH analysis for centromere regions of chromosomes 7 and 8 (CEP7 and CEP8) was performed on interphase nuclei and metaphase cells according to the manufacturer's instructions (Vysis/Abbott Laboratories, Des Plaines, IL).

Discussion

Our case demonstrates highly diverse karyotype changes appearing one after another in three unrelated clones during the treatment with tyrosine kinase inhibitors. Complex aberrations in the Ph-positive clone emerged during the management with imatinib, while +8 and -7 appeared separately in the Ph-negative clones during the nilotinib treatment.

Karyotype changes in the Ph+ clone emerged 40 months after the imatinib therapy was started as the only sign of disease relapse. This distinctive karyotype included complex translocation and the rare event of centromere fission, which were previously published [10, 11]. A negative search for mutations by Sanger sequencing in the kinase domain further contributed to the complexity of the case.

Only after the introduction of a more potent TKI treatment, nilotinib, the Ph+ clone slowly decreased, but trisomy 8 in the Ph-negative cells appeared. The CCgR was achieved after 30 months on nilotinib, while +8 remained and existed in up to 30% of analyzed cells during the next 2.5 years of follow-up.

Nota bene, the Ph-negative clones are less frequent in patients treated with second-generation TKIs and after the failure of imatinib due to higher pressure on leukemic and residual normal hematopoiesis [12]. However, when present, their type and frequency are very similar to those seen in patients on imatinib, as well as their incidence and effect in evolution to MDS/AML [12].

Our patient developed secondary AML after 66 months of the nilotinib treatment. The cytogenetic and FISH analysis revealed -7 in 60% of metaphases and 80% of interphase

nuclei, respectively, along with the absence of BCR-ABL and +8. The clone with -7 quickly progressed to 100% of the analyzed cells in two months, while RQ-PCR still showed a stable MR4. Despite introducing a high-dose therapy for AML, the patient died 6 months after the diagnosis of the secondary AML had been established.

The Ph-negative clones with -7 were described in the CML cases with a high propensity to evolve into MDS/AML [13]. However, there have been rare cases with -7 without disease evolution [4, 5, 14].

In several studies, factors contributing to the appearance of chromosomal aberrations in the Ph-negative clone have been discussed. The previous cytotoxic treatment [13], the negative effect of TKIs on DNA repair mechanisms [15–17], or the innate genetic instability in the CML marrow [18] are described as potential causes of the Ph-negative clone appearance. However, among all the abnormalities, only those involving chromosome 7 [del(7q) and/or -7] bear a higher risk of secondary malignancies [5, 19]. We can conclude that while the patient was in a stable chronic phase of CML, complex chromosomal aberrations in the Ph-positive cells might reflect a highly unstable genome, which could contribute to a further lower sensitivity to a subsequent alternative treatment and thus, negatively affect overall survival.

Other parameters that could lead to the development of MDS/AML are pretreatment with interferon or hydroxyurea, a persistent aberration in the Ph-negative clone, and clone size > 50% [19]. Unfortunately, our patient had all the negative features mentioned above in developing secondary malignant disease.

During the treatment with TKIs, it is highly important to reveal the biological diversity of the Ph-negative clones, which in some patients can lead to disease transformation (clone with -7), while in others, it does not have the propensity towards secondary hematological malignancy (clone with +8). Minimal investigations should include blood test results (cytopenia), bone marrow morphology (dysplastic changes and blast count), and cytogenetic (evidence of the Ph-negative clones and -7). In cases with the additional Ph-negative clones, further evaluation of changes with the FISH and real-time PCR analyses are highly recommended.

Conclusion

The evolution of karyotype and the occurrence of diverse clones arising from the stem cell level in our patients, warrants the need for thorough follow-up and evaluation of all related hematological and biological findings during the treatment with tyrosine kinase inhibitors, including the standard karyotype, although, some study groups tend to omit any bone marrow evaluation in the current monitoring schedule.

Acknowledgement

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R E F E R E N C E S

1. *Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF,* et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood 2013; 122(6): 872–84.
2. *Deininger MW, Cortes J, Paquette R, Park B, Hochhaus A, Baccarani M,* et al. The prognosis for patients with chronic myeloid leukemia who have clonal cytogenetic abnormalities in Philadelphia chromosome-negative cells. Cancer 2007; 110(7): 1509-19.
3. *Perel JM, McCarthy C, Walker O, Irving I, Williams B, Kennedy GA.* Clinical significance of development of Philadelphia chromosome negative clones in patients with chronic myeloid leukemia treated with imatinib mesylate. Haematologica 2005; 90 (Suppl): ECR25.
4. *Bacher U, Hochhaus A, Berger U, Hiddemann W, Hehlmann R, Haferlach T,* et al. Clonal aberrations in Philadelphia chromosome negative hematopoiesis in patients with chronic myeloid leukemia treated with imatinib or interferon alpha. Leukemia 2005; 19(3): 460–3.
5. *Kovitz C, Kantarjian H, Garcia-Manero G, Abruzzo LV, Cortes J.* Myelodysplastic syndromes and acute leukemia developing after imatinib mesylate therapy for chronic myeloid leukemia. Blood 2006; 108(8): 2811–3.
6. *Terre C, Eclache V, Rousselot P, Imbert M, Charrin C, Gervais C,* et al. Report of 34 patients with clonal chromosomal abnormalities in Philadelphia negative cells during imatinib treatment of Philadelphia-positive chronic myeloid leukemia. Leukemia 2004; 18(8): 1340-6.
7. *Andersen MK, Pedersen-Bjergaard J, Kjeldsen L, Dufva IH, Brøndum-Nielsen K.* Clonal Ph-negative hematopoiesis in CML after therapy with imatinib mesylate is frequently characterized by trisomy 8. Leukemia 2002; 16(8): 1390–1393.
8. *Chomczynski P, Sacchi N.* Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162(1): 156–9.
9. *Moravcová J, Lukásová M, Starý J, Haskovec C.* Simple competitive two-step RT-PCR assay to monitor minimal residual disease in CML patients after bone marrow transplantation. Leukemia 1998; 12(8): 1303–12.
10. *Denčić-Fekete M, Đorđević V, Storlazzi C. T, Janković G, Bogdanović A, Jovanović J,* et al. t(5;6;12) associated with resistance with imatinib mesylate in chronic myeloid leukemia. Int J Hematol 2009; 89(4): 508–12.
11. *Storlazzi CT, Albano F, Dencić-Fekete M, Djordjević V, Rocchi M.* Late-appearing pseudocentric fission event during chronic myeloid leukemia progression. Cancer Genet Cytogenet 2007; 174(1): 61–7.
12. *Baldazzi C, Luatti S, Marzocchi G, Stacchini M, Gamberini C, Castagnetti F,* et al. Emergence of clonal chromosomal abnormalities in Philadelphia negative hematopoiesis in chronic myeloid leukemia patients treated with nilotinib after failure of imatinib therapy. Leukemia Res 2009; 33(12): e218–20.
13. *Ribeiro de Mello Conchon M, Bendit I, Ferreira P, Lima W, Kumeda C, Dias L,* et al. Emergence of abnormal clone with monosomy 7 in Philadelphia negative cells of CML patients treated with tyrosine kinase inhibitors. Int J Hematol 2009; 89(1): 123–5.
14. *McMullin MF, Humphreys M, Byrne J, Russell NH, Cuthbert RJ, O'Dwyer ME.* Chromosomal abnormalities in Ph- cells of pa-

tients on imatinib. Blood 2003; 102(7): 2700–1; author reply 2701.

15. *Bumm T, Müller C, Al-Ali HK, Krohn K, Shepherd P, Schmidt E,* et al. Emergence of clonal cytogenetic abnormalities in Ph- cells in some CML patients in cytogenetic remission to imatinib but restoration of polyclonal hematopoiesis in the majority. Blood 2003; 101(5): 1941–9.
16. *Kharbanda S, Pandey P, Jin S, Inoue S, Bharti A, Yuan ZM,* et al. Functional interaction between DNA-PK and c-Abl in response to DNA damage. Nature 1997; 386(6626): 732–5.
17. *Fabarius A, Haferlach C, Müller M, Erben P, Lahaye T, Giehl M*, et al. Dynamics of cytogenetic aberrations in Philadelphia chromosome positive and negative hematopoiesis during dasatinib therapy of chronic myeloid leukemia patients after imatinib failure. Haematologica 2007; 92(6): 834–7.
18. *Tanaka H, Tanaka K, Oguma N, Ito K, Ito T, Kyo T,* et al. Effect of interferon-α on chromosome abnormalities in treated chronic myelogenous leukemia patients. Cancer Genet Cytogenet 2004; 153(2): 133–43.
19. *Groves MJ, Sales M, Baker L, Griffiths M, Pratt N, Tauro S*. Factors influencing a second myeloid malignancy in patients with Philadelphia-negative -7 or del(7q) clones during tyrosine kinase inhibitor therapy for chronic myeloid leukemia. Cancer Genet 2011; 204(1): 39–44.

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LETTER TO THE EDITOR

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Prevalence of depression in people over 65 years of age in Serbia – public health significance

Prevalencija depresije kod osoba starijih od 65 godina u Srbiji – javnozdravstveni značaj

To the Editor:

Depression, with 350 million affected people worldwide, is one of the leading mental health issues and a great public health concern. Depression is the second top medical condition among all illnesses and injuries based on Disability-Adjusted Life Year (DALY) [1]. It currently contributes to about 12% of the total number of years lived with disabilities, and if this trend continues, by 2030, depressive disorders will become the leading global diagnosis among the disease burden causes [2].

Depression is manifested as low mood, loss of interest and satisfaction, with feelings of guilt and inferiority, sleep and appetite disorders, loss of energy, and poor concentration [3]. In contrast to the "normal" feeling of sadness that can occur in any person, depression in its strength, duration, and degree of dysfunction deviates from everyday mood swings [4].

Depression, with its severe impacts on the personal, interpersonal, and social lives of the affected individuals, has been recognized as the most frequent mental health issue in the elderly (aged 65 and older) [5]. In addition to being a great source of disturbances in life quality and social functioning, depression increases morbidity, mortality, and disability; hence, it has significant social and economic outcomes [6]. Moreover, depressive symptoms among the elderly increase the risks of suicide [7]. Depressive episodes had been present in about 60% to 90% of those aged 65 and over who committed suicide [8].

According to the World Health Organization (WHO) data, total depression prevalence among the elderly ranges from 10%–20% [9]. The prevalence rates of late-life depression are estimated at 8%–23% in Europe [10], 13%–25% in India [6], 15%–19% in America [11], 23.6% in China [12], 30% in Brazil [13] and 8% in Australia [14], with more frequent occurrences in older women [15].

There are very few studies of late-life depression in African countries, thus the issue remains largely under-studied [16]. Huge variations in the prevalence of depression among the elderly stem from regional, racial, sociodemographic, and cultural diversity. However, they can also be seen as a result of the fact that the current surveys and studies have used different methods of data collection and geriatric depression assessment [9].

The results presented were obtained through the evaluation of depression prevalence during the third Serbian Population Health Survey conducted by the Ministry of Health of the Republic of Serbia in 2013. The questionnaires used as instruments in this study were created in accordance with the questionnaires of the European Health Interview Survey (EHIS) – Second Wave (EHIS wave 2) [17]. Depression was evaluated with the 8-item Patient Health Questionnaire Depression (PHQ-8) scale [18]. The target population for this particular analysis were the individuals aged 65 and over who lived in private households in Serbia at the time of the data collection. The number of participants who fulfilled this age criterion was 3,540. The final sample of this study thus comprised 3,540 elderly adults. Based on the PHQ-8 score, 10% of the Serbian population aged 65 and over had a depressive episode. The results showed that the depression prevalence was twice higher in women (12.7%) than in men (6.5%). Mild depression symptoms were present in every fifth woman (21.2%) and every eighth man (12.7%). The average PHQ-8 value in the population aged 65 and over was 3.5 and was higher in the female (4.1) than in male (2.6) respondents (Table 1).

These results indicate the urgency of solving the issues of depression among the elderly as one of the priorities of public health in order to reduce the burdens of disability and enhance the overall health of the elderly. Thus, identifying the elderly population as a subpopulation that is at significant risk of developing depression is crucial. Therefore, early diagnosis and early treatment are vital factors in reducing the negative consequences that depression leaves on individuals and the community as a whole.

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Table 1

Depression prevalence in the Serbian population aged 65 and over

PHQ-8 score	Sex		Total	*p*
	women	men		
	n (%)	n (%)	n (%)	
0–4 (no symptoms)	1,330 (66.1)	1,235 (80.8)	2,656 (72.5)	< 0.001
5–9 (subsyndromal depression)	427 (21.2)	194 (12.7)	621 (17.5)	
10–24 (depression)	255 (12.7)	99 (6.5)	354 (10.0)	
10–14 (moderate)	149 (7.4)	56 (3.7)	205 (5.8)	
15–19 (moderately severe)	65 (3.2)	28 (1.8)	93 (2.6)	
20–24 (severe)	41 (2.1)	15 (1.0)	56 (1.6)	
Total	2,012 (12.7)	1,528 (6.5)	3,540 (10.0)	
Average PHQ-8 score	4.1	2.6	3.5	< 0.001

PHQ – Patient Health Questionnaire.

Depression in the elderly can be controlled by providing opportunities for the elderly to be more involved and active in social events and activities. Besides, the financial support to the elderly and their financial independence would have a positive impact on their mental well-being. Moreover, raising public awareness of the mental struggles affecting older populations, early diagnostics, and the appropriate management of the most vulnerable groups would reduce their grief, enhance their life quality, and would eventually be beneficial for whole societies [19].

We expect the results of this research to be the starting point for decision-makers and health policy-makers in our country in creating strategies to improve mental health and reduce depressive disorders in the elderly population. This can be done by promoting active and healthy aging, which involves creating conditions and environments that encourage well-being and enable people to lead integrated, healthy, and high-quality lives.

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R E F E R E N C E S

1. *World Health Organization*. Depression and other common mental disorders: global health estimates. Geneva: World Health Organization; 2017.
2. *Buvneshkumar M, John KR, Logaraj M*. A study on prevalence of depression and associated risk factors among elderly in a rural block of Tamil Nadu. Indian J Public Health 2018; 62(2): 89–94.
3. *World Health Organization*. The World Health Report 2001. Mental Health: New Understanding, New Hope. Geneva: World Health Organization; 2001.
4. European Brain Council (EBC). Depression Fact Sheet. 2011 [cited 2019 February 14]. Available from: http://ebc-brussels.org/wp-content/uploads/2015/07/Depression-fact-sheet-July-2011.pdf
5. *Mirkena Y, Reta MM, Haile K, Nassir Z, Sisay MM*. Prevalence of depression and associated factors among older adults at ambo town, Oromia region, Ethiopia. BMC Psychiatry 2018; 18(1): 338.
6. *Behera P, Sharan P, Mishra AK, Nongkynrih B, Kant S, Gupta SK*. Prevalence and determinants of depression among elderly persons in a rural community from northern India. Natl Med J India 2016; 29(3): 129–35.
7. *Wright PP, Thorpe CW*. Triple Threat Among the Elderly: Depression, Suicide Risk, and Handguns. J Emerg Nurs 2016; 42(1): 14–8.
8. *Kopp-Bigault C, Walter M*. Prevention of suicide of the elderly in France. To a multimodal strategy against depression and isolation: CQFDi. Encephale 2019; 45 (Suppl 1): S35–7. (French)
9. *Rangaswamy SM*. World Health Report: Mental Health: New understanding New Hope. Geneva, Switzerland: World Health Organization; 2001.
10. *Barua A, Ghosh MK, Kar N, Basilio MA*. Prevalence of depressive disorders in the elderly. Ann Saudi Med 2011; 31(6): 620–4.
11. *Li D, Zhang DJ, Shao JJ, Qi XD, Tian L*. A meta-analysis of the prevalence of depressive symptoms in Chinese older adults. Arch Gerontol Geriatr 2014; 58(1): 1–9.
12. *Cahoon CG*. Depression in Older Adults. Am J Nurs 2012; 112(11): 22–30; quiz 31.
13. *Nogueira EL, Rubin LL, Giacobbo Sde S, Gomes I, Cataldo Neto A*. Screening for depressive symptoms in older adults in the Family Health Strategy, Porto Alegre, Brazil. Rev Saude Publica 2014; 48(3): 368–77. (England, Portuguese)
14. *Pirkis J, Pfaff J, Williamson M, Tyson O, Stocks N, Goldney R,* et al. The community prevalence of depressionin older Australians. J Affect Disord 2009; 115(1–2): 54–61.
15. *He G, Xie JF, Zhou JD, Zhong ZQ, Qin CX, Ding SQ*. Depression in left-behind elderly in rural China: Prevalence and associated factors. Geriatr Gerontol Int 2016; 16(5): 638–43.
16. *Reynolds CF 3rd, Dias A, Cohen A, Morse J, Anderson SJ, Cuijpers P,* et al. Preventing Late-Life Depression: Lessons in Intervention Development From Goa, India. Innov Aging 2018; 1(3): igx030.
17. European Health Interview Survey (EHIS wave 2) Methodological manual 2013. Available from:

http://ec.europa.eu/eurostat/documents/3859598/5926729/KS-RA-13-

18. *Kroenke K, Strine TW, Spritzer RL, Williams JB, Berry JT, Mokdad AH*. The PHQ-8 as a measure of current depression in the general population. J Affect Disord 2009; 114(1–3): 163–73.
19. *Mitchell PB, Harvey SB*. Depression and the older medical patient--when and how to intervene. Maturitas 2014; 79(2): 153–9.

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Suppurative gastritis in a HIV-positive patient: A case report
Vojnosanit Pregl 2020; 77(7): 751–753.

STOMATOGNATHIC DISEASES

Vladimir Sinobad, Ljiljana Strajnić, Tamara Sinobad
Skeletal changes in patients with mandibular prognathism after mandibular set back and bimaxillary surgery – A comparative cephalometric study
Vojnosanit Pregl 2020; 77(4): 395–404.

RESPIRATORY TRACT DISEASES

Slavica Konević, Nela Djonović, Dušan Djurić, Ljiljana Marković-Denić, Dobrila Vasić, Jelena Martinović
Impact of educational intervention for correct inhaler technique on the quality of life of children with asthma
Vojnosanit Pregl 2020; 77(8): 859–865.

Jovan Matijašević, Srdjan Gavrilović, Ilija Andrijević, Ana Andrijević, Svetislava Milić, Marija Vukoja
Inhalatory and intravenous colistin in treating ventilator-associated pneumonia due to *Acinetobacter species*: should we combine them?
Vojnosanit Pregl 2020; 77(8): 832–838.

Goran Koraćević, Dragana Ilić
Should anti-vitamin K be started on the first day in non-high risk pulmonary embolism?
Vojnosanit Pregl 2020; 77(12): 1336–1341.

Rade Milić, Boris Džudović, Bojana Subotić, Slobodan Obradović, Ivan Soldatović, Marina Petrović
The significance of C-reactive protein for the prediction of net-adverse clinical outcome in patients with acute pulmonary embolism
Vojnosanit Pregl 2020; 77(1): 35–40.

Djordje Taušan, Zoran Kostić, Damjan Slavković , Branimir Nešković , Dubravko Bokonjić, Sandra Šipetić-Grujičić, Nenad Ratković, Vesna Šuljagić
Incidence, in- hospital mortality and risk factors for hospital-acquired pneumonia in patients with intraabdominal surgical procedures hospitalized in a tertiary hospital in Belgrade, Serbia: A matched case-control study
Vojnosanit Pregl 2020; 77(5): 525–531.

OTORHINOLARYNGOLOGIC DISEASES

Aleksandar Trivić, Nada Tomanović, Sanja Krejović Trivic, Jovica Milovanović, Ivan Boričić, Ana Jotić, Miljan Folić, Ivana Ćolović Čalovski, Nikola Miković, Zoran Tatić
Nasal polyposis: a semiquantitative morphometric histopathological study
Vojnosanit Pregl 2020; 77(8): 704–788.

Nenad Baletić, Aleksandar Perić, Jelena Sotirović, Milan Erdoglija
Chronic rhinitis in glassblowers
Vojnosanit Pregl 2020; 77(10): 1011–1016.

NERVOUS SYSTEM DISEASES

Mehmet Güney Şenol, Hakan Şimşek
To do or don't, to take or don't take: STN-DBS therapy in young PD patient
Vojnosanit Pregl 2020; 77(9): 1000–1002.

Aleksandar J. Ristić, Aleksandra Arsić, Goran Trajković, Ivana Berisavac, Bojana Kisić
Electroclinical characteristics of MRI negative focal epilepsy: A video-EEG study
Vojnosanit Pregl 2020; 77(9): 901–907.

Jagoš Golubović, Djula Djilvesi, Tomislav Cigić, Vladimir Papić, Bojan Jelača, Petar Vuleković
Rare case of dural arteriovenous fistula presenting by spontaneous acute subdural hematoma – A case report and review of literature
Vojnosanit Pregl 2020; 77(2): 237–239.

Biljana Salak-Djokić, Tanja Stojković, Gorana Mandić-Stojmenović, Elka Stefanova
A profile of dementia patients in a Serbian sample – experience from the center for dementia and memory disorders
Vojnosanit Pregl 2020; 77(3): 271–281.

Svetlana Miletić-Drakulić, Dejan Zoran Aleksić
Recurrent painful ophthalmoplegic neuropathy: A report on the patient from the Romani population and 82-year-old patient
Vojnosanit Pregl 2020; 77(11): 1231–1234.

Galina Stevanović, Nebojša Jović, Miljana Kecmanović
Is adjunctive perampanel beneficial for Lafora disease?
Vojnosanit Pregl 2020; 77(5): 539–544.

Jadranka Miletić Vukajlović, Snežana Pejić, Ana Todorović, Ana Valenta Šobot, Dunja Drakulić, Ivan Pavlović, Aleksandra Stefanović, Milica Prostran, Tihomir V. Ilić, Marina Stojanov
Antioxidant status and clinicopathologic parameters in patients with Parkinson's disease
Vojnosanit Pregl 2020; 77(7): 724–730.

EYE DISEASES

Mirko Resan, Miroslav Vukosavljević, Elisaveta Stanić, Bojan Kovač, Dragana Ristić, Maja Petrović, Brigitte Pajić-Eggspuehler, Bojan Pajić
Steep keratometry and central pachymetry after corneal collagen cross-linking procedure in patients with keratoconus
Vojnosanit Pregl 2020; 77(2): 215–219.

Ivan Marjanović, Marjan Marjanović, Ranko Gvozdenović, Marija Marjanović, Vujica Marković, Marija Božić, Vesna Marić, Antonio Martinez
Pre-trabeculectomy intravitreal injections of bevacizumab for treating neovascular glaucoma in diabetic patients
Vojnosanit Pregl 2020; 77(6): 637–640.

Igor Kovačević, Aleksandra Radosavljević, Jelena Karadžić, Ivan Stefanović, Jovana Vukadinović
Clinical characteristics of posterior segment penetrating eye injuries treaed in tertiary referral hospital in Serbia
Vojnosanit Pregl 2020; 77(12): 1245–1251.

UROLOGIC AND MALE GENITAL DISEASES

Branka Gvozdić, Dragica Milenković Petronić, Nebojša Ladjević, Aleksandar Vuksanović, Otaš Durutović
The examination of the quality of life changes of patients with urolithiasis regarding different methods of treatment
Vojnosanit Pregl 2020; 77(2): 158–164.

Mirko Jovanović, Vladimir Bančević, Branko Košević, Predrag Aleksić, Vesna Šuljagić
Possible risk factors for postoperative urinary tract infection following ureteroscopic lithotripsy
Vojnosanit Pregl 2020; 77(11): 1135–1141.

Sandra Trivunić Dajko, Jovo Bogdanović, Aleksandra Fejsa Levakov, Mirjana Živojinov,Matilda Djolai
Immunohistochemical expression of androgen receptors in prostate carcinoma and benign prostatic hyperplasia
Vojnosanit Pregl 2020; 77(2): 426–430.

FEMALE UROGENITAL DISEASES AND PREGNANCY COMPLICATIONS

Mirjana Marjanović Cvjetićanin, Snežana Plešinac, Jelena Dotlić, Darko Plećaš, Dušica Kocijančić Belović, Slavica Akšam
Doppler middle cerebral artery peak systolic velocity measurement as diagnostic tool for fetal anemia after *in utero* transfusions in red blood cell alloimmunisation
Vojnosanit Pregl 2018; 77(1): 8–13.

Branko Košević, Ivica Nikolić, Vladimir Bančević, Predrag Marić, Mirko Jovanović, Dušica Stamenković, Aleksandar Spasić, Predrag Aleksić
Surgical outcome of the transobturator tape procedure for management of female urinary incontinence – A single center experience
Vojnosanit Pregl 2020; 77(12): 1304–1308.

Slavica Akšam, Snežana Plešinac, Jelena Dotlić, Dušica Kocijančić Belović, Mirjana Marjanović Cvjetičanin
Influence of chorionicity on healthy twin pregnancy outcome
Vojnosanit Pregl 2020; 77(10): 1080–1085.

Ljubinka Nikolić, Saša Čakić, Neda Perunović, Emina Čolak, Jelena-Kotur Stevuljević, Saša Janković, Milanko Djurić, Darko Plećaš
Salivary and plasma inflammatory mediators and secretory status in preterm delivery women with periodontitis – a cross sectional study
Vojnosanit Pregl 2020; 77(3): 247–255.

Relja Lukić, Nataša Karadžov-Orlić, Amira Egić, Barbara Damnjanović-Pažin, Željko Miković
Predictive value of extremely low PAPP-A, free βhCG and extremely high mean uterine artery pulsatility index in the first trimester for fetal growth restriction
Vojnosanit Pregl 2020; 77(3): 256–261.

CARDIOVASCULAR DISEASES

Dijana Djikić, Nebojša Mujović, Vojislav Giga, Milan Marinković, Goran Trajković, Snežana Lazić, Vedrana Pavlović, Vladan Perić, Dragan Simić
Interatrial conduction time is early marker of disturbed impulse propagation in adults with slightly elevated blood pressure
Vojnosanit Pregl 2020; 77(6): 569–574.
Sandra Šipetić Grujičić, Dragan Miljuš, Ivan Soldatović, Aleksandra Nikolić, Isidora Vujčić
Prehypertension and hypertension prevalence and risk factors among adult population in Republic of Serbia: A cross-sectional study
Vojnosanit Pregl 2020; 77(6): 590–600.

Miroslav Miličić, Ivan Soldatović, Duško Nežić, Miomir Jović, Vera Maravić Stojković, Petar Vuković, Predrag Milojević
Remote ischemic preconditioning in patients undergoing coronary bypass grafting following acute coronary syndrome without ST elevation
Vojnosanit Pregl 2020; 77(10): 1017–1023.

Ivan Ilić, Radosav Vidaković, Aleksandra Janićijević, Milica Stefanović, Srdjan Kafedžić, Milivoje Cerović, Dušan Milićević, Gojko Obradović, Vladimir Jovanović, Ivan Stanković, Biljana Putniković, Aleksandar N. Nešković
The increased main branch to stent diameter ratio is associated with side branch decreased coronary flow in "true" coronary artery bifurcations treated by "provisional" stenting – a two-dimensional quantitative coronary angiography study
Vojnosanit Pregl 2020; 77(1): 53–59.

Marija Mirković, Milan Nedeljković, Dušan Ružičić, Mira Vuković
Development of one-year major adverse cardiac events risk index in patients with acute coronary syndrome and diabetes mellitus who underwent percutaneous coronary intervention
Vojnosanit Pregl 2020; 77(11): 1192–1200.

Zoran Stanojković, Ana Antić, Bela Balint, Milena Todorović, Miodrag Vučić, Nebojša Vacić, Milan Lazarević
Evaluation of the anticoagulant effect of vitamin K antagonists in patients with non-valvular atrial fibrilation
Vojnosanit Pregl 2020; 77(12): 1260–1265.

Dragan Mitrović, Svetlana Lazarević
Iatrogenic pulmonary fat embolism after surgery in a patient with fatty liver
Vojnosanit Pregl 2020; 77(3): 340–343.

Aleksandar Kocijančić, Dragan Simić, Nebojša Mujović, Tatjana Potpara, Vladan Kovačević, Milan Marinković, Jelena Marić-Kocijančić, Dušica Kocijančić Belović
Long-term outcomes after catheter-ablation of atrioventricular nodal reentrant tachycardia: A ten-year follow-up
Vojnosanit Pregl 2020; 77(4): 382–386.

Vojislava C. Nešković, Slobodan D. Obradović, Ana V. Popadić, Nenad B. Nikolić, Dušica Stamenković, Zoran R. Kostić
Balloon coronary angioplasty and parenteral antiplatelet therapy for intraoperative myocardial infarction during general surgery: an attempt to balance benefits and risks – A case report
Vojnosanit Pregl 2020; 77(4): 445–449.

Petar Dabić, Saša Borović, Predrag Milojević, Jelena Kostić, Zoran Trifunović, Rade Babić
Evolution of concomitant moderate and moderate to severe functional mitral regurgitation, following aortic valve surgery for severe aortic stenosis
Vojnosanit Pregl 2020; 77(5): 479–486.

Rade Babić, Goran Grujić, Dejan Kojić, Jelena Kostić, Zoran Trifunović, Saša Borović
Primary percutaneous intervention of the right coronary artery in a setting of anomalous origination of left coronary artery from the opposite sinus of Valsalva
Vojnosanit Pregl 2020; 77(5): 549–555.

HEMIC AND LYMPHATIC DISEASES

Lidija Popović Dragonjić, Maja Jovanović, Miodrag Vrbić, Maja Stanojević, Miljan Krstić, Aleksandar Tasić, Nikola Živković
Castleman's disease associated with mixed connective tissue disorder and cerebral ischaemia and vasculitis: A rare case and a diagnostic challenge for an infectologist
Vojnosanit Pregl 2020; 77(8): 872–877.

Nada Kraguljac Kurtović, Dragana Vujić, Biljana Mihaljević
Influence of applied CD34+ cell dose on the survival of Hodgkin's lymphoma and multiple myeloma patients following autologous stem cell transplants
Vojnosanit Pregl 2020; 77(8): 844–851.

Dragan Katanić, Jovanka Kolarović, Danica Grujičić, Milica Skender Gazibara, Marija Knežević Pogančev, Katarina Koprivšek, Jovan Vlaški, Ivana Vorgučin, Jasmina Katanić
Brain histiocytosis with precocious puberty and growth hormone deficiency at early childhood – A case report
Vojnosanit Pregl 2020; 77(1): 92–96.

Aleksandar Lj. Djukić, Jovan P. Antović
Clinical significance of diagnostic algorithm in detection of mild hemostasis disorders in women with menorrhagia
Vojnosanit Pregl 2020; 77(6): 601–606.

Vesna Djordjević, Nataša Tošić, Marija Denčić-Fekete, Marijana Virijević, Jelica Jovanović, Ljubomir Jaković, Nada Kraguljac-Kurtović, Andrija Bogdanović, Tatjana Kostić, Sonja Pavlović
Acute promyelocytic leukemia lacking t(15;17): molecular evidence of atypical PML/RAR-α transcriptional variant by gene sequencing
Vojnosanit Pregl 2020; 77(1): 97–102.

Vesna Djorđević, Marija Denčić-Fekete, Jelica Jovanović, Ivan Soldatović, Gradimir Janković, Andrija Bogdanović
Significance of cytogenetic-risk categories and refined international prognostic scoring system for overall survival in primary myelofibrosis: A single-center experience
Vojnosanit Pregl 2020; 77(5): 516–524.

Igor Pilić, Darko Plećaš, Jelena Dotlić, Snežana Plešinac
Twin pregnancies conceived by assisted reproduction – Early prediction of preterm birth
Vojnosanit Pregl 2020; 77(2): 174–181.

CONGENITAL, HEREDITARY AND NEONATAL DISEASES AND ABNORMALITIES

Ivan Pavlović, Darko Plećaš, Snežana Plešinac, Jelena Dotlić, Nemanja Stojanović
Congenital anomalies: occurrence and potential risk factors
Vojnosanit Pregl 2020; 77(3): 317–323.

Katarina Koprivšek, Mladen Bjelan, Miloš Lučić, Olivera Šveljo, Dejan Kostić, Duško Kozić
Unilateral, frontal polymicrogyria and supratentorial white matter microcysts in fetus with Joubert syndrome and related disorders: Prenatal diagnosis with magnetic resonance imaging
Vojnosanit Pregl 2020; 77(10): 1093–1096.

Danijela Pavićević, Jelena Milošević, Ivana Petrović Marković, Zoran Milenković, Katarina Parezanović Ilić
The importance of physical treatment in children underwent craniosynostosis surgery in the first year of life
Vojnosanit Pregl 2020; 77(3): 324–329.

Milena Šaranović, Suzana Matejić, Nebojša Matejić, Ivan Radić, Goran Trajković
Significance of hysteroscopy in diagnosis and treatment of congenital uterine anomalies
Vojnosanit Pregl 2020; 77(11): 1161–1168.

SKIN AND CONNECTIVE TISSUES DISEASE

Predrag Vidaković, Nemanja Damjanov
The impact of altered hands function on working ability of patients with systemic sclerosis
Vojnosanit Pregl 2020; 77(1): 70–78.

NUTRITIONAL AND METABOLIC DISORDERS

Branko Srećković, Igor Mrdović, Ivan Soldatović, Mirko Resan, Nenad Janeski, Emina Čolak, Hristina Janeski, Mirjana Šumarac-Dumanović, Miloš Joković, Nebojša Ivanović, Jasna Gačić,Vesna Dimitrijević-Srećković
The correlation between metabolic syndrome quantification scores and numerous laboratory parameters related to this syndrome
Vojnosanit Pregl 2020; 77(8): 789–795.

Veljko Crnobrnja, Branislava Ilinčić, Edita Stokić, Marijana Basta Nikolić, Sonja Slankamenac, Anastazija Stojšić Milosavljević, Radmila Žeravica, Velibor Čabarkapa
Association between ultrasonographically measured visceral fat tissue thickness and proinflammatory adipokines in obesity
Vojnosanit Pregl 2020; 77(11): 1146–1153.

ENDOCRINE SYSTEM DISEASES

Ksenija Bubnjević, Dušan Ugarković, Jelena Kovačević
Aerobic physical exercise and prolactin levels in blood during breastfeeding in woman with Hashimoto's thyroiditis – A case report
Vojnosanit Pregl 2020; 77(2): 225–228.

Ivan Marjanović, Marjan Marjanović, Ranko Gvozdenović, Marija Marjanović, Vujica Marković, Marija Božić, Vesna Marić, Antonio Martinez, Zvezdan Stefanović, Branislav Donfrid, Tomislav Jovanović, Zoran Zorić, Radmila Radojević-Popović, Uroš Zoranović
Hyperbaric oxygenation in prevention of amputations of diabetic foot
Vojnosanit Pregl 2020; 77(4): 363–372.

Petar Ristić, Tamara Dragović, Saša Kiković, Dragana Ristić, Marija Zdravković, Saša Hinić, Tijana Durmić, Zoran Hajduković
Hypokalemic thyrotoxic periodic paralysis in a young Serbian male
Vojnosanit Pregl 2020; 77(4): 440–444.

Nedim Hamzagić, Marija Andjelković, Marijana Stanojević Pirković, Petar Čanović, Vesna Ignjatović, Amra Ramović, Dejan Petrović
Influence of secondary hyperparathyroidism in management anemia in patients on regular hemodialysis
Vojnosanit Pregl 2020; 77(5): 508–515.

Marijana Petrović, Tamara Dragović, Stanko Petrović, Katarina Obrenčević, Nemanja Rančić, Tatjana Djurašinović, Dejan Petrović, Ljiljana Ignjatović, Violeta Rabrenović, Nemanja Nenezić, Dejan Marinković, Djoko Maksić
Effect of Vitamin D on proteinuria, lipid status, glycoregulation and C-reactive protein in patients with type-2 diabetes mellitus
Vojnosanit Pregl 2020; 77(6): 582–589.

Branko Barać, Sanja Stanković, Milika Ašanin, Zorana Vasiljević-Pokrajčić, Svetlana Vujović
The level of endogenous testosterone and its correlation with lipid profile in men older than 40 years with acute myocardial infarction
Vojnosanit Pregl 2020; 77(8): 804–810.

Milena S. Pandrc, Andjelka Ristić, Vanja Kostovski, Violeta Randjelović Krstić, Jelena Milin Lazović, Biljana Nedeljković Beleslin, Jasmina Ćirić
Evaluation of a three-month trial of thyroxine replacement in symptomatic subclinical hypothyroidism: An impact on clinical presentation, quality of life and adoption of long-term therapy
Vojnosanit Pregl 2020; 77(9): 893–900.

Dara Stefanović, Milan Petrović, Sanja Dugonjić
The accuracy of ultrasonography for detection of enlarged parathyroid glands in patients with different forms of hyperparathyroidism
Vojnosanit Pregl 2020; 77(12): 1277–1288.

PATHOLOGICAL CONDITIONS, SIGNS AND SYMPTOMS

Nataša Savić, Vesna Živković, Ivona Stanković, Tatjana Stojković, Marina Milenković, Zorica Stojanović, Biljana Balov
The role of physical therapy in the treatment of children with chronic functional constipation
Vojnosanit Pregl 2020; 77(1): 87–91.

Zoran Hajduković, Tamara Dragović, Slavica Radjen, Dejan M. Marinković, Nenad Ratković, Saša Kiković
Significance of the pulsatility index in the evaluation of hemodynamic changes in peripheral arterial circulation in obese persons treated with orlistat
Vojnosanit Pregl 2020; 77(10): 1067–1072.

Ivana Joksić, Thomas Liehr, Mina Toljić, Nataša Karadzov-Orlić, Zagorka Milovanović, Željko Miković, Amira Egić
Prenatal ultrasonographic manifestations of partial trisomy 12q(12q24.2→qter) and partial monosomy 2q (2q37.3→qter)
Vojnosanit Pregl 2020; 77(7): 754–757.

Jovana Manevski, Ivana Stojšin, Karolina Vukoje, Ognjenka Janković
Dental aspects of purging bulimia
Vojnosanit Pregl 2020; 77(3): 300–307.

CHEMICALLY-INDUCED DISORDERS

Jovan Jovanović, Dragan R. Milovanović, Predrag Sazdanović, Maja Sazdanović, Milan Radovanović, Ljiljana Novković, Vladimir Zdravković, Nemanja Zdravković, Ivan Simić, Dejana Ružić Zečević, Slobodan M. Janković
Risk factors profile for liver damage in cardiac inpatients
Vojnosanit Pregl 2020; 77(9): 934–942.

Miloš Danilović, Jelena Isailović, Ivan Aleksić, Jelena Džambas, Nadica Marinković
Accidental colchicine poisoning with fatal outcome after ingestion of meadow saffron (*Colchicum autumnale L.*)
Vojnosanit Pregl 2020; 77(10): 1104–1108.

Ena Grbović, Boban Mugoša
Social support in recovery former users of psychoactive substances in Montenegro: A survey study
Vojnosanit Pregl 2020; 77(5): 494–499.

WOUNDS AND INJURIES

Nataša Vešović, Aleksandar Ristanović, Vlado Cvijanović, Dejan Stojković, Nebojša Marić, Vanja Kostovski, Ljubinko Djenić, Aleksandar Nikolić
Penetrating neck injury with consequential thoracic complications managed with use of video-assisted thoracoscopic surgery – A case report
Vojnosanit Pregl 2020; 77(3): 330–334.

Vladimir Harhaji, Ivica Lalić, Miodrag Vranješ, Milena Mikić, Vladimir Djan
Isolated avulsion fracture of the fibular head
Vojnosanit Pregl 2020; 77(5): 545–548.

CHEMICALS AND DRUGS CATEGORY

BIOLOGICAL FACTORS

Aleksandar Sekulić, Goran Barišić, Duško Dundjerović, Svetislav Tatić, Zoran Krivokapić
Vascular endothelial growth factor as a potential prognostic factor for T3N0 rectal cancer
Vojnosanit Pregl 2020; 77(2): 189–195.

PHARMACEUTICAL PREPARATIONS

Sanja M. Uzelac, Radica S. Živković Zarić, Milan R. Radovanović, Goran Ž. Ranković, Slobodan M. Janković
Efficacy and safety of triazoles versus echinocandins in the treatment of invasive aspergillosis: A meta-analysis
Vojnosanit Pregl 2020; 77(9): 974–985.

Nevena Vidović, Slavica Ranković, Zorana Oreščanin-Dušić, Jasmina Debeljak-Martačić, Tamara Popović, Mirko Tomić, Marija Glibetić
The effects of fish-based and milk-based diets on liver tissue antioxidant enzymes and lipid peroxidation in female Wistar rats: A pilot study
Vojnosanit Pregl 2020; 77(6): 641–646.

CHEMICAL ACTIONS AND USES

Tatjana Ćebović, Dunja Jakovljević, Zoran Maksimović, Snežana Djordjević, Sanja Jakovljević, Dragana Četojević-Simin
Antioxidant and cytotoxic activities of curly dock (*Rumex crispus L.*, Polygonaceae) fruit extract
Vojnosanit Pregl 2020; 77(3): 308–316.

Milan Zarić, Radica Živković Zarić, Marina Mitrović, Ivana Nikolić, Petar Čanović, Zoran Milosavljević, Danijela Jovanović, Ivanka Zelen
***Teucrium polium* induces apoptosis in peripheral blood lymphocytes isolated from human chronic lymphocytic leukemia**
Vojnosanit Pregl 2020; 77(12): 1252–1259.

ANALYTICAL, DIAGNOSTIC AND THERAPEUTIC TECHNIQUES AND EQUIPMENT CATEGORY

DIAGNOSTICS

Snežana Lukić, Lukas Rasulić, Vojin Kovačević, Filip Vitošević, Andrija Savić, Milan Mijailović
Radiation exposure during neurointerventional procedures in modern angiographic systems: A single center experience
Vojnosanit Pregl 2020; 77(9): 962–966.

Živko Krivokuća, Željka Tatomirović, Gordana Cvetković, Jelena Džambas, Vesna Škuletić, Saša Ristić
Validity of cytology in the diagnosis of small cell lung carcinoma
Vojnosanit Pregl 2020; 77(9): 954–961.

Igor Ivanov, Vladimir Veselinov, Dejan Ćelić, Jadranka Dejanović, Dušanka Obradović, Violeta Knežević
Lung ultrasound for volume status assessment in chronic hemodialysis patients
Vojnosanit Pregl 2020; 77(9): 943–949.

Nevena Manevska, Siniša Stojanoski, Irfan Ahmeti, Toni Tripunoski, Daniela Pop Gjorčeva, Venjamin Majstorov, Gordana Pemovska
Lower limb perfusion scintigraphy with 99mTc-MIBI scintigraphy and determination of endothelin in diabetic and nondiabetic patients
Vojnosanit Pregl 2020; 77(7): 673–679.

Ljiljana Šoškić, Mladen Kočica, Dragan Cvetković, Biljana Miličić, Nebojša Ladjević, Ivan Palibrk, Milica Karadžić, Miloš Grujić, Milica Vještica-Mrdak, Arsen Ristić
Correlation between central venous and mixed venous oxygen saturation in the elective abdominal aortic aneurysm surgery
Vojnosanit Pregl 2020; 77(7): 697–703.

Nataša Janović, Gorica Marić, Marija Dušanović, Aleksa Janović, Tatjana Pekmezović, Marija Djurić
Introducing Nasal Obstruction Symptom Evaluation (NOSE) scale in clinical practice in Serbia: validation and cross-cultural adaptation
Vojnosanit Pregl 2020; 77(7): 704–709.

Marija Stupar, Djordje Špreng, Aleksandar Perić
Application of questionnaires in the assessment of clinical severity of chronic rhinosinusitis
Vojnosanit Pregl 2020; 77(2): 182–188.

Branislava Milenković, Sanja Dimić Janjić, Jelena Kotur-Stevuljević, Ivan Kopitović, Jelena Janković, Mihailo Stjepanović, Marija Vukoja, Snežana Ristić, Žaklina Davičević-Elez
Validation of Serbian version of chronic obstructive pulmonary disease assessment test
Vojnosanit Pregl 2020; 77(3): 294–299.

Jelena Mitić, Nikola Vitković, Miodrag Manić, Miroslav Trajanović, Sladjana Petrović, Stojanka Arsić
Reverse modeling of the human mandible 3D geometric model
Vojnosanit Pregl 2020; 77(3): 262–270.

Biljana Majstorović, Mladen Pešta
Factors predicting rehabilitation outcome in patients after unilateral transtibial amputation due to peripheral vascular disease

Vojnosanit Pregl 2020; 77(4): 357–362.

THERAPEUTICS

Jelena Stevanović, Maja Vulović, Danijela Pavićević, Mihailo Bezmarević, Andjelka Stojković, Aleksandar Radunović, Miljana Aksić, Bojan Milošević, Aleksandar Cvetković, Milan Jovanović, Anita Ivošević
Physical therapy improves motion in a patient with inclusion body myositis – A case report
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Zoran Paunić, Milica Dekleva-Manojlović, Nataša Marković-Nikolić, Nemanja Rančić, Nada Dimković
Impact of active fluid management on cardiac hemodynamics and mechanics in patients on maintenance hemodialysis
Vojnosanit Pregl 2020; 77(1): 60–69.

Dejan Škorić, Ivan Milovanović, Jovana Dimić, Dragana Bogićević
Three obstetric factors should be considered in umbilical cord blood donor selection
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Zoran Matić, Tijana Bojić
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Nebojša Vacić, Ana Antić, Zoran Stanojković, Miodrag Vučić, Milan Lazarević
Biochemical and functional quality assessment of platelet concentrates
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Goran Čitlučanin, Miomira Ivović, Milina Tančić-Gajić, Fadil Canović, Miloš Stojanović, Ljiljana Marina, Zorana Arizanović, Aleksandar Djogo, Vladimir Gerginić, Svetlana Vujović
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Vojnosanit Pregl 2020; 77(4): 418–425.

Dušica Mirković, Svetlana Ibrić
Investigation of short-term stability of parenteral nutrition nanoemulsions prepared under laboratory conditions
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Heart transplantation in a patient with left ventricular assist device after pump thrombosis –The first case report in Serbia
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Aleksandar Radunović, Vesna Radunović, Srdjan Starčević, Goran Lekić, Maja Vulović
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Dragan Milić, Saša Živić, Mladjan Golubović, Dragan Bogdanović, Milan Lazarević, Konstansa Lazarević
A randomized trial of surgery alone versus surgery plus compression in the treatment of venous leg ulcers in patients with primary venous insufficiency
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The influence of temporary occlusion of parent vessel on outcome of surgical treatment of ruptured cerebral aneurysms
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Dragan Cvetković, Mladen Kočica, Ljiljana Šoškić, Filip Vučićević, Olga Petrović, Ivana Jovanović, Snežana Jovičić, Jelena Trifković, Saša Kostovski, Biljana Miličić, Milica Karadžić, Arsen Ristić, Dragutin Savić
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Andrej Preveden, Mila Kovačević, Stamenko Šušak, Aleksandar Redžek, Lazar Velicki
Coronary embolism causing myocardial infarction after heart valve surgery
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Vladimir Jovanović, Lukas Rasulić, Vojin Kovačević, Aleksandar Janićijević, Filip Vitošević, Andrija Savić, Marko Djurović, Goran Tasić
Unruptured distal anterior cerebral artery mirror aneurysms associated with ruptured middle cerebral artery aneurysm: A case report
Vojnosanit Pregl 2020; 77(7): 746–750.

Ivan Tušek, Miroslav P. Ilić, Jasmina Tušek, Stojan Ivić, Branislav Tušek
Pediatric mandibular fracture therapy – A case report
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INVESTIGATIVE TECHNIQUES

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Teledentistry in dental care of children
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Evaluation of chronological age based on third-molar development in the Serbian population
Vojnosanit Pregl 2020; 77(10): 1054–1059.

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Danijela Staletović, Ibrachim Kannosh, Meliha Šehalić, Vladanka Vukićević, Zoraida Milojković, Aleksandra Ilić, Verica Pavlić, Zlata Brkić
Presence of *Tannerella forsythia* in patients with chronic periodontal disease and atherosclerosis
Vojnosanit Pregl 2020; 77(6): 614–619.

Marija Igić, Ljiljana Kesić, Radmila Obradović, Gordana Filipović, Branislava Stojković, Kosta Todorović
Comparative clinical evaluation of the therapeutic effects of low-level laser and hyaluronic acid on *gingivitis catarrhalis* in children
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Shahabe Saquib Abullais, Gore Anoop, Nitin Dani, Saad Al-qahatani, Ashfaq Yaqoob, Abdul Ahad Khan, Mohammed Abdul Kader
Assessment of supracrestal tissue attachment variation in patients with chronic periodontitis before and after treatment: A clinical-radiographic study
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Public trust and media influence on anxiety and depression levels among skilled workers during the COVID-19 outbreak in Serbia
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Principal characteristics of patients acutely poisoned by ethanol in the region of Belgrade (Serbia)
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Petar Vojvodić, Ana Andonov, Dejan Stevanović, Ivana Peruničić Mladenović, Goran Mihajlović, Jovana Vojvodić
Montgomery–Asberg depression rating scale in clinical practice: Psychometric properties on Serbian patients
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Mirjana Djordjević, Nenad Glumbić, Branislav Brojčin
Irony, deception and theory of mind in people with intellectual disabilities and dual diagnoses
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Sanja Vukadinović Stojanović, Zlatan Stojanović
Association of severity of depression, paroxetine use and markers of liver damage with QT interval duration in patients with alcohol dependence
Vojnosanit Pregl 2020; 77(7): 680–687.

MENTAL DISORDERS

Vladan Djordjević, Mila Jovanović, Sanja Čolić, Milena Stašević, Amina Asotić, Saša Čakić, Ivana Stašević Karličić, Ljubomir Todorović
Evaluation of dental health among adolescents with mental disorders
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Vesna Petrović, Dragan Žuljević, Zorica Knežević
Corelation between somatic complaints, personality traits and positivity
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Bratislav Živić, Danilo Joković, Marija Vranić, Zvezdana Stojanović
Post-traumatic stress disorder psychotic subtype or comorbid psychotic disorder and evaluation of military service ability
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Danilo Pešić, Tara Adžić, Olivera Vuković, Marko Kalanj, Dušica Lečić Toševski
Analysis of personality disorder profiles obtained by five-factor personality model
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Miodrag M. Stanković, Jelena Stevanović, Aleksandra Stojanović, Sandra Stanković
Psychogenic diabetes insipidus – A case report of behavioral psychotherapy
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Association of PAX3 and TMTC2 genes polymorphism with the face morphology changes after excision of skin Tumors
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Ljiljana Stojković, Aleksandra Stanković, Ivan Životić, Evica Dinčić, Dragan Alavantić, Maja Živković
Gene expression of chemokines CX3CL1 and CXCL16 and their receptors, CX3CR1 and CXCR6, in peripheral blood mononuclear cells of patients with relapsing-remitting multiple sclerosis – A pilot study
Vojnosanit Pregl 2020; 77(9): 967–973.

Milica Ćućuz Jokić, Vesna Ilić, Bojana Cikota Aleksić, Slobodan Obradović, Zvonko Magić
Association of factor II G20210A, factor V G1691A and methylenetetrahydrofolate reductase C677T gene polymorphism with different forms of myocardial infarction: ST segment elevation and non-ST segment elevation
Vojnosanit Pregl 2020; 77(10): 1041–1047.

METABOLISM

Aleksandra Arsić, Snježana Petrović, Nikola Čikiriz, Danijela Ristić Medić, Vesna Vučić
Effect of long-term strenuous training on the plasma phospholipid fatty acid composition in handball players
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The effects of acutely and subchronically applied DL-methionine on plasma oxidative stress markers and activity of acetylcholinesterase in rat cardiac tissue
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Work-related stress among primary healthcare workers
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Assessment of oral health of the Serbian Armed Forces members
Vojnosanit Pregl 2020; 77(6): 575–581.

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Psychometric evaluation of the short version of the Defense Style Questionnaire on Serbian healthy adult men
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The use of mobile-aided learning in education of local anesthesia for the inferior alveolar nerve block
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Application of a geographic information system in the study of spatial aspects of cervical cancer incidence in Belgrade
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Aleksandar S. Nedok
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DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG*, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

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Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure **– Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi **– Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

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Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa *Interneta* citiraju se uz navođenje datuma pristupa tim podacima.

Primeri referenci:

Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG*, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

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