YU ISSN 0042-8450

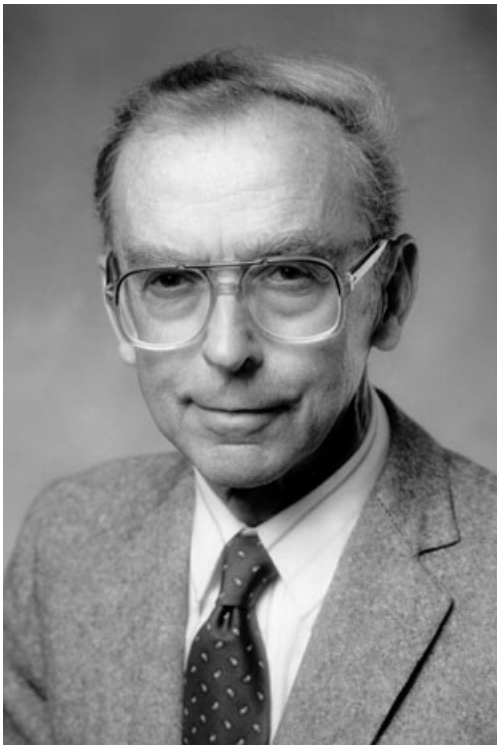
ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2022; January Vol. 79 (No. 1): pp. 1–102.



Vojnosanitetski Pregled 2022 January Vol. 79 (No. 1): pp. 1–102.

vol. 79, No. 1, 2022

VOJNOSANITETSKI PREGLED

The first issue of *Vojnosanitetski pregled* was published in September 1944
The Journal continues the tradition of Vojno-sanitetski glasnik which was published between 1930 and 1941

PUBLISHER
University of Defence, Ministry of Defence of the Republic of Serbia, Belgrade, Serbia

ISSN 0042-8450
eISSN 2406-0720

Editorial Office: University of Defence, Faculty of Medicine of the Military Medical Academy, Center for Medical Scientific Information, Crnotravska 17, 11 040 Belgrade, Serbia. E-mail: **vsp@vma.mod.gov.rs**

Papers published in the Vojnosanitetski pregled are indexed in: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica, Serbian Citation Index (SCIndex), DOAJ. Contents are published in Giornale di Medicine Militare and Revista de Medicina Militara. Reviews of original papers and abstracts of contents are published in International Review of the Armed Forces Medical Services.

The Journal is published monthly. Subscription: Giro Account No. 840-19540845-28, refer to number 122742313338117. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 5,000.00 RSD, institutions 10,000.00 RSD, and foreign subscribers 150 €.

Printed by: Vojna štamparija, Beograd, Resavska 40b

vol. 79, br. 1, 2022

VOJNOSANITETSKI PREGLED

Prvi broj *Vojnosanitetskog pregleda* izašao je septembra meseca 1944. godine
Časopis nastavlja tradiciju *Vojno-sanitetskog glasnika*, koji je izlazio od 1930. do 1941. godine

IZDAVAČ
Ministarstvo odbrane Republike Srbije, Univerzitet odbrane, Beograd, Srbija

ISSN 0042-8450
eISSN 2406-0720
Open Access
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Adresa redakcije: Univerzitet odbrane, Medicinski fakultet Vojnomedicinske akademije, Centar za medicinske naučne informacije, Crnotravska 17, 11 040 Beograd, Srbija. Informacije o pretplati (tel.): +381 11 3608 997. E-mail (redakcija): **vsp@vma.mod.gov.rs**

Radove objavljene u „Vojnosanitetskom pregledu" indeksiraju: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica, Srpski citatni indeks (SCIndeks), DOAJ. Sadržaje objavljuju Giornale di Medicine Militare i Revista de Medicina Militara. Prikaze originalnih radova i izvoda iz sadržaja objavljuje International Review of the Armed Forces Medical Services.

Časopis izlazi dvanaest puta godišnje. Pretplate: Žiro račun br. 840-19540845-28, poziv na broj 122742313338117. Za pretplatu iz inostranstva obratiti se službi pretplate na tel. +381 11 3608 997. Godišnja pretplata: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za pretplatnike iz inostranstva. Kopiju uplatnice dostaviti na gornju adresu.

Štampa: Vojna štamparija, Beograd, Resavska 40b

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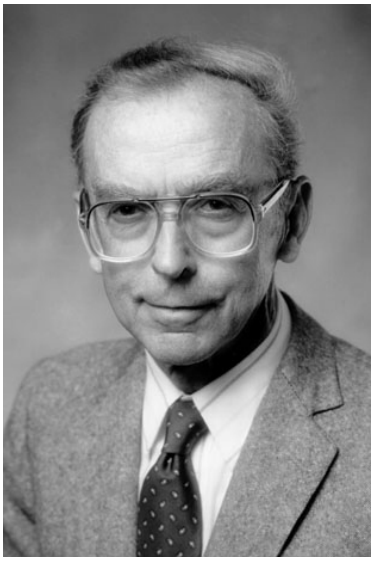

Robert William Holly (January 28, 1922–February 11, 1993) was an American biochemist who shared the 1968 Nobel Prize in Physiology and Medicine with Har Gobind Horan and Marshall Warren Nuremberg for describing the structure of alanine transfer RNA, which made understanding the connection between DNA and protein synthesis possible.
This year, January 28 marks the 100th anniversary of the birth of this scientist.

Robert Vilijam Holi (28. januar 1922–11. februar 1993.) bio je američki biohemičar koji je sa Harom Gobindom Horanom i Maršalom Vorenom Nirenbergom podelio Nobelovu nagradu za fiziologiju i medicinu 1968. Godine. Nobelovu nagradu su dobili za opisivanje strukture transportne RNK koja prenosi alanin, što je omogućilo razumevanje povezanosti DNK sa sintezom proteina.
Ove godine, 28. januara, obeležava se 100 godina od rođenja ovog naučnika.

E D I T O R I A L

UDC: 655.411
https://doi.org/10.2298/VSP2201005V

The *Vojnosanitetski pregled* in the second year of the COVID-19 pandemic

Vojnosanitetski pregled u drugoj godini pandemije COVID-19

Dragana Vučević

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

Welcome to the first 2022 issue of the *Vojnosanitetski pregled* (VSP). First of all, on behalf of the Editorial Staff I am very pleased to express our sincere gratitude to authors, reviewers, members of the Editorial Board, and readers for their supportive and dedicated participation in the Journal in 2021, because, without these contributions, we would not be able to produce it.

The last two years have been very challenging for all of us. Many of us lost loved ones in the pandemic caused by the SARS-CoV-2. We have all faced a unique feeling of anxiety for the future of ourselves and the whole community, and, more than ever, we have become aware of the importance of science for fighting COVID-19. Fortunately, we have been saved by the outstandingly fast development of COVID-19 vaccines, which have helped us in the face of this challenge. Therefore, we would like to take the opportunity to thank and send a word of respect to the scientists developing the COVID-19 vaccines. We are now far better prepared with vaccine stocks, but we should not loosen up too much because COVID-19 cases are on the rise again, and a far more contagious strain of the new Omicron strain of the SARS-CoV-2 virus is spreading across the globe. Fortunately, this strain of the virus appears to be less dangerous than the Delta variant.

In spite of the SARS-CoV-2 storm that has damaged the world, we are ready to start the new year with the belief that we will have a more normal life in 2022.

Last year, we were pleased to receive very informative manuscripts on diverse topics in the fields of medicine, dentistry, and pharmacy. A total of 278 manuscripts were received, which was almost identical to the number from 2020 when 280 manuscripts had been submitted to our Journal. As usual, the largest number was from the category Original articles (196 or 70.50%) and Case reports (52 or 18.71%) (Table 1).

Table 1

Categories and the number of manuscripts submitted to the *Vojnosanitetski Pregled* in 2021

Category	Manuscripts n (%)
Original Articles	196 (70.50)
Case Reports	52 (18.71)
Current Topics	8 (2.88)
General Review	4 (1.44)
Short Communications	3 (1.08)
History of Medicine	5 (1.80)
Letter to the Editor	6 (2.16)
Editorial	1 (0.36)
Practical Advice for Physicians	1 (0.36)
Meta-analysis	1 (0.36)
Book Review	1 (0.36)
Total	278 (100)

Out of all the submitted manuscripts, 65.11% were from domestic authors, primarily from the so-called civilian health and academic institutions (81.22%). The authors of the remaining 18.78% of the manuscripts were from the University of Defence in Belgrade, Serbia.

The VSP is proud to be an international journal with about 35% of its submitted manuscripts being from abroad, which was more compared to the last year's number of papers by foreign authors (27.14%). Last year, we were pleased to receive manuscripts from Bosnia and Herzegovina, China, India, Indonesia, Iran, Iraq, Lithuania, Mongolia, Montenegro, Northern Macedonia, Pakistan, Romania, Saudi Arabia, and Turkey (in alphabetical order). We express our greatest respect to all of the authors from abroad because the manuscripts of these authors have

Correspondence to: Dragana Vučević, University of Defence, Faculty of Medicine of the Military Medical Academy, Center for Medical Scientific Information, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: vsp@vma.mod.gov.rs

significantly contributed to the growth of the VSP's impact in the scientific and academic community.

Of the 278 papers received in 2021, 91 (32.73%) manuscripts were rejected and 187 (67.27%) were accepted for publication. The structure of published papers is given in Table 2. As in 2021, most of the published papers were from the category Original Articles, which is expected because most of the submitted papers belong to that category. In addition, a total of 105 articles were published last year as Online first in electronic form with an assigned DOI number.

Table 2
Categories and the number of articles published in the *Vojnosanitetski Pregled* in 2021

Category	Articles
	n (%)
Editorial	1 (0.54)
Original Articles	116 (62.03)
Case Reports	40 (21.39)
General Review	1 (0.54)
Current Topic	6 (3.21)
Short Communications	10 (5.35)
Preliminary report	1 (0.54)
Practical advices for physicians	2 (1.07)
History of Medicine	5 (2.67)
Letter to the Editor	4 (2.14)
Book Review	1 (0.54)
Total	187 (100)

The quality of a scientific journal heavily relies on high-quality peer review. We thank our reviewers for taking their time to make the journal as successful as it is. The following experts provided reviews of papers submitted to VSP in 2021 (Table 3).

Members of the Editorial Staff are working hard to enhance the status of the Journal and its position among journals indexed in the Science Citation Index Expanded database. In 2022, we will continue improving the impact and quality of the VSP. We welcome submissions of better-quality manuscripts in 2022, hoping you will enjoy reading VSP throughout the year.

As mentioned above, in 2021, given that VSP is a multidisciplinary biomedical journal, papers with topics from all fields of medicine, dentistry, and pharmacy were submitted to our journal. Among them were papers on COVID-19, mostly in the form of Case Reports, and Letters to the Editor, which confirmed what had already been published about the SARS-CoV-2 and COVID-19. Therefore, a relatively small number of such papers were accepted to be considered for publication, and then published.

In these challenging times of the pandemic, we encourage authors to submit research findings concerning the role of the immune system in the protection against viruses. Looking ahead, we look forward to receiving your suggestions in the future, in anticipation that VSP will continue to grow and make a place for itself among the most valued journals from the biomedical science field.

We wish you all a fruitful and scientifically productive 2022, better coping with the healthcare challenges we confront worldwide, and personal and family happiness.

Table 3
Reviewers of the *Vojnosanitetski pregled* in 2021

Aćimović Slobodan	Čutović Tatjana	Elez Marija	Jovanović Vesna
Andrejic Višnjić Bojana			Jović Miomir
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Bezmarević Mihajlo	Dominicus Husada	Ivanović Mirjana	Konstantinović Vitomir
Biočanin Vladimir	Dragović Tamara		Koračević Goran
Bogdanović Jovo	Drapšin Miodrag	Jakovljević Miro	Kosanović Rade
Bokonjić Dubravko	Drobac Milan	Jakovljević Vladimir	Kostić Dejan
Borovčanin Milica	Dubljanin Raspopović Emilija	Jakšić Vesna	Kostić Smiljana
Božić Milena	Dukanac Vesna	Janković Borisav	Kotur-Stevuljević Jelena
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Cvetković Gordana		Jovanović Verica	Lakić Aneta

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Lalošević Dušan
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Latas Milan
Lorigo Margarida
Lukić Ljiljana

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Obradović Slobodan
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Palibrk Ivan
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Pavlović Siniša
Perić Aleksandar
Perić Stojan
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Tušek Ivan

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ORIGINAL ARTICLES

UDC: 613.281:577.115.3
DOI: https://doi.org/10.2298/VSP200212054D

Lipid profile and health benefit of commonly consumed fresh water and sea water fish species in the population of Serbia

Lipidni profil i zdravstveni značaj najčešće konzumiranih rečnih i morskih riba u populaciji Srbije

Ivana Djuričić*, Tamara Gojković†, Biljana Antonijević‡, Sladjana Šobajić*

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Abstract

Background/Aim. Dietary intake of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) is important in prevention and treatment of different diseases. In general population, the average intake of n-3 LC-PUFA is often significantly lower than recommended levels. Fish lipids are rich sources of these fatty acids, of which the most important are eicosapentaenoic (20:5 n-3, EPA) and docosahexaenoic (22:6 n-3, DHA) fatty acids. This study was designed to determine and compare fat, fatty acids and lipid quality indices in 10 commercial fish species available on the Serbian market, as well as relation between their price and nutritional value. **Methods.** Freshwater fish originated from the Danube River in the Belgrade Region, while seawater fish were mostly from the Adriatic Sea. A gas chromatography method was used to define fatty acids in 40 fish samples after lipid extraction. Cost-minimization analysis was conducted to assess the economic utility. **Results.** Seawater fish had a significantly higher value of flash lipid quality compared to the freshwater fish ($p < 0.05$). Value of hypercholesterolaemic fatty acids (OFA) for the freshwater group was 18.70 (17.40–21.30) while the seawater group had a similar range of values 18.90 (17.55–22.75). Hypocholesterolaemic fatty acids (DFA) also showed similar ranges for both groups: 68.80 (66–70.20) for freshwater and 68.40 (64.85–73.05) for seawater group. The ratio of DHA/EPA ranged from 1.8 for sardine samples and up to 10 for tuna samples, indicating that the amount of DHA in natural samples exceeds the amount of EPA in many cases. The values of atherogenic (AI) and thrombogenic index (TI) were lower than 1 for all analysed samples. **Conclusion.** Sardine and mackerel had the highest content of n-3 LC-PUFA and presented the least expensive sources of EPA and DHA. The low values of AI and TI obtained from studied fish indicate its benefits from a health point of view.

Key words:
economic factors; fatty acids, unsaturated; food; health; oceans and seas; rivers; serbia.

Apstrakt

Uvod/Cilj. Unos n-3 polinezasićenih masnih kiselina (PMK) je od velike važnosti u prevenciji i tretmanu različitih oboljenja. Generalno posmatrajući, prosečan unos n-3 PMK je obično značajno niži od utvrđenih preporuka. Lipidi riba sadrže masne kiseline n-3 serije od kojih su najvažnije eikozapentaenska (20:5 n-3, EPA) i dokozaheksaenska (20:6 n-3, DHA) masna kiselina. Cilj ove studije bio je odrediti i uporediti lipidni profil i lipidne indekse u 10 različitih vrsta riba dostupnih na tržištu Srbije. Takođe, određen je odnos cene i nutritivne vrednosti odabranih vrsta. **Metode.** Ispitivane su rečne ribe Dunava iz Beogradskog regiona, dok su morske ribe uglavnom vodile poreklo iz Jadranskog mora. Gasna hromatografija sa jonskim detektorom je korišćena za određivanje masnih kiselina u 40 uzoraka nakon lipidne ekstrakcije. *Cost-minimization* analiza je korišćena za procenu ekonomske koristi. **Rezultati.** Morske ribe su imale značajno veće vrednosti za parametar *flash lipid quality* u odnosu na rečne ribe ($p < 0,05$). Vrednosti hiperholesterolemijskih masnih kiselina za grupu rečnih riba [18,70 (17,40–21,30)] bile su slične vrednostima dobijenim za morske ribe [18,90 (17,55–22,75)]. Hipoholesterolemijske masne kiseline su takođe pokazale sličan raspon vrednosti za rečne [68.80 (66–70.20)] i morske ribe [68.40 (64.85–73.05)]. Odnos DHA/EPA kretao se od 1,8 za uzorke sardine, do 10 za uzorke tune, što potvrđuje činjenicu da DHA prevazilazi vrednosti za EPA u svim ispitivanim uzorcima. Vrednosti za aterogeni i trombogeni indeks su bile niže od 1 za sve analizirane uzorke. **Zaključak.** Sardine i skuša su imale najveći sadržaj n-3 PMK i predstavljale su najekonomičniji izvor EPA i DHA. Niske vrednosti za aterogeni i trombogeni indeks ukazuju na potencijalno povoljan zdravstveni efekat ispitivanih vrsta.

Ključne reči:
ekonomski faktori; masne kiseline, nezasićene; hrana; zdravlje; okeani i mora; reke; srbija.

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Introduction

Fish and seafood are the only significant sources of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) in the modern human diet. Alfa-linoleic acid (ALA) is also found in foods of plant origin, but the process of converting this fatty acid (FA) into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in humans is not effective enough. Due to the importance of fish as a source of n-3 LC-PUFA in the past decades, numerous investigations have been carried out on the health impact of their consumption. The Danish scientists established a link between the high intake of sea fish and the low incidence of cardiovascular diseases in the Greenland Inuit [1]. Further research has shown that the consumption of fish in everyday diet has numerous beneficial effects. A study including subjects from 36 countries found a significant correlation between fish consumption and a reduced risk of general mortality, as well as the mortality caused by ischemic heart disease and cerebrovascular disease [2]. The Hordaland Health Study among middle-aged men and women confirmed an inverse relation between total fish intake and parameters of metabolic syndrome [3]. Also, very recent review revealed results from interventional and prospective cohort studies, with mostly beneficial effects of fish consumption on cardiovascular diseases and metabolic syndrome [4]. In addition to the protective effect on the cardiovascular system, it is considered that the intake of fish can positively influence the development and proper functioning of the central nervous system, especially in children. Fish intake and n-3 LC-PUFA are associated with favourable effects in neurodegenerative and retinodegenerative diseases [5, 6]. In 2012, European Food Safety Authority (EFSA) established dietary recommendations for the intake of 250–500 mg/day of EPA + DHA based on cardiovascular risk for European adults [7]. Fish consumption of at least twice a week (recommended fatty fish – salmon, herring and mackerel) and the use of vegetable oils with ALA for preparing food (soybean oil) has been recommended by the American Heart Association (AHA). For the purpose of secondary preventions, the AHA recommends 1 g EPA + DHA/day [8]. According to other governing bodies, at least 2 servings of fish per week are set by Australian Dietary Guidelines [9], while 280–525 g of fish is the appropriate weekly intake recommended by Dietary Guidelines for Chinese Residents [10].

Intake of n-3 LC-PUFA in a population can vary significantly according to dietary and supplementation habits, age group, and gender, but in general, the average intake is often significantly lower than recommended levels [11–13]. For the Serbian population, there are still no published data on EPA and DHA intake, but it is deemed that dietary fish intake is low and infrequent, and our previous study showed that n-3 LC-PUFA status in middle-aged healthy individuals was inadequate, confirming low fish consumption [14, 15]. Additionally, patients with cardiometabolic risk factors in Serbia have abnormal n-3 LC-PUFA profile compared to healthy subjects [16].

When increased fish intake is recommended, attention should be paid to the fact that both the total fat and PUFA content vary considerably among species. The fat content and the fatty acid composition of fish can vary significantly due to species, variations in their diet, life cycle, temperature, location, gender and environmental conditions [17, 18]. Moreover, fish can be an important source of aquatic contaminants such as heavy metals and polychlorinated organic compounds with high persistence, bio-accumulative properties and potential harmful human health effects [19].

Thus, the aim of this study was to determine and compare the fat, fatty acids and lipid quality indices in common freshwater and seawater fish species available on the Serbian market and to evaluate the most economical fish species as sources of dietary long-chain n-3 fatty acids. In addition, the potential health risks due to the toxicity of contaminants present in fish were also taken into account.

Methods

Chemicals and reagents

All chemicals used were of analytical, high pressure liquid chromatography (HPLC or gass chromatography (GC grades (≥ 99.8%, puriss. pa., Sigma Aldrich). Fatty Acid Methyl Esters (FAME) mix standard (SupelcoTM FAME Mix C4-C24) was provided by Supelco (Bellefonte, PA, USA).

Sample preparation

Five seawater fish species commonly consumed in the Serbian diet were chosen and purchased from the local fish market (salmon, tuna, mackerel, sardines, and hake). The origin of salmon was the North Sea (Norway), while other four species originated from the Adriatic Sea around Montenegro and Croatia. On the other hand, freshwater fish species were caught on the Sava and Danube (rivers) in the vicinity of Belgrade. These are carp, catfish, perch and bream. Trout was the only freshwater cultivated species analyzed in this study. The season chosen for investigation was winter. Skin, head, gills, fins, and bones from each fish species were removed; meat was cut into pieces and homogenized. Three to five specimens from each species were used for the analyses.

Total fat content

The total lipids content from each homogenized sample was determined by the gravimetric method after Soxhlet extraction. Total fats were extracted according to Soxhlet method with chloroform as an organic solvent [20]. About 10 g of the homogenized sample was extracted for 4–5 hrs. Fat content per 100 g of sample was expressed in %.

Fatty acid methyl esters (FAMEs) preparation and determination

Approximately 0.2 g of extracted lipids from each specimen was transferred into glass cuvettes and 1.5 mL of 3 mol/L HCl/methanol was added for conversion of lipids to volatile FAMEs. The cuvettes were mixed, heated in the water bath at 85 °C for 45 min, and cooled. One mL of hexane was added for FAMEs extraction. After centrifugation for 10 minutes at 3,000 rpm, the hexane (upper layer) containing the FAMEs was transferred into vials and immediately analysed [21]. FAMEs were further analyzed using an Agilent Technologies 7890A Gas Chromatograph with a flame ionization detector (FID). Separation of the FAMEs was performed on a CP-Sil88 capillary column (100 m x 0.25 mm x 0.2 μm). The oven temperature program started at 80 °C, and increased by 4 °C/ min up to 220 °C (hold time 5 min), then by 4 °C/min up to 240 °C and held at 240 °C for 10 min. Injector temperature was 250 °C and the detector temperature was 270 °C. The carrier gas was helium with a constant flow of 1 mL/min and makeup gas was nitrogen, with a flow of 25 mL/min. Fatty acids were identified by their retention time in comparison with reference fatty acids standard. The results were expressed as a percentage of individual fatty acid in total fatty acids content.

The lipid quality indices

From the data on the fatty acid composition, the atherogenic and thrombogenic indices were calculated using modified equations by Ulbricht and Southgate [22] and Garaffo et al [23]:

Index of atherogenicity (AI):

AI = [C12:0 + (4 × C14:0) + C16:0]/ (n-3PUFA + n-6PUFA + MUFA)] (1)

Index of thrombogenicity (TI):

TI = [C14:0 + C16:0 + C18:0]/[(0.5 × C18:1) + (0.5 × sum of other MUFA) + (0.5 × n-6PUFA) + (3 × n-3PUFA) + n-3PUFA/n-6PUFA)] (2)

Myristic acid (C14:0) is considered to be 4-times more atherogenic than the other fatty acids; thus a coefficient of 4 was assigned to it. Monounsaturated fatty acids (MUFA) and n-6 PUFA were assigned coefficients of 0.5 since they are less antiatherogenic than n-3 PUFA, which was assigned a coefficient of 3 [24].

Flesh-lipid quality (FLQ) was calculated following the formulae by Abrami et al. [25] and Senso et al. [26]:

FLQ = 100 × (EPA + DHA)/(% of total fatty acids) (3)

Hypercholesterolaemic fatty acids (OFA):

OFA = C12:0 + C14:0 + C16:0 (4)

Hypocholesterolaemic fatty acids (DFA):

DFA = C18:0 + UFA (unsaturated fatty acids) (5)

Statistical analysis

All descriptive variables were shown as mean ± standard error (SE). The Shapiro-Wilk test was used for testing the distribution. Asymmetrically distributed variables were shown as median (interquartile range). Continuous variables were compared by the Mann-Whitney *U*-test. All data were analyzed using IBM® SPSS® Statistics version 22 software. A *p*-value less than 0.05 was considered statistically significant. Cost-minimization analysis was conducted to assess the economic utility.

Results

Table 1 shows the total lipid content of homogenized tissues of freshwater and seawater species. Among freshwater fish, it was observed that fat content varied the most in common carp (2.2–6.5%) and bream (1–4.2%), while perch (0.8–2.1%) and catfish (0.4–1%) showed less variability, comparatively. There were no significant differences in fat content between mackerel, sardine and salmon (p = 0.183). After the analysis of fatty acids composition in the lipid extracts, the proportional content of each FA was expressed as a percentage of the total amount of FA (data not shown). The most abundant saturated and monounsaturated fatty acids (SFA and MUFA) in freshwater

Table 1

Lipid content of fish species

Fish species	Total lipids (%), mean ± standard error
Freshwater fish	
carp (*Cyprinus carpio*), n = 5	3.76 ± 0.74
catfish (*Siluris glanis*), n = 5	0.63 ± 0.12
perch (*Sander lucioperca*) n = 5	1.51 ± 0.24
bream (*Abramis brama*) , n = 5	2.91 ± 0.25
trout (*Salmo irideus*), n = 3	7.69 ± 1.56
Seawater fish	
mackerel (*Scomber scombrus*), n = 3	13.20 ± 0.21
sardines (*Sardina pilchardus*), n = 3	11.03 ± 2.21
tuna (*Thunnus thynnus*), n = 3	1.17 ± 0.14
hake (*Merluccius merluccius*), n = 3	1.50 ± 0.35
salmon (*Salmo salar*), n = 5	10.60 ± 0.65

and seawater fish were palmitic (16:0) and oleic acid (18:1 n-9). Palmitic acid was most present in carp (16%) and in sardines (21.5%), while oleic acid predominated in salmon (22.1%) and carp (18%). Essential linoleic acids (18:2 n-6) were found in the highest percentage in carp and salmon samples (14.9 and 8.25%, respectively), while the content of arachidonic acid (20:4 n-6) was highest in mackerel (12%). EPA and DHA were the most abundant LC-PUFA ranging from only 3.2% in tuna to 14.7% in sardine samples for EPA and from 12.9% in carp up to 36.8% in hake samples for DHA. Figure 1 shows a relationship between SFA and UFA in the analyzed fish samples. In all freshwater fish species, the ratio was about 75% for unsaturated and 25% for saturated fatty acids. A similar ratio was observed in seawater fish. The content of PUFA was especially uniform, except for salmon which had a lower percentage of SFA (15%) and a higher percentage of MUFA (32%). No difference was found in terms of PUFA/SFA ratio between freshwater and seawater fish ($p > 0.05$).

Fatty acid content per 100 g of edible fish was calculated using the analyzed lipid content of each fish species with the equation: FA in g per 100 g muscle = (% FA of total FA/100) × (Muscle fat % × 0.9). The results are shown in Table 2 in relation to the total content of saturated, monounsaturated, polyunsaturated n-6 and n-3 fatty acids, as well as to the content of the most important long-chain n-3 fatty acids in fish meat. Among the freshwater and seawater fish species studied, the highest average content of SFA was found in sardine and mackerel samples. Salmon had the highest content of MUFA, whereas mackerel was with the highest content of PUFA. The highest values of total n-3 fatty acids and EPA were found in mackerel and sardines samples. Mackerel, sardines, and salmon had the highest content of DHA among seawater species, as well as trout among freshwater species. The ratio of DHA/EPA ranged from 1.8 for sardine samples and up to 10 for tuna samples, indicating that the amount of DHA in natural samples exceeds the amount of EPA in many cases. The content of n-3 fatty acids, EPA, and DHA was significantly higher in seawater fish compared to freshwater fish group (Table 3).

Results in Table 4 are related to the amount of weekly intake of fish in grams or portions necessary for achieving the mean value of the EFSA dietary recommendations of 400 mg EPA+DHA/day. The selected portion was similar to the

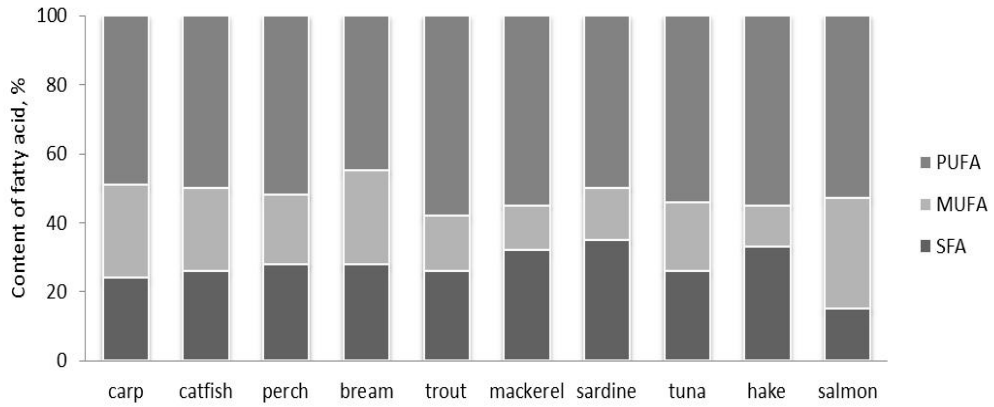


Fig. – 1. The average distribution of saturated and unsaturated fatty acids (MUFA and PUFA) in lipids of freshwater and seawater fish. For abbreviations see Table 2 below.

Table 2

Fatty acid content of each edible freshwater and seawater fish species

Fish species	Fatty acid content (g/100 g), mean ± standard error						
	SFA	MUFA	PUFA	n-3 FA	n-6 FA	EPA	DHA
Freshwater fish							
carp, n = 5	0.73 ± 0.15	0.79 ± 0.13	1.49 ± 0.33	0.74 ± 0.21	0.75 ± 0.15	0.14 ± 0.04	0.48 ± 0.17
catfish, n = 5	0.13 ± 0.02	0.14 ± 0.05	0.25 ± 0.04	0.18 ± 0.03	0.06 ± 0.01	0.05 ± 0.01	0.10 ± 0.02
perch, n = 5	0.35 ± 0.06	0.25 ± 0.04	0.66 ± 0.11	0.54 ± 0.09	0.12 ± 0.02	0.14 ± 0.02	0.33 ± 0.06
bream, n = 5	0.73 ± 0.20	0.77 ± 0.25	1.18 ± 0.36	0.90 ± 0.28	0.27 ± 0.07	0.27 ± 0.08	0.52 ± 0.18
trout, n = 3	1.70 ± 0.41	0.98 ± 0.10	3.79 ± 0.85	3.26 ± 0.85	0.54 ± 0.14	0.46 ± 0.14	2.57 ± 0.66
Seawater fish							
mackerel, n = 3	2.67 ± 0.16	1.51 ± 0.34	6.56 ± 0.30	4.87 ± 0.33	1.69 ± 0 09	1.18 ± 0.06	2.68 ± 0.46
sardines, n = 3	2.72 ± 0.61	1.18 ± 0.57	4.41 ± 1.04	4.01 ± 0.99	0.39 ± 0.05	1.34 ± 0.47	2.45 ± 1.05
tuna, n = 3	0.27 ± 0.04	0.14 ± 0.03	0.48 ± 0.06	0.40 ± 0.04	0.08 ± 0.01	0.04 ± 0.00	0.35 ± 0.03
hake, n = 3	0.39 ± 0.13	0.21 ± 0.05	0.66 ± 0.11	0.62 ± 0.11	0.04 ± 0.00	0.11 ± 0.01	0.48 ± 0.09
salmon, n = 5	2.04 ± 0.13	2.79 ± 0.23	4.47 ± 0.31	3.42 ± 0.28	1.04 ± 0.07	0.74 ± 0.08	2.15 ± 0.22

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; FA – fatty acids; EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid.

Table 3

Comparison of fatty acid content between freshwater and seawater fish groups

Fish species	Fatty acid content (g/100 g), median (interquartile range)						
	SFA	MUFA	PUFA	n-3 FA	n-6 FA	EPA	DHA
Freshwater fish	0.47 (0.19–0.96)	0.39 (0.16–0.97)	0.87 (0.37–1.65)	0.65 (0.27–0.99)	0.18 (0.09–0.48)	0.15 (1.07–0.29)	0.32 (0.16–0.53)
Seawater fish	1.99 (0.34–2.60)	0.86 (0.20–2.37)	4.19 (0.64–5.76)	3.06 (0.56–4.28)	0.47 (0.07–1.19)	0.74 (0.09–1.02)	1.61 (0.46–2.41)
p-value	< 0.01	0.073	< 0.01	< 0.01	0.194	< 0.05	< 0.001

For abbreviations see Table 2 above.

Table 4

Weekly intake of fish necessary for achieving EFSA daily dietary recommendations and EPA + DHA weekly intake cost

Fish	Weekly intake (400 mg EPA + DHA/day), g (portions)	Mean cost of EPA + DHA weekly intake, €
Bream	300 (~2)	3.3
Catfish	1,570 (~10)	5.9
Carp	459 (~3)	4.2
Perch	600 (~4)	5.2
Hake	410 (~2.5)	1.7
Trout*	93 (~0.5)	0.46
Sardine*	67 (~0.5)	0.12
Mackerel*	75 (~0.5)	0.23
Salmon*	95 (~0.5)	1.4
Tuna	636 (~4)	11.8

***Fish with high content of lipid.**

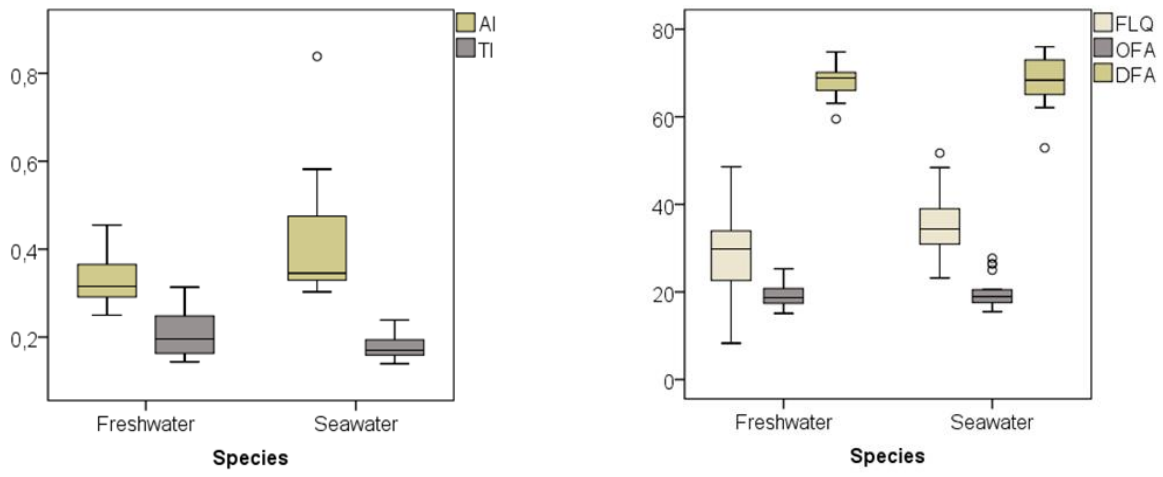
EFSA – European Food Safety Authority; for other abbreviations see Table 2 above.

middle portion of the fatty fish defined by the United Kingdom Scientific Advisory Committee on Nutrition, which is ~ 150 g. The evaluation of the costs of EPA + DHA using average prices of raw fish from the Serbian market was also presented.

Median values (interquartile range) for lipid quality indices are shown in Figure 2. TI, OFA, and DFA within the freshwater and seawater fish groups did not differ significantly ($p > 0.05$). TI for freshwater and seawater fish groups were 0.20 (0.16–0.25) and 0.17 (0.15–0.19), respectively. OFA for the freshwater group was 18.70 (17.40–21.30) while the seawater group had a similar values [18.90 (17.55–22.75)]. DFA also showed similar ranges for both groups 68.80 (66–70.20) for freshwater and 68.40 (64.85–73.05) for seawater group. AI for the freshwater fish group was 0.32 (0.29–0.38) and 0.34 (0.33–0.49) for the seawater fish group ($p < 0.01$). Seawater fish had a significantly higher value for flash lipid quality 34.40 (30.25–39.95) compared to the freshwater fish [29.80 (22.60–34.60)], ($p < 0.05$).

Discussion

It was noted that all species of freshwater fish (except trout) had a similar content of lipids and the obtained values were very low compared to tested species of seawater fish.



a) **b)**

Fig. – 2. Lipid quality indices: a) Atherogenic index (AI) and Thrombogenic index (TI); b) Flesh-lipid quality (FLQ); Hypercholesterolaemic fatty acids (OFA); Hypocholesterolaemic fatty acids (DFA).

Ljubojević et al. [27] reported high variability (6.3–15%) in lipid content of common carp in Serbia. On the other hand, Łuczyńska et al. [28] showed lower values of the fat content in common carp found on Polish market (0.21–1.47%). However, according to the Unated States Department of Agriculture (USDA) database [29], where data come from a variety of sources, the mean value for common carp lipid content is 5.6%. Ljubojević et al. [27] and Stancheva et al. [30] found about 4% of total lipid in catfish, while the USDA database records value of 2.8%. These results are higher compared to our results for catfish. Previous study on fat quality of marketable freshwater fish species in Serbia have indicated that total lipids obtained for perch ranged from 1.5 up to 2.2% [27], which is in agreement with our results as well as with the results given by the USDA database. Lipid content for bream obtained in this study is similar to previous data published by Łuczyńska et al. [31]. The muscles of trout contained more lipids than the other freshwater fish species. Our results are in accordance with the data obtained by Łuczynska et al. [31] and with the USDA database value (6.6%). In a recent study conducted by Bandarra et al. [32], similar results for sardine fat content were obtained as in our research (14 ± 2.9%), while in the study conducted by Zorica et al. [33], the fluctuations in fat content were also reported for the same species of sardine from the middle eastern Adriatic Sea Region. According to McCance and Widdowson's The Composition of Foods Integrated Dataset 2019 on the nutrient content of the United Kingdom food supply, fat content from tuna and salmon is similar to the results in this study, while results for mackerel are slightly higher (17.9 g/100 g of raw fish) [34]. The USDA database shows similar results for mackerel (12%) compared to ours. Ozyilmaz et al. [35] found the total lipid content of European hake from the northeastern Mediterranean of 1.21 ± 0.4%. According to Ackman [36], fish can be classified by its lipid content. He graded the fish in four categories based on lipid levels: lean (< 2%), low-fat (2–4%), medium-fat (4–8%), and high-fat (> 8%). Our results confirmed catfish, perch as lean, bream and carp as low-fat, trout as medium-fat freshwater fish, while within analysed sea fish only tuna and hake were lean fish, and mackerel, salmon, and sardines all belonged to the high-fat category. The fish classification into lean, low-fat, medium-fat, and high-fat can be significant for making tailored dietary programs depending on the dietary goals – either lower fat and energy intake or higher n-3 fatty acid intake.

Wood et al. [37] have suggested that the ratio of PUFA/SFA in food should be above 0.4, and according to that, all the fish species examined revealed a favourable PUFA/SFA ratio from 1.4 to 3.5. The DHA/EPA ratio for freshwater fish and most seawater fish analyzed in this study are in agreement with the literature data [27, 28, 30–33, 35]. Only in carp, the pattern n-3/n-6 ratio was less than 1 in present study, while in all other fish samples, the content of n-3 fatty acids was several times higher than content of n-6 fatty acids. According to the research conducted by Buchtova et al. [38] and Ćirković et al. [39], the carp grown on natural food had a high content of both n-6 and n-3 fatty acids. Ljubojević et al. [27] also reported n-3/n-6 ratio lower than 1 (0.48 ± 0.18) for common carp. Sardine had the highest ratio of these fatty acids at 10.2. The n-6/n-3 ratio should not exceed 4 for the prevention of cardiovascular, heart, and certain chronic diseases [40]. All studied species meet this suggestion.

As it was expected, the content of n-3 fatty acids, EPA and DHA, was significantly higher in seawater fish compared to freshwater fish group. The content of n-3 fatty acids should always be observed in relation to the lipid content of fish and in relation to the origin of fish (river, marine, cultivated). This fact justifies the dietary recommendation of a desirable intake of fatty fish in the diet, as only the fatty fish can provide a significant amount of EPA and DHA. For individuals who do not prefer eating fish, weekly intake of properly chosen species even in a small amount (about 10 g *per* day) may be sufficient to reach the mean value of the EFSA recommendation (400 mg EPA + DHA/day). Taking into account the limitation of the study, related to the small sample size and lack of evidence for seasonal variation of fat and fatty acid composition of fish species, we can only hypothetically suggest portions for achieving dietary recommendations. Having in mind insufficient data of fish consumption in Serbian population, further study should take into account more specific needs among different groups in relation to health benefits. The results of a 2015 cross-sectional study obtained from the Food frequency questionnaire (FFQ) among Serbian women in reproductive age indicated that freshwater fish (trout, catfish and carp) and salmon were consumed by 71% and 41% of examinees, respectively [41]. Increasing of California trout farming in Serbia, compared to previous years may be an indicator of new trends in fish consumption [15]. In the present study, the costs of EPA + DHA in the two fish categories were also calculated and this calculation proved there were few inexpensive sources of EPA + DHA on Serbian market. Sardine and mackerel were the least expensive and very affordable fish sources of EPA and DHA.

Index of atherogenicity indicates the relationship between the sum of the main saturated and the main unsaturated FA, the former being considered pro-atherogenic and the latter anti-atherogenic. Index of thrombogenicity shows the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic FA (MUFA, n-6 PUFA, and n-3 PUFA) [22, 26]. Flesh-lipid quality indicates the percentage correlation between the main n-3 LC-PUFA (EPA + DHA) and the total lipids. The higher value of this index is an indicator of the higher quality of the dietary lipid source [25, 26]. Ouraji et al. [42] and Stancheva et al. [30] reported that higher values of AI and TI (> 1.0) are detrimental to human health. The value of these parameters in the present study were lower than 1. The low values of AI and TI indicates that the tissue of all the studied fish is beneficial from a health point of view.

When considering the optimal fish intake there is one more aspect that should be taken into account. Due to the presence of various contaminants in the aquatic environment,

fish may be contaminated with persistent chemicals resulting in potential risks to human health. Health benefit and health risk of fish consumption is generally known as the nutritional-toxicological conflict [43]. Contaminant concentrations in fish species depend strongly on the species itself, its metabolism and feeding habits, environmental conditions, chemical contaminations of the sediment and suspended particulate matter of the region where it was living before catch or during its production. In a recently published study [44], the content of metals (Hg, Cd, Pb, As) in three fish species from the Danube River in the Belgrade Region did not reach maximum levels established by The Commission of European Communities (EC 1881/2006, 2006) [45]. Also, a recent data on the mercury content in 9 fish species from the Adriatic sea have indicated that concentrations did not exceed the maximum level of 500 μg/kg [46]. In the study conducted by Janković et al. [47], total concentrations of Hg were measured in fish muscle and canned fish products available on Serbian market. Total of 651 samples were analyzed: 350 samples of marine fish, 34 samples of freshwater fish and 267 samples of canned fish products (tuna and sardines). Mercury concentrations in marine fish were in the range of 0.005-0.208 μg/g; in freshwater fish 0.005–0.099 μg/g and in canned products they were in the range of 0.005–0.642 μg/g. All analyzed samples contained mercury below the maximum level laid down by the European Union and Serbian regulation. Additionally, in a study on the Serbian population, the insignificant risk was demonstrated due to mercury, dichlorodiphenyltrichloroethane and polychlorinated biphenyls associated with fish consumption [48]. All mentioned data suggest that fish consumption in Serbia should not pose health risk derived from contaminants commonly found in fish. Beside the level of contaminants in fish, the frequency of fish consumption, type of fish consumed, as well as the size of the meal have to be taken into consideration for balancing the health benefits and risks of fish intake [49]. For example, hybrid of the Mediterranean-Dietary Approaches to Stop Hypertension (DASH) diets, called MIND (Mediterranean-DASH Intervention for Neurodegenerative Delay) emphasizes an optimal serving of just one meal of fish per week linked to neuroprotection and dementia prevention. This is opposed to 6 meals per week specified by the cardiovascular Mediterranean diet [50]. Our results on n-3 fatty acid content in freshwater and seawater fish indicated that weekly intake of some of the analyzed fish species that would enable achieving dietary recommendation is in accordance with MIND diet approach (sardine, mackerel, salmon, trout).

Conclusion

Among the results of analyses within species, significant variations in the content of certain classes of fatty acids were observed, which confirms that a large number of factors affect the lipid content. Although there were differences in lipid content and fatty acid composition, it was shown that both fish categories are good sources of PUFA. Among the investigated freshwater and seawater fish species, sardine and mackerel had the highest content of n-3 LC-PUFA and represented economically viable sources of EPA + DHA. Besides the fish, other dietary sources of n-3 fatty acids should be considered in further studies.

Acknowledgement

This research was supported by the Serbian Ministry of Education, Science and Technological Development, Project No 46009.

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Received on February 12, 2020
Revised on May 21, 2020
Accepted on June 1, 2020
Online First June, 2020

ORIGINAL ARTICLE

UDC: 616.31-08
DOI: https://doi.org/10.2298/VSP200304060A

Clinical and microbiological effects of photodynamic therapy applied in non-surgical treatment of periodontitis

Klinički i mikrobiološki efekti primene fotodinamske terapije u nehirurškom lečenju parodontopatije

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Abstract

Background/Aim. Treatment of periodontitis undergoes several treatment phases. Non-surgical periodontal treatment (NSPT) represents the basic treatment stage, and it is applied to all the patients undergoing periodontal treatment. Adjunctive antimicrobial photodynamic therapy (aPDT) is one of several contemporary and relatively new possibilities with a role to inactivate microorganisms responsible for the occurrence and progression of the disease. The aim of this study was to comparatively analyze the clinical and microbiological effects of the NSPT alone, as well as combined with aPDT. **Methods.** A split-mouth method design was used in a prospective randomized controlled trial. The following clinical parameters were registered and monitored: plaque index (PI), bleeding on probing, probing depth (PD), and clinical attachment level (CAL). The presence of microorganisms *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Treponema denticola* was detected by the polymerase chain reaction (PCR) method. Samples were tested before the therapy, as well as three months after the therapy. Therapeutic modalities of NSPT and NSPT with adjunct aPDT were examined. **Results.** All of the analyzed clinical parameters proved statistically significant improvement after the application of both treatment modalities ($p < 0.001$). Microbiological analyses showed that the total number of microorganisms was statistically significantly lower after the application of both methods ($p < 0.001$). Following the treatment, there was a statistically significantly reduced number of microorganisms *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Treponema denticola* ($p < 0.001$). NSPT combined with aPDT led to a statistically significant improvement of both clinical parameters and microbiological status compared to NSPT applied on its own. **Conclusion.** The study showed improvement of all clinical indicators after the application of both treatment modalities. The total number of microorganisms was reduced as well as the number of specific microorganisms. Combining aPDT with NSPT led to a statistically significantly higher reduction in the number of microorganisms compared to NSPT alone.

Key words:
aggregatibacter actinomyctemcomitans; periodontitis; photochemotherapy; polymerase chain reaction; porphyromonas gingivalis; treponema denticola; treatment outcome.

Apstrakt

Uvod/Cilj. Lečenje parodontopatije prolazi kroz nekoliko faza. Kauzalna (inicijalna, bazična) faza lečenja parodontopatije predstavlja osnovnu terapijsku fazu kroz koju prolaze svi pacijenti. Antimikrobna fotodinamska terapija (aPDT) predstavlja jednu od savremenih i relativno novih mogućnosti, čija je uloga inaktivacija mikroorganizama odgovornih za nastanak i progresiju oboljenja. Cilj istraživanja bio je uporedna analiza kliničkih i mikrobioloških efekata primene samo kauzalne terapije i njene kombinacije sa aPDT. **Metode.** U prospektivnoj randomizovanoj studiji bila je primenjena metoda podeljenih usta. U istraživanju su bili praćeni sledeći klinički parametri: plak indeks (PI), krvarenje na provokaciju, dubina sondiranja (DS) i nivo pripojnog epitela (NPE). Metodom lančane reakcije polimeraze (PCR) praćeno je prisustvo mikroorganizama *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* i *Treponema denticola*. Testirani su uzorci pre, kao i tri meseca nakon terapije. Ispitivani su terapijski modaliteti kauzalne terapije i njene kombinacije sa aPDT. **Rezultati.** Analizirani klinički parametri: PI, krvarenje gingive, DS i NPE pokazali su statistički značajno poboljšanje nakon primene oba modaliteta lečenja ($p < 0,001$). Mikrobiološke analize pokazale su da je ukupni broj mikroorgnizama

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bio statistički značajno manji primenom obe metode lečenja ($p < 0{,}001$). Mikroorganizmi *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* i *Treponema denticola* nalazili su se u statistički značajno manjem broju nakon primenjenih terapija ($p < 0{,}001$). Kauzalna terapija u kombinaciji sa aPTD je dovela do statistički značajnog poboljšanja kako kliničkih parametara, tako i mikrobiološkog statusa u odnosu na kauzalnu terapiju primenjenu samostalno. **Zaključak.** Istraživanje je pokazalo da je došlo do poboljšanja svih kliničkih pokazatelja nakon primene oba modaliteta lečenja. Ukupni broj mikroorganizama, kao i broj specifičnih mikroorganizama je bio smanjen. Kombinacija aPDT sa kauzalnom terapijom dovela je do statistički značajno većeg smanjenja broja mikroorganizama u poređenju sa samom kauzalnom terapijom.

Ključne reči:
aggregatibacter actinomyctemcomitans; periodontitis; fotohemioterapija; polimeraza, reakcija stvaranja lanaca; porphyromonas gingivalis; treponema denticola; lečenje, ishod.

Introduction

Periodontitis is a chronic inflammatory disease with bacterial infection playing one of the key roles in the etiopathogenesis of the disease [1]. Socransky et al. [2] and Socransky and Haffajee [3] have classified periodontal pathogens into six complexes based on pathogenesis and subgingival biofilm colonization in adults.

Red complex bacteria are dominant in subgingival dental plaque among patients who suffer from chronic periodontitis with deep periodontal pockets. It is known that *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* are able to invade certain periodontal tissues. Their persistence over a long period of time and after the treatment leads to the reactivation of the disease [4, 5].

Periodontal treatment consists of several phases. Nonsurgical treatment of periodontitis (NSPT) presents a basic and inevitable treatment phase. In certain cases, further chemical plaque control and/or antibiotic therapy are performed [6, 7].

The removal of dental plaque and calculus from the root surface, elimination of necrotic cementum, and root planning are achieved by mechanical scaling and root planning (SRP). The above-mentioned procedures result in the activation of reparatory and regenerative processes in periodontium [8]. However, SRP alone cannot completely remove subgingival periodontal pathogens in inaccessible or hardly accessible areas. Incomplete elimination of periodontal pathogens leads to a relapse of the infection. Germ removal and infection elimination, present in dental apparatus, stand as crucial factors that create conditions for possible regeneration of lost tissues. This is the aim of contemporary periodontal therapy [9–11].

The use of systemic antibiotics has shown positive effects on the periodontium when treating periodontitis [12]. However, additional research is needed in order to evaluate real effects on the periodontium due to increased microbial resistance on antibiotics [13]. Local or systematic antibiotic therapy is commonly used as additional treatment in initial active periodontal treatment (APT), aimed at bacterial removal for a longer period of time [14]. Mentioned pharmacological therapy has many side effects, therefore, it is necessary to look for alternative methods. There is an ongoing search for ideal alternative methods for eliminating microorganisms, thus improving the effects of periodontal treatment [15].

Antimicrobial photodynamic therapy (aPDT) represents one of the several contemporary and relatively new possibilities for the inactivation of microorganisms responsible for the pathological occurrences in the periodontium. Antimicrobial photodynamic therapy is defined as an oxygen-based photochemical reaction that involves photosensitizer (PS), light source, and oxidative molecules. Owing to its high antibacterial potential, aPDT is suggested as a potential adjunctive treatment for periodontitis, peri-implantitis [16], as well as in endodontic therapy [17–19]. The advantage of aPDT over the use of antibiotics is in the fact that microorganisms do not develop resistance, therefore, the procedure can be repeated as many times as needed without any negative effects on the patient's organism and general health [20].

The aim of this research was to conduct a comparative analysis of clinical and microbiological effects of NSPT alone and NSPT supplemented by adjunctive use of aPDT.

Methods

This was a prospective, randomized study with a split-mouth method. This method means that one half of both jaws represents a test group, while the other half of both jaws served as a control group. The test group was treated by NSPT combined with aPDT, while the control group was treated by NSPT applied alone. The research was approved by the Ethics Committee of the Faculty of Medical Sciences in Kosovska Mitrovica (number 05-1691, from September 13, 2016). Patients were included in the study from October 2016 until December 2018. The research was conducted at the Periodontology and Oral Medicine Clinic, Faculty of Medical Sciences, University of Priština/Kosovska Mitrovica. The research included 25 patients. Patients who participated in this study were 30 to 70 years old; they suffered from chronic periodontitis and had not used local or systemic antibiotic therapy at least three months prior to the inclusion in the study. The participants had to be healthy non-smokers and with at least three teeth in each quadrant except for the third molar, as well as with at least two teeth with probing depth of 5 and more millimeters in each quadrant. Criteria for exclusion were the presence of chronic diseases, smoking, periodontal therapy in the last six months, pregnancy, and lactation. Determining which side of the mouth will be tested and which control was done by means of randomization envelopes.

First stage – clinical parameters followed in the study

After the anamnesis, clinical examination, and analysis of additional diagnostic tools (orthopantomographic image – OPG), the following parameters were registered: plaque index (PI) according to Silness Löu, bleeding on probing (BOP) according to Mühlemann, probing depth (DP), and clinical attachment level (CAL). The amount of dental plaque in one-third of the gingival dental crown was examined, with a periodontal probe, on four tooth surfaces (distobuccal, buccal, bucco-mesial, and oral surface) and graded from 0 to 3 [21]. Determining BOP was performed by interdental papilla bleeding provocation with Williams periodontal probe (Hu-Friedy, USA). Probing was performed from the papilla base to its highest point and graded from 0 to 4 [22]. PD is the measured distance from the gingival margin to the bottom of the periodontal pocket. CAL is the measured distance from the cemento-enamel junction (CEJ) to the coronal part of the junctional epithelium. DP and CAL measurements were performed using a periodontal probe according to Williams (Hu-Friedy, USA) in six points at each tooth (three points on the vestibular side and three points on the oral side). Values were expressed in millimeters, and numbers were rounded towards a higher value. Periodontal condition assessment was done before the therapy as well as three months after the therapy.

Examiner calibration

One examiner (ZA) recorded all the clinical parameters. A standard calibrated probe was used for measuring. Periodontal pocket depth, CAL, and BOP were tested on three patients and measured in two separate sessions with an interval of seven days. Calibration was accepted if the percentage between the measuring at the beginning and the measuring after seven days was higher than 90%.

Second stage – Microbiological procedure

The samples of subgingival dental plaque were collected from all patients after the second visit and three months after the therapy. The sampling of subgingival dental plaque for microbiological analysis was performed 24 hours after the clinical parameters' registration (in order to avoid blood contamination). DP value determined the choice of periodontal pocket used for sampling, meaning that sampling was performed in the region with the highest value. Sterile paper points (four in total, one for each quadrant) from PET–diagnostic set (MIP Pharma GmbH, Germany) were placed until the first mild resistance and held in place for twenty seconds. They were placed in separate plastic test tubes (Eppendorf) and kept at room temperature until microbiological analysis.

The presence of the following microorganisms: *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Treponema denticola* was analyzed by means of polymerase chain reaction (PCR). Samples were tested prior to the treatment and three months after it.

Third stage – non-surgical periodontal treatment (NSPT)

Samples for microbiological analysis were taken during the second visit, after which NSPT had been applied. This study utilized Full Mouth Disinfection protocol (FMD), meaning that NSPT was performed within 24 hours. Quirynen et al. [23] introduced a one-session approach in treating diseased periodontium, with the main goal being elimination or reduction of all periodontopathogens from the oropharynx's region (periodontal pocket, all of the oral mucous membrane, spit, and tonsils) within 24 hours. Removal of supragingival and subgingival layers while treating periodontal pockets was conducted under local infiltration anaesthesia (2% lidocaine with adrenaline in scale 1:100,000). Heavy dental deposits were removed with an ultrasonic device (Mini Piezon, EMS, Nyon, Switzerland). NSPT was applied in all quadrants at once due to possible contamination of other periodontal pockets with residual microorganisms, which might diminish the effects of the therapy using Gracey's curettes (Gracey curettes, Hu-Friedy, Chicago, IL, USA). Pockets were rinsed deeply with sterile physiological solution. Removal of soft deposits and final polishing of the teeth surfaces was performed by using abrasive paste (Proxyt RDA 36), middle-sized particles (Liechtenstein) without fluoride, and a rotating brush.

Fourth stage – application of antimicrobial photodynamic therapy

After NSPT (0–24 hours in minor and 24–48 hours in greater gingiva bleeding), aPDT was applied. Procedure for aPDT application included creating a relatively dry workspace, using aspiration tools and cotton rolls, followed by photosensitizer placement in the periodontal pocket. The photosensitizer used was phenothiazine chloride (HELBO® Blue photosensitizer, Bredent Medical GmbH & Co KG) which was thoroughly rinsed with the physiological solution for periodontal pockets up to 5 millimeters after one minute and for pockets over six millimeters after three minutes according to the manufacturer manual. Photosensitizer was activated with a 660 nm diode laser powered by 100 mW (HELBO® TheraLite Laser, HELBO® Zenden, Germany). Fibers 450 μm (3D Pocket Probe; Bredent Zenden Germany) with activated laser light were applied on periodontal pockets for 1 minute (10 seconds at the time on 3 points on vestibular and on 3 points on oral side).

Statistical analysis

In order to analyze primary data, descriptive statistical methods were used, methods for testing statistical hypotheses, and methods for analyzing outcomes and potential predictor relations. Depending on variable type and type of distribution, data description was shown as n (%), mean ± standard deviation (SD), or median (minimum-maximum). When testing the statistical hypothesis, we used the follow-

ing methods: the *t*-test, Wilcoxon's test, χ^2 test, and variance analyses of repeated measurements. The statistical hypothesis was tested at the statistically significant level (alpha level) of 0.05. All the data were processed in IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) software package or R programming environment (R Core Team, 2018).

Results

All participants completed the study successfully, and there were no side effects after the treatment or during the research period. The research was conducted on 25 subjects. Their average age was 35 years (30–64 years). There was a total of 13 male (52%) and 12 female (48%) participants.

One hundred samples were gathered from 25 subjects (four samples each) using the split-mouth method. Fifty samples were analyzed for the test group and 50 samples for the control group.

There was no statistically significant difference in clinical values such as PI, BOP, and DP for both treatment modalities before the treatment. However, there was a statistically significant difference in the values of CAL before the treatment.

Three months after the therapy, all clinical parameters monitored (PI, BOP, PD, and CAL) were statistically significantly reduced in comparison to their initial values. The results showed continual clinical improvement three months after the therapy, especially in clinical parameter values where additional aPDT was applied. Overall, statistically significant changes in PI, PD, and CAL for both treatment modalities were observed ($p < 0.001$). For the given parameters concerning therapy models, a statistically significant difference in values was obtained after the treatment ($p < 0.001$) (Table 1).

There was no statistically significant difference in the values of total bacteria number in relation to therapy modalities before the treatment ($p = 0.836$), while after the treatment, that difference was statistically significant ($p < 0.001$) (Table 2).

Statistically significant differences in the total number and prevalence of microorganisms *Aggregatibacter actinomycetemcomitans* and *Treponema denticola* were not shown before the treatment, while for *Porphyromonas gingivalis* a statistically significant difference was shown ($p < 0.001$). By observing bacterial microorganisms individually after the treatment, it was demonstrated that there was a statistically significant difference in the values for *Aggregatibacter actinomycetemcomitans* ($p = 0.035$), *Porphyromonas gingivalis* ($p < 0.001$), and *Treponema denticola* ($p < 0.001$) in relation to therapy modalities (Table 3).

The clinical status of periodontium before and after applied treatments is shown in Figure 1.

Table 1

Clinical parameters' values before and after the therapy

Parameter	Treatment	Values (mean ± SD)		Overall *p*-value	
		before	after	in time	between treatment
Plaque index	C	1.53 ± 0.44	0.83 ± 0.29	< 0.001	0.685
	E	1.54 ± 0.42	0.74 ± 0.28		
	p (C vs. E)	0.621	< 0.001		
Gingiva bleeding index	C	2.03 ± 0.98	1.17 ± 0.60	< 0.001	0.861
	E	2.04 ± 0.97	1.08 ± 0.59		
	p (C vs. E)	0.496	< 0.001		
Depth sounding	C	3.78 ± 0.75	2.92 ± 0.61	< 0.001	0.652
	E	3.75 ± 0.70	2.79 ± 0.61		
	p (C vs. E)	0.536	< 0.001		
Junctional epithelium level	C	2.88 ± 0.51	2.41 ± 0.49	< 0.001	0.714
	E	2.86 ± 0.50	2.33 ± 0.57		
	p (C vs. E)	0.040	0.058		

C – control group treated with causal therapy alone; E – test group treated with causal therapy combined with antimicrobial photodynamic therapy; SD – standard deviation.

Table 2

Total bacteria number before and after the treatment

Treatment	Total bacteria number, median (minimum– maximum)	
	before	after
C	1.7×10^8 (4.5×10^6–8.3×10^9)	2.3×10^6 (5.3×10^4–9.2×10^7)
E	1.1×10^8 (3.8×10^6–7.7×10^9)	5.6×10^4 (3.7×10^3–1.9×10^7)
p (C vs. E)	0.836	< 0.001

C – control group treated with causal therapy alone; E – test group treated with causal therapy combined with antimicrobial photodynamic therapy.

Table 3

Prevalence of microorganisms before and after the treatment

Bacterium	Treatment	Prevalence, n (%)		Overall *p*-value	
		before	after	in time	between treatment
Aggregatibacter actinomycetemcomitans	C	18 (36.0)	9 (18.0)	< 0.001	0.998
	E	17 (34.0)	2 (4.0)		
	p (C vs. E)	0.655	0.035		
Porphyromonas gingivalis	C	28 (56.0)	20 (40.0)	< 0.001	< 0.001
	E	22 (44.0)	4 (8.0)		
	p (C vs. E)	0.025	< 0.001		
Treponema denticola	C	49 (98.0)	33 (67.3)	< 0.001	0.001
	E	47 (94.0)	12 (24.0)		
	p (C vs. E)	0.317	< 0.001		

C – control group treated with causal therapy alone; E – test group treated with causal therapy combined with antimicrobial photodynamic therapy.

Fig. 1– A) Clinical status before therapy; B) Non-surgical periodontal treatment (NSPT); C) HELBO® Blue photosensitizer application; D) Diode laser activation; E) Clinical status after therapy.

Discussion

Present clinical data unambiguously proved that mechanical treatment of the root surface provides satisfying results with improvements of clinical parameters, as shown in other studies [24–27]. However, complete elimination of the present subgingival periodontogens is not possible [26, 27]. The present study showed that the application of both treatment modalities significantly improved clinical parameters after the therapy. The results showed continuous clinical im-

provements three months after the therapy, especially in the group where additional aPDT was applied. Results gathered by Husejnagic et al. [28] also correlate with our research (PI was 58.83 ± 25.31 at baseline and was reduced to 31.32 ± 15.12 at the point of reevaluation).

This study followed up several clinical parameters, but it also analyzed microbiological parameters through the application of the PCR technique, quantitative-qualitative analysis of subgingival periodontal pathogens, showing reduction of certain examined pathogens. Our research, using the quantification method (PCR), showed more accurate results in examining pathogens in relation to a study conducted by Petrovic et al. [29]. The use of the PCR method showed that the following bacteria predominated in our samples: *Porphyromonas gingivalis* and *Treponema denticola*, which is a characteristic finding in patients affected by chronic periodontitis. Kumawat et al. [30] established that the prevalence of these two microorganisms correlates with the progression of pathological processes and that their presence is connected with the level of periodontal tissue damage. By following each of the three microorganisms, our study proved that the total number of *Aggregatibacter actinomycetemcomitans* was reduced after the treatment by both therapy modalities. However, antimicrobial photodynamic therapy gave better results. Corrêa et al. [31] have followed this reduction of *Aggregatibacter actinomycetemcomitans* during a period of 3, 7, 14, and 90 days, and proved that antimicrobial photodynamic therapy significantly affects the reduction of microorganisms in a shorter period of time, while improving clinical parameters in longer time periods.

Examining microbiological status during the assessment of the same pathogens as in this study, Husejnagic et al. [28] have also found out that the number of *Porphyromonas gingivali*s and *Treponema denticola* significantly declined, while the number of *Aggregatibacter actinomycetemcomitans*, after two applications of aPDT during the therapy, also declined, but not significantly. Unlike our study, the abovementioned study also included smokers. In relation to microbiological status, our achieved results showed superior effects with only one application of aPDT. This could be explained by the fact that smokers were not included in our study. Smoking plays an important role in periodontitis etiology, as well as in its further development. Smoking can change the microbial status of oral flora, leading to increased growth of pathological periopathogens *Porphyromonas gingivalis* and *Treponema denticola* in the subgingival region and consequently to the destruction of the periodontal tissue over time [32, 33].

NSPT alone cannot achieve long-term results in improving clinical parameters and reducing subgingival periodontopathogens in smokers [34]. Xue et al. [35] have examined the clinical efficiency of aPDT as a supplement to SRP in treating chronic periodontitis and have not achieved significant improvements in the values of periodontal probing depth decrease nor in the clinical attachment level increase in groups of patients which included smokers. Multiple applications of aPDT using the "split-mouth" method did not achieve clinical, microbiological, or immunological improvements during the treatment [36]. There are few studies able to prove that aPDT application results in bacterial species' reduction in smokers affected by chronic periodontitis.

The number of laser applications, as well as the time of exposure of aPDT, is not crucial in achieving clinical results during the treatment. Applying aPDT in 3 sessions (0, 7, and 14 days) and with two-minute exposure time has not achieved better results compared to our study [37]. Some studies also imply that there is no long-term method that may be able to completely remove microorganisms from periodontal pockets. The results of Lulic et al. [38] have shown that recolonization of deep periodontal pockets happens even though the numbers of microorganisms are reduced in a short period of time. Comparative analysis of therapy modalities after the therapy, conducted in our study, proved that initial periodontal therapy combined with aPDT led to a significant reduction in microorganism number compared to NSPT alone. Moreira et al. [39] conducted a study with an immunological analysis and proved that the application of aPDT combined with NSPT therapy gives promising results in the long-term prognosis of treating patients with aggressive forms of periodontitis. Similar results have also been reported by other authors [38, 40].

Given the above, it may be concluded that aPDT produces a positive effect only in combination with previously applied mechanical treatment of the root canal. However, many studies are dealing with these questions and there is no consensus concerning the approach and treatment of patients affected by periodontitis. This is primarily related to the antibiotic application in patients who smoke and to the question of which antibiotic is more efficient in treating non-smokers and *vice versa* [41]. There is also the question of the number of aPDT applications and the choice of photosensitizers. Over the last decade, several clinical studies have concluded that repeated aPDT application gives better results in treating periodontitis [31, 36, 42]. On the other hand, some other authors reduce the number of applications or apply only one aPDT treatment [43].

Conclusion

This research showed that all clinical parameters significantly improved after the application of both treatment modalities. The total number of microorganisms was significantly reduced, as well as the number of specific microorganisms: *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Treponema denticola*. The combination of antimicrobial photodynamic therapy with nonsurgical periodontal therapy showed improvement of all clinical parameters and the reduction of the number of microorganisms compared to NSPT therapy alone.

Conflict of interest

There is no conflict of interest.

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Received on March 4, 2020
Revised on May 26, 2020
Accepted on June 2, 2020
Online First June, 2020

ORIGINAL ARTICLE

UDC: 616.833.5-009.7
DOI: https://doi.org/10.2298/VSP181002069V

Assessment of the neuropathic component in a chronic low back pain syndrome

Ispitivanje neuropatske komponente bola kod hroničnog lumbalnog bolnog sindroma

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Abstract

Background/Aim. Chronic low back pain syndrome (CLBPS) is the most common cause of functional disability and loss of working ability in developed countries. Some research shows that neuropathic pain (NP) is present in almost 50% of patients with CLPBS. The aim of this study was to determine the characteristics of NP and its impact on quality of life (QoL) in patients with CLBPS. **Methods.** Patients were tested using three questionnaires for NP: Pain Detect Questionnaire, Leeds Assessment of Neuropathic Symptoms and Signs, and *Douleur Neuropathique 4 questions*. Thirty-two patients diagnosed with NP based on current clinical criteria and with positive results for NP on all three NP questionnaires formed an experimental group. A control group consisted of 32 patients with CLBPS who did not fulfill clinical criteria for NP and were negative for NP on all three questionnaires. Hamilton depression and anxiety rating scales (Ham-D and Ham-A, respectively) and Short Form (SF)-36 questionnaire were also applied. **Results.** According to magnetic resonance imaging (MRI), disc herniation was typically detected in the experimental group, while degenerative changes were commonly found in the control group. Patients from the experimental group had significantly greater intensity of pain, pain radiation in the legs, and the pain was usually presented as episodes of sudden attacks with mild pain between them. The most distinctive features of NP were allodynia, electric shock sensation, and hypoesthesia to prick. Patients from the experimental group also had significantly higher depression and anxiety scores, as well as worse QoL compared to the control group, especially in mental domains. Predictors of worse QoL in the patients with CLBPS were a higher level of anxiety and depression. **Conclusion.** The presence of allodynia, electric shock-like sensations, and hypoesthesia to prick in patients with CLBPS suggest NP. CLBPS patients with NP had worse scores in mental domains of QoL compared to CLPBS patients without NP.

Key words:
anxiety; back pain; depression; neuralgia; quality of life.

Apstrakt

Uvod/Cilj. Hronični lumbalni bolni sindrom (HLBS) je najčešći uzrok funkcionalne onesposobljenosti i gubitka radne sposobnosti u razvijenim zemljama. Neka istraživanja pokazuju da je neuropatski bol (NB) prisutan u gotovo 50% bolesnika sa HLBS. Cilj rada bilo je određivanje karakteristika NB i njegovog uticaja na kvalitet života (KŽ) kod bolesnika sa HLBS. **Metode.** Bolesnici sa HLBS bili su testirani pomoću tri upitnika za procenu NB (*Pain Detect Questionnaire*, *Leeds Assessment of Neuropathic Symptoms and Signs* i *Douleur Neuropathique 4 questions*). Trideset dva bolesnika koja su imala kliničku dijagnozu NB prema važećim kriterijuma i koji su imali pozitivne rezultate na sva tri upitnika za procenu NB svrstani su u eksperimentalna grupu, a 32 bolesnika sa HLBS koji nisu ispunili kliničke kriterijume za NP i bili negativni i prema kliničkim kriterijumima i prema korišćenim upitnicima činili su kontrolnu grupu. Takođe, u ispitivanju su korišćene Hamiltonove skale za procenu depresije i anksioznosti (Ham-D i Ham-A), kao i *Short Form* (SF)-36 upitnik za procenu KŽ. **Rezultati.** Prema rezultatima magnetne rezonance, diskus hernija je bila češće prisutna kod bolesnika u eksperimentalnoj grupi, dok su u kontrolnoj grupi najčešće zabeležene degenerativne promene. Bolesnici iz eksperimentalne grupe su imali znatno veći intenzitet bola, bol sa propagacijom u nogama, koji se obično javljao u obliku epizoda iznenadnih jakih

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napada sa blagim bolom između epizoda. Najspecifičnije karakteristike NB bile su alodinija, senzacija nalik električnom udaru i hipestezije pri bockanju. Ovi bolesnici su imali znatno veće skorove depresije i anksioznosti, kao i lošiji KŽ u odnosu na bolesnike iz kontrolne grupe. Prediktori lošijeg KŽ kod bolesnika sa HLBS su bili veći stepen anksioznosti i depresije. **Zaključak**. Prisustvo alodinije, osećaja strujnih udara i hipestezija na bockanje ukazuju na HLBS. Bolesnici sa HLBS i NB imaju lošiji KŽ u odnosu na bolesnike sa HLBS bez NB.

Ključne reči:
anksioznost; leđa, bol; depresija; neuralgija; kvalitet života.

Introduction

Chronic low back pain syndrome (CLBPS) is defined as a presence of pain lasting for at least 12 weeks and is located in the back area between the lower edge of the ribs and gluteal region, with or without radiation to the legs [1]. The prevalence of CLBPS is 4–10%, and it has been increasing over the years [2]. CLBPS typically leads to a significant reduction of a patient's quality of life (QoL). It is the most common cause of functional disability and loss of working ability in developed countries. Increasing treatment costs, loss of productivity, and decrease in working days are associated with CLBPS [3].

Baron et al. [4] reported in their review that neuropathic pain (NP) was present in 16–55% of patients with CLBPS. NP in CLBPS commonly occurs because of the damage of the nerve fibers due to degenerative changes of the intervertebral disc (local NP), the release of local inflammatory mediators in the area of the degenerated disc (inflammatory, root NP), and, finally, because of the mechanical root compression (root NP) [5]. Compared to nociceptive pain, NP seems to be associated with higher pain intensity, larger number of comorbidities, more severe comorbidities, reduced QoL, and higher treatment costs [4]. One recent study noted that the treatment costs for patients who have the neuropathic component of pain in the lower part of the back were 67% higher than for those who had only nociceptive pain [6].

The aim of this study was to determine the frequency, characteristics, and impact of NP on QoL in patients with CLBPS.

Methods

This study was approved by the Ethics Committee of the University Clinical Center of the Republic of Srpska, Banja Luka, Bosnia and Herzegovina. Prior to research, informed consent was obtained from all patients. At the Neurology Clinic in Banja Luka, 168 patients with CLBPS were examined from January 2015 until December 2015. Nine patients were excluded because of the presence of another disorder that could significantly affect their QoL and the results of this research. Thus, 159 patients were included in the further examination. Among them, a definite clinical diagnosis of NP according to the NP criteria proposed by Haanpää et al. [7] was made in 59 patients. We tested all 59 patients with three questionnaires for NP diagnosis: Pain Detect Questionnaire (PD-Q), Leeds Assessment of Neuropathic Symptoms and Signs (LANSS), and *Douleur Neuropathique 4 questions* (DN4). The questionnaires were filled in by all patients in the presence of a neurologist, who was available for them in case of difficulties in understanding certain questions. Score ≥19 on the PD-Q, score ≥12 on the LANSS, as well as score ≥ 4 on the DN4, indicated the presence of NP [8–10]. Finally, for further analysis, we selected 32 patients who had a diagnosis of NP based on criteria by Haanpää et al. [7] and had positive results for NP on all three NP questionnaires (experimental group). The control group consisted of 32 patients with CLBPS who did not fulfill clinical criteria for NP diagnosis given by Haanpää et al. [7] and were negative for NP on all three questionnaires.

We examined sociodemographic characteristics of our patients including gender, current age, education, occupation, marital and employment status. Following features of the CLBPS were also examined: age at onset of the disease, disease duration, degree of disability, presence of comorbid disorders, and current therapy.

Identification of the affected nerve root and the severity of the nerve injury were established by electromyography (EMG) [11]. All examinations were performed by the same examiner (ZV) on the Oxford Synergy equipment. The temperature of the tested limb was above 31ºC. Nerve conduction study (NCS) was performed using surface stimulation and registration electrodes. The following parameters were assessed: motor conduction velocity (MCV), the amplitude of the compound muscle action potentials (CMAP) and minimal F wave latency of motor nerves (peroneal and tibial nerves), sensory conduction velocity (SCV), and amplitude of sensory nerve action potentials (SNAP) of sensory nerves (sural nerve). Using the needle electrode, *extensor digitorum brevis*, *flexor hallucis brevis*, *tibialis anterior*, *gastrocnemius* and *vastus medialis* muscles were examined on both sides. We also performed magnetic resonance imaging (MRI) in all CLBPS patients.

Hamilton depression rating scale (Ham-D) was used to assess symptoms of depressiveness, where a score > 8 indicated the presence of depression [12]. Hamilton anxiety rating scale (Ham-A) was used to estimate anxiety, where a score > 18 indicated the presence of anxiety [13].

As a measure of health-related QoL, each patient filled in the Serbian version of the Short Form (SF)-36 questionnaire [14], which is a generic measure that combines eight general health domains: physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE), and mental health (MH). Besides the total SF-36 score, physical composite score (PCS) and mental composite score (MCS) are two main scores to summarize these eight domains. All scores are interpreted with a 0-100 scale, where higher numbers represent better QoL.

All examined variables were analyzed using the Kolmogorov-Smirnov test in order to determine whether they were distributed normally. For the comparison of nominal and ordinal variables, the χ^2 test or Fisher test was used. The difference between two continuous nonparametric variables was investigated using the Mann-Whitney *U*-test, while the Student's *t*-test was used for continuous parametric variables. All parameters that differed between patients with and without NP were included in the multiple linear regression analysis (stepwise method) as independent variables, while the SF-36 score was considered a dependent variable. The level of statistical significance was 0.05 for a statistically significant difference and 0.01 for a highly statistically significant difference.

Results

Sociodemographic and clinical characteristics of patients included in the study are given in Table 1. According to magnetic resonance imaging (MRI), disc herniation was typically detected in the experimental group, while degenerative changes were commonly found in the control group.

Patients from the experimental group had significantly greater intensity of pain, pain radiation in the legs, and the pain was usually presented as episodes of sudden attacks with mild pain between them (Table 2). The most distinctive features of NP were allodynia, electric shock sensation, and hypoesthesia to prick. Patients from the experimental group also had significantly higher depression and anxiety scores

Table 1

Sociodemographic and clinical characteristics of patients with chronic low back pain (CLBP) syndrome with (experimental group) and without (control group) neuropathic pain

Characteristic	Experimental group (n = 32)	Control group (n = 32)
Gender (% of males)	53.1	56.2
Age (years), mean ± SD	46.6 ± 8.5	47.2 ± 9.5
Education (% of patients)		
lower	18.8	18.8
middle	56.2	59.4
higher	25.0	21.9
Employment (% of patients)		
physical work	50.0	59.4
intelectual work	50.0	40.6
Employment status (% of patients)		
employed	75.0	81.2
unemployed	25.0	18.8
Marital status (% of patients)		
lives with a partner	81.2	81.2
lives alone	18.8	18.8
Age at CLBPS onset (years), mean ± SD	42.2 ± 8.2	43.0 ± 8.6
Disease duration (years), mean ± SD	4.4 ± 1.4	4.2 ± 2.0
CLBP (% of patients)		
unilateral	78.1	78.1
bilateral	21.9	21.9
Root involve according to EMG (% of patients)*		
none	0.0	21.9
L4	21.9	9.4
L5	34.4	31.2
S1	43.8	37.5
Severity of radiculopathy according to EMG (% of patients)**		
absent	0.0	21.9
mild	9.4	62.5
moderate	56.2	15.6
severe	34.4	0.0
Root involvement according to MRI (% of patients)		
L3	21.9	12.5
L4	34.4	34.4
L5	43.8	53.1
Type of the lesion according to MRI (% of patients)*		
disc herniation	100.0	0.0
degenerative changes	0.0	100.0

SD – standard deviation; EMG – electromyography; MRI – magnetic resonance imaging. * $p < 0.05$; ** $p < 0.01$.

(Table 3), as well as worse QoL compared to the control group, especially in mental domains (Table 4). Predictors of worse QoL in the patients with CLBPS were a higher level of anxiety and depression (Table 5).

Table 2

Main characteristics of pain in patients with chronic low back pain (CLBP) syndrome with (experimental group) and without (control group) neuropathic pain

PDQ questionnaire	Experimental group (n = 32)	Control group (n = 32)
Actual pain (mean ± SD) **	4.5 ± 0.8	3.5 ± 1.0
Strongest pain (mean ± SD) **	7.2 ± 1.0	5.9 ± 1.2
Average pain (mean ± SD) **	4.8 ± 0.7	3.9 ± 1.0
Course of pain (% of patients) **		
persistent pain with slight fluctuations	21.9	62.5
persistent pain with pain attacks	15.6	31.2
pain attacks with pain between them	62.5	6.2
Pain radiation (% of patients) **	100.0	46.9
Pain localization (% of patients) **		
leg only	71.8	6.2
leg and the lower back	28.1	93.8

PDQ – Pain Detect Questionnaire; SD – standard deviation.
**** $p < 0.01$.**

Table 3

The presence of anxiety and depression in patients with chronic low back pain (CLBP) syndrome with (experimental group) and without (control group) neuropathic pain

Scale	Experimental group (n = 32)	Control group (n = 32)
Ham-D score (mean ± SD)*	7.9 ± 5.2	5.6 ± 3.4
Depressive patients (% of patients)	34.4	21.9
Ham-A score (mean ± SD)*	11.5 ± 8.3	7.7 ± 6.4
Patients with anxiety (% of patients)	31.2	18.8

Ham-D – Hamilton depression rating scale; Ham-A – Hamilton anxiety rating scale; SD – standard deviation.
**** $p < 0.05$.**

Table 4

Quality of life measured by Short Form (SF)-36 questionnaire in patients with chronic low back pain (CLBP) syndrome with (experimental group) and without (control group) neuropathic pain

Domain of the SF-36 scale	Experimental group (n = 32)	Control group (n = 32)
Physical functioning (PF) score (mean ± SD)	72.7 ± 9.8	73.1 ± 8.2
Role physical (RP) score (mean ± SD)	12.5 ± 19.1	13.3 ± 19.0
Bodily pain (BP) score (mean ± SD)**	41.7 ± 8.0	48.3 ± 6.9
General health (GH) score (mean ± SD)	32.7 ± 7.7	34.0 ± 10.0
Vitality (VT) score (mean ± SD)**	50.2 ± 15.4	62.7 ± 18.0
Social functioning (SF) score (mean ± SD)**	50.6 ± 15.4	62.3 ± 11.6
Role emotional (RE) score (mean ± SD)	51.0 ± 50.1	66.6 ± 44.8
Mental health (MH) score (mean ± SD)*	57.1 ± 22.5	69.5 ± 19.9
Physical composite score (PCS) (mean ± SD)*	41.8 ± 8.7	46.1 ± 7.6
Mental composite score (MCS) (mean ± SD)**	48.3 ± 20.1	59.1 ± 19.0
Total SF-36 score (mean ± SD)*	46.1 ± 14.8	53.8 ± 13.3

SD – standard deviation.
**** $p < 0.05$; ** $p < 0.01$.**

Table 5

Predictors of the total Short Form (SF)-36 score in patients with chronic low back pain (CLBP) syndrome – multiple regression analysis (stepwise method)

Varables included	Beta	*p*
Ham-D **	-0.81	< 0.01
Ham-A **	-0.39	< 0.01
R^2 adjusted	0.75	

***Note*: Excluded variables were: the presence of neuropathic pain, nerve root involvement according to electromyography (EMG), severity of nerve root injury according to EMG and magnetic resonance maging (MRI).**
Ham-D – Hamilton depression rating scale; Ham-A – Hamilton anxiety rating scale.
**** $p < 0.01$.**

Discussion

The prevalence of NP in patients with CLBPS in our study was 35%, which is in accordance with the results of most previous studies [4]. All of our patients had a definite clinical diagnosis of NP according to the criteria of Haanpää et al. [7], which included the patient's history, neurological examination, EMG testing, and MRI of the lumbosacral spine. The wide range of NP prevalence in patients with CLBPS in the literature (16–55%) could be the result of methodological differences between studies (especially in the definition of NP, pain assessment tools, and examined body parts) [4]. One previous study underlined that the prevalence of NP in CLBPS was even 90% [15].

Significant differences in sociodemographic characteristics of patients with CLBPS and NP vs. CLBPS without NP were not noted. These results are very similar to the findings of other studies [16]. NP may appear in patients with CLBPS independently of gender and age. According to our EMG results, patients with NP usually had at least one affected nerve root and more severe nerve root injuries. All patients with NP had a disc herniation in contrast to the control group, where degenerative spine changes predominated. Accordingly, pain radiation and the presence of pain in one or both legs were significantly more frequent in patients with NP. These findings are in accordance with the research conducted by Attal et al. [17]. It was suggested that the percentage of patients with NP increased with the degree of distal pain radiation. Around 8% of patients with localized back pain, 15% of patients with pain limited above knees, 39% of patients with the presence of pain under the knees, and 80% of patients with pain radiation to the foot/feet had NP [17].

Patients with NP had significantly more severe pain intensity compared to the control group, which corresponds to the results of previous publications [18, 19]. Except for the mentioned difference in pain intensity, significant differences were also noted in the course of the disease, pain radiation, and localization. Patients with NP were most likely to have pain in the form of severe pain attacks with mild pain between them, while patients without NP more often had persistent pain with slight fluctuations. Using three questionnaires for NP diagnosing, we observed that the most significant features of NP were allodynia, electric shock-like sensations, and hypoesthesia to prick. To date, only a few studies have been published in which two or more questionnaires were applied at the same time and on the same group of patients, but even in these papers, patients with different etiology of peripheral NP have been included in the trials [20–22]. It seems that standardized questionnaires for NP seem to be insufficiently sensitive and specific for detecting NP in patients with CLBPS. These results imply the need for further development of the specific NP questionnaire for CLBPS.

The worst QoL score in both groups of patients was RP, which is in accordance with some previous studies [23–26]. These studies also reported slightly lower scores for physical domains in contrast to mental ones, which is in line with our results. According to the SF-36 questionnaire, patients with CLBPS and NP had worse QoL compared to the patients without NP [18, 27, 28]. Lower scores on mental domains in patients with NP and the fact that patients with NP had significantly higher rates of depression and anxiety than the control group underline the significance of the bidirectional relationship between NP and anxiety/depression. The presence of depression and anxiety was reported to be in a positive correlation with the presence of pain and functional disability and a negative correlation with patients' QoL [27, 29–31]. Multiple linear regression analysis showed that the presence of depression and anxiety was a predictor of worse QoL in our patients with CLBPS and that these two variables could explain as much as 75% of the total SF-36 score variability. Several studies indicated that QoL in patients with CLBPS is more impaired due to psychosocial factors than due to pain intensity and functional disability [28, 32]. All these results support the biopsychosocial model of the development of CLBPS, where the psychosocial factors tend to be more important than the initial injury of anatomical structures [28]. Depression and anxiety should be an important treatment "target" in CLBPS patients in order to significantly improve their QoL. Although depression and anxiety are the main psychosocial factors that play an important role in the occurrence and maintenance of CLBPS, social factors like those related to the work environment (demanding work conditions, job dissatisfaction) should not be underestimated. However, the association between NP and type of patients' work was not found in our cohort.

The main limitation of our study could be a relatively small sample size. However, this study is well defined and represents the first study where frequency and characteristics of NP in CLBPS were examined by three questionnaires, as well as its influence on QoL. Furthermore, the control group was included (patients with CLBPS without NP). Another advantage is the fact that patients who had comorbid disorders, which could affect their QoL, were excluded from the study.

Conclusion

The presence of allodynia, electric shock-like sensations, and hypoesthesia to prick in patients with CLBPS suggest NP. CLBPS patients with NP had worse scores in mental domains of QoL compared to patients without NP.

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Received on October 2, 2018
Revised on January 11, 2020
Accepted on July 9, 2020
Online First July, 2020

ORIGINAL ARTICLE

UDC: 617.741-004.1-089
DOI: https://doi.org/10.2298/VSP200421081M

Predictors of intraocular pressure change after cataract surgery in patients with pseudoexfoliation glaucoma and in nonglaucomatous patients

Prediktori promene intraokularnog pritiska nakon operacije katarakte kod obolelih od pseudoeksfolijativnog glaukoma i kod bolesnika bez glaukoma

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Abstract

Background/Aim. The cataract surgery in eyes with and without glaucoma results in the sustained intraocular pressure (IOP) reduction but it is still unknown which glaucomatous patients will achieve clinically significant reduction. The preoperative IOP and some ocular biometric parameters have been shown as potential predictors of the postoperative IOP reduction. The aim of our prospective intervention study was to evaluate that relationship in medically controlled patients with the pseudoexfoliation glaucoma (PXG) and in the nonglaucomatous patients. **Methods.** Thirty-one PXG patients (31 eyes) and 31 nonglaucomatous patients (31 eyes), all with clinically significant cataract, were enrolled. The preoperative IOP, anterior chamber depth (ACD), axial length (AL), lens thickness (LT), lens position (LP) [LP = ACD + 0.5 LT], relative lens position (RLP) [RLP = LP / AL] and the pressure-to-depth ratio (PD ratio) [PD ratio = preoperative IOP/preoperative ACD] were evaluated as potential predictors of the IOP change in the 6th postoperative month. **Results.** In the 6th postoperative month, in the PXG group, the IOP reduction was -3.23 ± 3.41 mmHg (-17.67 ± 16.86%) from the preoperative value of 16.27 ± 3.08 mmHg and in the control group, the reduction was -2.26 ± 1.71 mmHg (-15.06 ± 10.93%) from the preoperative value of 14.53 ± 2.04 mmHg. In the PXG group, the significant predictors of the absolute and the percentage IOP reduction were the preoperative IOP, AL, and PD ratio. In the same group, RLP was shown as a significant predictor of absolute change in the IOP in multivariate analysis, and the percentage IOP change in both the univariate and the multivariate analyses. In the control group, the preoperative IOP and the PD ratio were the only significant parameters that could predict absolute change in the postoperative IOP. **Conclusion.** The cataract surgery leads to the IOP reduction both in the PXG and nonglaucomatous eye. Predictors monitored in this study are widely available and simply calculable parameters that can be potentially used in managing glaucoma.

Key words:
cataract surgery; glaucoma; intraocular pressure; ophthalmologic surgical procedures; postoperative complications.

Apstrakt

Uvod/Cilj. Operacija katarakte rezultira održivim sniženjem intraokularnog pritiska (IOP) u očima sa ili bez glaukoma, ali je još uvek nejasno kod kojih će se bolesnika sa glaukomom postići klinički značajna redukcija IOP. Preoperativni IOP i neki okularni biometrijski parametri su se pokazali kao potencijalni prediktori postoperativnog sniženja IOP. Stoga je cilj naše prospektivne intervencijske studije bio da ispitamo taj odnos kod medikamentozno lečenih bolesnika sa pseudoeksfolijativnim glaukomom (PXG) i kod onih bez glaukoma. **Metode.** Ispitan je 31 bolesnik sa PXG (31 oko) i 31 bolesnik bez glaukoma (31 oko), svi sa klinički značajnom kataraktom. Preoperativni IOP, dubina prednje očne komore (ACD), aksijalna dužina (AL), debljina sočiva (LT), pozicija sočiva (LP) [LP = ACD + 0.5 LT], relativna pozicija sočiva (RLP) [RLP = LP/ AL] i indeks pritisak-dubina (PD indeks) [PD indeks = preoperativni IOP/preoperativni ACD] su ispitani kao potencijalni prediktori promene IOP u 6. postoperativnom mesecu. **Rezultati.** U 6. postoperativnom mesecu u grupi sa PXG, sniženje IOP je iznosilo -3.23 ± 3.41 mmHg (-17.67 ± 16.86%) u odnosu na preoperativnu vrednost IOP od 16.27 ± 3.08 mmHg, a u kontrolnoj grupi, sniženje je iznosilo

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-2.26 ± 1.71 mmHg (-15.06 ± 10.93%) u odnosu na preoperativnu vrednost IOP od 14.53 ± 2.04 mmHg. U grupi sa PXG, značajni prediktori apsolutnog i relativnog sniženja IOP su bili preoperativne vrednosti IOP, AL i PD indeksa. U istoj grupi, RLP se pokazao kao značajan prediktor apsolutne promene IOP u multivarijantnoj analizi, a procentualne promene IOP i u univarijantnoj i multivarijantnoj analizi. U kontrolnoj grupi, preoperative vrednostii IOP i PD indeksa su bili jedini značajni parametri koji su mogli da ukažu na apsolutnu promenu IOP posle operacije katarakte.

Zaključak. Operacija katarakte dovodi do sniženja IOP kod obolelih od PXG i kod bolesnika bez glaukoma. Prediktori ovog sniženja su široko dostupni parametri, jednostavni za izračunavanje i potencijalno se mogu koristiti u donošenju odluka o lečenju glaukoma.

Ključne reči:
katarakta, ekstrakcija; glaukom; intraokularni pritisak; hirurgija, oftalmološka, procedure; postoperativne komplikacije.

Introduction

Glaucoma is the leading cause of irreversible blindness and the only treatment is lowering the intraocular pressure (IOP) to a level on which the disease does not progress [1]. The pseudoexfoliation glaucoma (PXG) is one of the most complicated forms of glaucoma for treatment because of the high IOP at the onset, poor response to medical therapy and faster progression. The PXG is characterized by the pathological production and accumulation of an abnormal extracellular fibrillar material mainly visible on the anterior lens capsule, the pupillary margin, corneal endothelium, lens zonules, trabecular meshwork and it often correlates with an increased incidence of cataract formation usually in the lens nucleus or center. A loss of zonules support and poor pupillary dilation make the cataract surgery challenging and with potential complications like vitreous loss, subluxation or luxation of the lens. Studies have shown that the cataract surgery leads to an IOP reduction in glaucomatous and nonglaucomatous eyes, and the IOP reduction effects vary, depending on the type of glaucoma and monitoring period [2]. However, a clinically significant IOP reduction does not occur in every patient [3, 4]. More recent studies are trying to identify the factors that can indicate which patients will achieve a clinically significant IOP reduction after a cataract surgery.

The preoperative value of IOP has been found to be a significant predictor of the IOP reduction after a cataract surgery. Patients with higher levels of preoperative IOP obtain greater postoperative IOP reduction [2, 3, 5]. Also, some ocular biometric parameters, such as anterior chamber depth (ACD), axial length (AL), lens thickness (LT), lens position (LP) [defined as: ACD + 0.5 LT], relative lens position (RLP) [defined as: LP/AL] and the pressure-to-depth ratio (PD ratio) [defined as: preoperative IOP/preoperative ACD] have been recognized as potential predictors of postoperative IOP reduction [2, 6–11].

To our knowledge, there have been no prospective studies which evaluated these clinical variables in the PXG patients. Accordingly, the aim of this study has been to determine if the preoperative values of IOP, ACD, AL, LT, LP, RLP and PD ratio are related to the postoperative IOP changes in the PXG patients and to compare these findings with those of nonglaucomatous patients.

Methods

Thirty-one (31 eyes) PXG patients and thirty-one (31 eyes) nonglaucomatous patients (controls), who underwent cataract surgery by phacoemulsification (PHACO) and posterior chamber intraocular lens (IOL) implantation at the Clinic for Eye Diseases of the University Clinical Center of the Republic of Srpska, Bosnia and Herzegovina, were included in this prospective intervention study between December 2016 and December 2018. The study was approved by the institutional Ethics Committee, conducted in accordance with the Helsinki Declaration and the informed consent was obtained from all the patients.

General exclusion criteria were: ocular trauma, inflammation, retinal disorder, nonglaucomatous optic neuropathy, long-term use of corticosteroids (systemic or topical), previous intraocular surgery or laser intervention, and any other type of glaucoma except PXG.

General inclusion criteria were: age ≥ 18 years, presence of clinically significant cataract, operative and postoperative course without complications and participation until the study completion.

All PXG patients were with previously diagnosed structural and functional glaucomatous changes, with the presence of pseudoexfoliation material (PXM) at the pupillary margin and/or the lens surface and treated with topical antiglaucomatous medications. The control group consisted of the nonglaucomatous patients referring to elective surgery of the senile cataract, with bilaterally gonioscopically determined angle openness classified as the Shaffer's and Tour's [12] grade 3 or 4 and with the bilateral IOP ≤ 21 mmHg.

The data we collected consisted of the patient's age, gender, the best corrected visual acuity (BCVA) measured by the Snellen's charts (decimal system), gonioscopy (the Shaffer's and Tour's [12] grade scale), the diurnal IOP curve test (the IOP measurement at 07:30, 13:30 and 19:30 with the Goldmann applanation tonometer), the number and type of glaucoma therapy. Eye selection criteria were based on worse BCVA.

The measurements of the biometric parameters ACD, LT and AL were obtained with non-contact optical biometer IOLMaster 500 (Carl Zeiss Meditec, Inc., Dublin, CA) and contact applanation the A-scan ultrasonic biometer (Tomey, AL-100 Biometer, Japan).

According to the following formulas, the calculations were as follows:

$IOP = (IOP_{07:30}+IOP_{13:30}+IOP_{19:30}) / 3$

$LP = ACD + 0.5\ LT$ [13]

$RLP = LP / AL$ [13]

PD ratio = preoperative IOP / preoperative ACD [9].

The surgery consisted of the standard clear corneal incision phacoemulsification by using the phaco-chop technique in all patients under topical anesthesia, using the Stellaris Vision Enhancement System (Bausch & Lomb) and the IOL (Akreos Adapt AO, Bausch & Lomb) was implanted in-the-bag. The antibiotic prophylaxis was provided as a subconjunctival and intracameral injection. Phacoemulsification parameter, Absolute phaco time was recorded at the end of each surgery.

The postoperative therapy included topical antibiotics/steroid 8 times daily during the first postoperative week, followed by 6 times daily, 4 times daily and 2 times daily in the next three weeks, respectively.

In order to avoid the effect of topical antiglaucoma therapy change on postoperative values of IOP, all the PXG patients postoperatively continued to use preoperatively administered antiglaucoma therapy, except for the prostaglandin analogs. After 1 month, the same prostaglandin analogs were resumed and the studied eyes were then back on the same glaucoma medication regimen.

The postoperative checkups were performed on the first and seventh day as well as in the 1st, 3rd and 6th month.

The clinical examinations, diagnostic measurements and all surgeries were performed by the same ophthalmologist (BM).

We compared the absolute IOP change and the IOP change percentage in the 6th postoperative month as the main outcomes for the predictors of interest.

Continuous variables were reported as the mean ± standard deviation (SD). The Mann-Whitney *U* test was used to assess differences between the groups for ordinal or continuous variables, and the χ^2 test for categorical variables.

We used the linear mixed-effects regression analysis in order to determine the correlation between the outcome variables and the preoperative factors, including the preoperative values of IOP, age, gender and biometric parameters (ACD, AL, LT, LP, RLP, PD ratio). The multivariate linear mixed-effects regression models were created in order to adjust to potential confounders, including gender, age and the preoperative values of IOP.

In all models assessing the PD ratio and the IOP change, the preoperative IOP value was not included because it is a part of the PD ratio calculation.

The regression coefficients (B), the coefficients of determination (r^2) and the statistical significance (*p*-value) were reported. *P* values ≤ 0.05 were considered significant. All the statistical analyses were performed using the SPSS software V.21 (SPSS, Inc., Chicago, IL, USA).

Results

The group of 31 patients with the PXG, and 31 patients with the senile cataract as the control group were included in this prospective study. The demographic characteristics, preoperative biometric measurements, preoperative IOP, postoperative IOP (measurements in the 1st, 3rd and 6th month) and the postoperative IOP changes of each group are shown in Table 1. According to the gender, there were no significant differences between the groups. The mean age was significantly higher in the PXG group. There was a significant difference between the two groups in patients' age (76 ± 6 years vs. 71 ± 7 years; $p < 0.01$), preoperative values of IOP (16.27 ± 3.08 mmHg vs. 14.53 ± 2.04 mmHg; $p < 0.01$), LT (4.65 ± 0.50 mm vs. 4.34 ±, 56 mm; $p < 0.05$), PD ratio (5.23 ± 0.25 vs. 4.77 ± 0.82; $p < 0.01$), the absolute IOP change in the 1st month (-2.96 ± 2.65 mmHg vs. -1.00 ± 1.73 mmHg; $p < 0.01$) and percentage IOP change in the 1st month (-16, 86 ± 13.05%; -6.54 ± 12.04%; $p < 0.01$), with all of the above

Table 1

Demographic characteristics and examined parameters in patients subjected to cataract surgery

Characteristics	PXG group (n = 31)	Control group (n = 31)	*p*
Gender (male/female), n (%)	20/11 (65/35)	15/16 (48/52)	0.153
Age (years), mean ± SD	76 ± 6	71 ± 7	< 0.01
Pre-op IOP (mmHg), mean ± SD	16.27 ± 3.08	14.53 ± 2.04	< 0.05
ACD (mm), mean ± SD	2.90 ± 0.34	3.07 ± 0.31	0.066
AL (mm), mean ± SD	23.80 ± 0.84	23.45 ± 0.93	0.213
LT (mm), mean ± SD	4.65 ± 0.50	4.34 ± 0.56	< 0.05
LP (mm), mean ± SD	5.23 ± 0.25	5.25 ± 0.32	0.490
RLP, mean ± SD	0.22 ± 0.01	0.22 ± 0.02	0.150
PD ratio, mean ± SD	5.66 ± 1.15	4.77 ± 0.82	< 0.01
Post-op IOP (mmHg), mean ± SD			
1st month	13.32 ± 2.34	13.53 ± 2.22	0.531
3rd month	13.19 ± 2.21	12.09 ± 2.03	0.099
6th month	13.04 ± 1.95	12.27 ± 1.89	0.147
Ab IOP change 1st month (mmHg), mean ± SD	-2.96 ± 2.65	-1.00 ± 1.73	< 0.01
% IOP change 1st month, mean ± SD	-16.86 ± 13.05	-6.54 ± 12.04	< 0.01
Ab IOP change 3rd month (mmHg), mean ± SD	-3.08 ± 2.73	-2.44 ± 1.76	0.426
% IOP change 3rd month	-17.57 ± 13.43	-16.40 ± 11.40	0.741
Ab IOP change 6th month (mmHg), mean ± SD	-3.23 ± 3.41	-2.26 ± 1.71	0.281
% IOP change 6th month, mean ± SD	-17.67 ± 16.86	-15.06 ± 10.93	0.356

PXG – pseudoexfoliation glaucoma; Pre-op – preoperative; IOP – intraocular pressure; ACD – anterior chamber depth; AL – axial length; LT – lens thickness; LP – lens position; RLP – relative lens position; PD ratio – the ratio of Pre-op IOP and ACD; Post-op – postoperative; Ab – absolute; SD – standard deviation.

variables being larger in the PXG group. Postoperatively, in both groups, compared to the preoperative IOP values, the reduction of the IOP was observed in each measurement time point. The absolute and the percentage reduction of the IOP in the 6th month in the PXG group was -3.23 ± 3.41 mmHg (-17.67 ± 16.86%), and in the control group, -2.26 ± 1.71 mmHg (-15.06 ± 10.93%). There was no statistically significant difference between the groups for either the absolute or the percentage IOP reduction (Table 1).

The linear mixed-effects regression models were used to show the association between the absolute IOP change in the 6th month and gender, age, preoperative IOP value and ocular biometric parameters for both groups (Table 2).

In the univariate mixed-effects models, for both groups, the preoperative IOP value was found to be a significant predictor of the absolute IOP reduction. In the PXG group, in the univariate and multivariate models, the AL and PD ratio were associated with the significant absolute IOP change 6 months after the cataract surgery, and the RLP in the multivariate model. In the control group, PD ratio was the significant predictor of the absolute IOP change both in the univariate and multivariate analyses (Table 2). The predictability of the potential predictors was shown through the coefficient of determination and according to the r^2 value, in the PXG group, in the univariate model, the preoperative IOP (r^2 = 47.3%) was the best predictor of the absolute IOP change, followed by the PD ratio and the AL (r^2 = 18.3% and 12.5%, respectively). In the multivariate model, in the PXG group, among significant predictors, the AL, RLP and PD ratio were the best predictors of the absolute IOP change (r^2 = 57.7%, 51.9% and 14.7%, respectively). In the control group, the best predictor was the preoperative IOP (r^2 = 22.5%), followed by the PD ratio (r^2 = 16.3% in the univariate and r^2 = 13.7% in the multivariate analyses) (Table 2).

Table 3 shows the association between the percentage IOP change in the 6th postoperative month and gender, age, preoperative IOP and ocular biometric parameters for the PXG and control groups, using the linear mixed-effects regression models. The coefficient of determination was also explored.

In the univariate analysis, in the PXG patients, the significant predictors of the percentage IOP change were the preoperative IOP and in the univariate and multivariate analyses, the AL, RLP and PD ratio. Among the significant predictors in the univariate model, the preoperative IOP, followed by the AL, PD ratio and RLP (r^2 = 26.7%, 14.7%, 12.6% and 10.5%, respectively) had the best predictability of the percentage IOP change. In the multivariate model, the order of the best predictability among the parameters was as follows: AL, RLP, PD ratio (r^2 = 37.9%, 35.2% and 8.8%, respectively).

Neither the univariate nor the multivariate analysis identified statistically significant predictors of the percentage IOP change in the 6th postoperative month in the control group (Table 3).

Discussion

The majority of studies, mainly the retrospective ones, examining the effect of a cataract surgery on the IOP reduction, have been conducted in patients with primary open angle glaucoma (POAG), primary angle-closure glaucoma (PACG), and nonglaucomatous patients, and only a few in patients with PXG [2, 14]. A possible explanation for this is the lower PXG incidence compared to other glaucoma forms, as well as its wide variations in incidence and prevalence among different countries globally and in different geographical areas within the same country [15]. Although there has been no organized data collection at the Clinic for Eye Diseases of the Republic of Srpska University Clinical Center so far, our clinical observation is that there is a significantly higher number of patients with pseudoexfoliation syndrome (PXS) and the PXG in the municipalities of Šipovo and Mrkonjić Grad compared to patients from other municipalities in the Krajina region. According to the data from the operative protocol of the Clinic for Eye Diseases in Banja Luka, out of 100 patients who undergo a cataract surgery, 11% also suffer from the PXG. This data significantly differs from the data of the study by Kovač et al. [15], where the PXG was present in 6.5% of the totally 674 patients planned for the cataract surgery. This discrepancy is undoubtedly influenced by the sample size, but certainly also by the geographical areas with the higher PXG incidence and their distance from the medical centers where the cataract surgery is performed, which is the case here.

Our research was inspired by the need to advance our day-to-day clinical practice in the PXG patient treatment. Aware of the diurnal IOP fluctuations [16], and in order to obtain the most recent values for IOP and PD ratio parameters, the IOP daily curve was performed preoperatively and at each measurement time point postoperatively.

Our study results show that the cataract surgery led to the IOP decrease in both PXG patients treated with medicaments and in nonglaucomatous patients. In patients with PXG, the reduction was recorded as early as in the first postoperative month and showed a tendency towards further reduction in the third and sixth month. In nonglaucomatous patients, the IOP decrease was the biggest in the third month, and the effect of the decrease began to weaken in the sixth month (Table 1; the absolute and the percent changes in IOP).

In the 6th postoperative month in the PXG group, the absolute IOP reduction was -3.23 ± 3.41 mmHg (-17.67 ± 16.86%), and in nonglaucomatous patients, -2.26 ± 1.71 mmHg (-15.06 ± 10.93%), with no significant difference between the groups.

According to the report of American Academy of Ophthalmology (AAO), among 5 studies that included only PXG patients (3 with level II evidence and 2 with level III evidence; totally 132 patients) and examining the effect of phacoemulsification on IOP, only 3 studies were prospective (totally 58 patients) [2]. The sample size in these five studies ranged from 4 to 51 patients and depending on the study, the follow-up period was 12 to 60 months. For the total sample

Table 2

Association between various predictors of absolute intraocular pressure (IOP) change after cataract surgery (using absolute IOP change at 6 months as the dependent variable)

Predictor	PXG group						Control group					
	Univariate			Multivariate			Univariate			Multivariate		
	B ± SE	*p*	r^2 (%)	*B* ± SE	*p*	r^2 (%)	*B* ± SE	*p*	r^2 (%)	*B* ± SE	*p*	r^2 (%)
Gender	1.03 ± 1.02	0.318	0.1				0.17 ± 0.62	0.830	3.3			
Age	0.11 ± 0.10	0.298	0.4				-0.03 ± 0.04	0.499	1.8			
Pre-op IOP	-0.73 ± 0.14	< 0.01	47.3				-0.42 ± 0.13	< 0.01	22.5			
ACD	-1.25 ± 1.53	0.421	1.2	-0.86 ± 1.20	0.482	41.3	0.10 ± 1.04	0.920	3.4	0.03 ± 0.93	0.974	20.4
AL	-1.41 ± 0.63	< 0.05	12.5	-1.48 ± 0.50	< 0.01	57.7	-0.38 ± 0.33	0.269	0.9	0.14 ± 0.38	0.711	20.8
LT	1.86 ± 0.97	0.065	8.8	1.44 ± 0.74	0.065	49.7	0.43 ± 0.56	0.448	1.4	0.38 ± 0.52	0.475	22.0
LP	1.42 ± 1.96	0.476	1.7	1.68 ± 1.73	0.340	44.1	0.75 ± 0.98	0.451	1.4	0.59 ± 0.91	0.522	21.7
RLP	7.66 ± 4.03	0.068	8.5	6.81 ± 3.04	< 0.05	51.9	2.56 ± 1.90	0.189	2.6	0.86 ± 1.86	0.648	21.1
PD ratio	-1.11 ± 0.41	< 0.01	18.3	-1.13 ± 0.47	< 0.05	14.7	-0.91 ± 0.35	< 0.05	16.3	-0.96 ± 0.36	< 0.05	13.7

PXG – pseudoexfoliation glaucoma; Pre-op IOP – preoperative intraocular pressure; ACD – anterior chamber depth; AL – axial length; LT – lens thickness; LP – lens position; RLP – relative lens position; PD ratio – the ratio of preoperative IOP to ACD; *B* – regression coefficient; SE – standard error; r^2 – coefficient of determination.

Table 3

Association between various predictors of percentage intraocular pressure (IOP) change after cataract surgery (using % IOP change at 6 months as the dependent variable)

Predictor	PXG group						Control group					
	Univariate			Multivariate			Univariate			Multivariate		
	B ± SE	*p*	r^2 (%)	*B* ± SE	*p*	r^2 (%)	*B* ± SE	*p*	r^2 (%)	*B* ± SE	*p*	r^2 (%)
Gender	-2.04 ± 5.52	0.714	3.3				1.90 ± 3.98	0.637	2.6			
Age	0.44 ± 0.57	0.447	1.5				-0.32 ± 0.28	0.265	1.7			
Pre-op IOP	-2.98 ± 0.90	< 0.01	26.7				-1.76 ± 0.94	0.072	7.7			
ACD	-0.61 ± 8.54	0.944	3.8	1.04 ± 7.89	0.896	19.8	-0.03 ± 6.64	0.997	3.4	-1.04 ± 6.43	0.873	6.6
AL	-7.81 ± 3.34	< 0.05	14.7	-8.44 ± 3.25	< 0.05	37.9	-2.12 ± 2.15	0.331	0.1	1.03 ± 2.60	0.695	7.0
LT	5.71 ± 5.69	0.325	0.0	5.16 ± 5.11	0.323	23.1	2.41 ± 3.60	0.508	1.9	2.92 ± 3.62	0.427	8.8
LP	8.55 ± 10.37	0.417	1.2	13.38 ± 10.62	0.220	24.9	3.62 ± 6.29	0.569	2.3	3.30 ± 6.29	0.604	7.5
RLP	43.52 ± 21.09	< 0.05	10.5	43.83 ± 18.68	< 0.05	35.2	13.67 ± 12.27	0.275	0.9	4.51 ± 12.91	0.725	6.9
PD ratio	-5.00 ± 2.26	< 0.05	12.6	-5.67 ± 2.56	< 0.05	8.8	-3.74 ± 2.37	0.126	4.7	-4.15 ± 2.41	0.097	4.1

IOP – intraocular pressure; PXG – pseudoexfoliation glaucoma; Pre-op – preoperative; ACD – anterior chamber depth; AL – axial length; LT – lens thickness; LP – lens position; RLP – relative lens position; PD ratio – the ratio of preoperative IOP to ACD; *B* – regression coefficient; SE – standard error; r^2 – coefficient of determination.

of 132 patients, the preoperative IOP was 20.7 ± 4.4 mmHg and in the follow-up period of 34.2 ± 20.8 months the IOP reduction of -4.1 mmHg (-20.0%) occurred. The largest IOP reduction was found in one study and was -13.6 mmHg (-43.0%) in the follow-up period of 12 months. The sample included 16 patients with uncontrolled PXG with the preoperative IOP value of 32.0 mmHg. A significant IOP reduction of -11.6 mmHg (-51.0%) was also found in one study in 4 patients who experienced secondary angle closure due to the zonular weakness. In the other three analyzed studies, the IOP reduction ranged from -1.1 mmHg (-6.0%) to -5.6 mmHg (-27.0%) [2].

Elgin et al. [17] determined, after a one-month period, the IOP reduction from the preoperative value of 18.3 ± 2.5 mmHg to the postoperative value of 15.2 ± 1.2 mmHg in 29 patients with PXG.

Jimenez-Roman et al.[18] retrospectively examined cataract surgery impact on IOP in 44 medically controlled patients with PXG, and with respect to the preoperative IOP (17.00 ± 2.75 mmHg) in the 6th postoperative month, the IOP reduction of -3.65 mmHg (-20.3%) was observed, which remained unchanged in the 12th month.

Abdelghany et al. [19] have recently conducted a prospective study on the impact of a cataract surgery on the IOP changes, ganglion cell complex, and peripapillary retinal nerve fibers layer in medically controlled patients with PXG. Eighty five patients were divided into two groups. The first group consisted of 40 patients with PXG and cataract who underwent cataract surgery. The control group consisted of 45 non-operated patients with PXG and no cataract. The controls were performed in the 3rd, 6th, 12th and 18th month. The preoperative IOP was significantly different between the groups (20.42 ± 0.90 mmHg in the pseudophakic group and 16.62 ± 1.00 mmHg in the control group), which may be due to cataract presence and changes in lens thickness. Compared with the preoperative IOP, a significant reduction in IOP was found postoperatively during each control, where the biggest one was in the third month (15.35 ± 1.03 mmHg), and the reduction effect gradually weakened till the end of the research in the 18th month (17.00 ± 2.75 mHg). In the 6th month, the reduction was -5.02 mmHg. Numerous studies have identified the IOP reduction in the nonglaucomatous patients after the cataract surgery by extracapsular extraction and phacoemulsification, ranging from 1.1 mmHg to 4.0 mmHg [3, 5, 19, 20].

In a study by Hsu et al. [21] on the cataract surgery impact on IOP in 75 nonglaucomatous patients (75 eyes), in the fourth postoperative month, the IOP reduction of -2.03 ± 2.42 mmHg (-12.74%) was noticed versus the preoperative value of 14.5 ± 3.05 mmHg.

Based on the above, we conclude that our results are consistent with the previous studies suggesting an IOP decrease after the cataract surgery in the PXG patients and nonglaucomatous patients, as well as the extent of its reduction.

In our study, regression analysis in the univariate model for both groups has shown the significant negative correlation of the preoperative IOP (PXG group: B = - 0.73 ± 0.14; $p < 0.01$; Control group: B = - 0.42 ± 0.13; $p < 0.01$) and its absolute postoperative reductions in the 6th month in the sense that the preoperatively higher IOP values are associated with the greater postoperative reductions, which is consistent with the results of other studies [2, 3, 5]. In our sample, this would mean that if the IOP is preoperatively increased by 1 mmHg (relative to the average IOP for the observed group), the absolute postoperative reduction will be greater by the additional 0.73 ± 0.14 mmHg in the PXG patients and in nonglaucomatous patients by the additional 0.42 ± 0.13 mmHg (Table 2). For the percentage IOP change in the 6th month, this was only the case for the PXG group (B = -2.98 ± 0.90; $p < 0.01$), whereas in the control subjects, this parameter had no significance (B = -1.76 ± 0.94; $p = 0.072$) (Table 3).

Since the preoperative IOP higher values tend to result in its greater absolute reduction compared to the lower basal values, we also examined the relative (percentage) IOP change in our study, because the percentage change may be similar in eyes with different initial IOP measurements.

The preoperative IOP values in our study proved to be a significant predictor of both the absolute and the percentage postoperative IOP changes in the PXG patients with predictive ability of $r^2 = 47.3\%$ and $r^2 = 26.7\%$, respectively, and in the control group only for the absolute change with predictive ability of $r^2 = 22.5\%$ (Tables 2 and 3).

Pradhan et al. [22] examined the impact of the cataract surgery on the IOP in 77 patients (70 POAG; 4 POAG suspect; 3 with ocular hypertension) and found that the preoperative IOP indicates a change in the postoperative IOP values in the range of 13% to 20% and that these percentages depend on the number of the preoperative IOP measurements (one preoperative IOP measurement, prediction of 13% change in the postoperative IOP; the average value of two measurements, prediction 17%; the average value of three measurements, prediction 15%; the average value of up to four measurements, prediction 20%). It is worth mentioning that in that study, the preoperative IOP data were collected up to five years prior to cataract surgery and reflect the long-term fluctuations in the IOP. Also, the regression analysis was performed only with respect to the absolute but not to the percentage IOP change.

Moghimi et al. [23] examined 33 nonglaucomatous patients with PXS and found a moderate IOP decrease of 3.3 mmHg (18%) three months after the cataract surgery (the preoperative IOP: 18.1 ± 3.4 mmHg) with a predictive ability of the preoperative IOP of $r^2 = 39\%$ for its postoperative change.

According to a study by Shingleton et al. [24], the prediction of the preoperative IOP value for the postoperative IOP change in people with PXS was 40%.

The slightly higher percentage of predictability of the preoperative IOP obtained in our study for the absolute IOP change in the PXG group ($r^2 = 47.3\%$) may reflect the analysis of the three preoperative IOP measurements in the daily IOP curve test and “improve” the result of a prediction of the postoperative IOP change relative to a single

measurement. The statistical phenomenon of "regression to the mean value", which is a consequence of an inadequate number of the basic preoperative IOP measurements, was minimized thanks to a daily curve test conducted preoperatively in our study.

The exact mechanisms of the IOP reduction after cataract surgery are still not clear.

So far, in patients with the preoperatively narrow iridocorneal angle, shallow anterior chamber, and greater natural lens thickness, cataract surgery results in major changes in the anterior segment configuration, resulting in clinically significant reduction and a good long-term IOP control [25]. Thanks to these observations, cataract surgery or clear lens extraction are the procedures that have often become the first choice or an integral part of treating the narrow-angle glaucoma [26].

Several theories have hypothesized about the occurrence of the postoperative IOP reduction in people with open angle, whether with POAG, PXG, or healthy subjects.

When it comes to people with the PXS or PXG, according to one theory, the removal of a portion of the anterior lens capsule during capsulorhexis also removes the source of pseudoexfoliation material [27]. A correlation between the intraoperatively used fluid volume and the postoperative IOP change was also found, regarding the higher flow, greater IOP reduction, which supports the idea that surgery increases the clearance of the exfoliative material and pigmentary debris from the anterior eye segment and trabeculum [2, 27, 11]. The removal of the natural lens leads to the anterior ocular chamber deepening (enlargement of the aqueous humor "reservoir") and the displacement of the ciliary body backward and, consequently, its smaller compression into the trabeculum and Schlemm's canal, thereby improving draining. Some other theories are that the chamber angle deepening, low grade inflammatory response caused by the delivered ultrasound energy and the trabecular meshwork microscopic remodeling lead to the aqueous increased draining [24]. Whatever the mechanism, the question is if any other way, except for the preoperative IOP measurement, is possible for determining which patient will benefit from the cataract surgery in terms of achieving a clinically significant IOP reduction? This is especially important for glaucoma patients who have low IOP (low-teen) by the medical or the laser therapy, but the disease progression is still present. In such patients, it is not easy to make a decision about a filtering operation known to be frequently accompanied by a range of serious intraoperative or postoperative complications and frequent failure.

In this regard, numerous biometric parameters are observed as possible IOP change predictors after cataract surgery. Recently, papers have been published, where in order to obtain biometric measurements, the optical coherence tomography for the anterior eye segment (AS-OCT) has been used, but due to the cost of the equipment, such diagnostics is unavailable for most public health institutions, especially in the economically underdeveloped countries. In our study, we have analyzed the parameters whose values are easily obtained as a part of the patient's preoperative preparation for the cataract surgery using optical biometry and ultrasound A-scans available at all centers where the cataract surgery is performed. Also, most of the research has so far been done using such equipment, so that our results can be compared with those of other authors.

The results of the biometric measurements obtained from our subjects indicate an average shallower anterior chamber in the PXG group relative to the control group (PXG ACD: 2.90 ± 0.34 mm; control ACD: 3.07 ± 0.31 mm; $p = 0.066$), which is in line with the results of other studies [17, 28]. The probable reason for this is the increased zonular laxity in patients with PXG and lens anteposition. The lens thickness also plays a significant role in this, and was on average higher in the PXG group (PXG LT: 4.65 ± 0.50 mm; control LT: 4.34 ± 0.56 mm; $p < 0.05$). The LP parameter, represented by the sum of ACD and half LT, is expected to be uniform between the groups given that the ACD is higher in the control group. As the AL parameter is also uniform across groups, so is the same case for the RLP parameter represented by the LP and AL relationship. The preoperatively higher IOP and the lower ACD resulted in the significantly higher PD ratio in the PXG group compared to the control group (PXG PD ratio: 5.66 ± 1.15; control PD ratio: 4.77 ± 0.82; $p < 0.01$) (Table 1). Examining the correlation of the absolute postoperative IOP change in the 6th month and the biometric parameters, in the PXG group univariate model, a significant inverse correlation was present for the parameters AL ($B = -1.41 \pm 0.63$; $p < 0.05$) and PD ratio ($B = -1.11 \pm 0.41$; $p < 0.01$). When in the multivariate analysis parameters such as gender, age and the preoperative IOP were added, a significant correlation between the absolute postoperative IOP change and parameters AL ($B = -1.48 \pm 0.50$; $p < 0.01$), RLP ($B = 6.81 \pm 3.04$; $p < 0.05$) and PD ratio ($B = -1.13 \pm 0.47$; $p < 0.05$) was found. Parameters AL and PD ratio had small predicative value for the absolute postoperative IOP change ($r^2 = 12.5\%$; $r^4 = 18.3\%$, respectively) in the univariate model. In the multivariate model, AL and RLP had standard predictability ($r^2 = 57.7\%$; $r^2 = 51.9\%$, respectively), while PD ratio parameter had low predictability ($r^2 = 14.7\%$) (Table 2).

Examining the correlation between the percentage postoperative IOP change in the 6th month and the biometric parameters in the PXG group, both in the univariate and multivariate models, it was significant for the parameters: AL, RLP and PD ratio. The parameters AL ($r^2 = 37.9\%$) and RLP ($r^2 = 35.2\%$) had the highest prediction value for the percentage IOP change in the multivariate model, while for the same parameters it was low in the univariate analysis. The predictability of the PD ratio was low in both univariate and multivariate models (Table 3).

For the control group, no parameter in the analysis model was found to be significant to indicate the percentage postoperative IOP change (Table 3).

A systematic review of the peer-reviewed literature shows that the largest number of studies addressing biometric parameters as possible predictors of the postoperative IOP change have been performed in PACG, POAG and nonglaucomatous patients. Since one of the inclusion crite-

ria in our research was the degree of angle openness according to Shaffer's grade 3 or 4, our results are more appropriate to compare with the previous studies in this field that included patients with POAG, in the absence of published studies with PXG subjects. Thus, a significant correlation between the AL parameter and the absolute and percentage reduction of the postoperative IOP was also found by Yoo et al. [29] in individuals with suspected POAG but not in patients with POAG. Hsu et al.[21], Coh et al. [8], Bilak et al.[30] and Moghimi et al.[7] found this correlation in nonglaucomatous patients in both the univariate and multivariate analyses. The above studies did not calculate the prediction calculations for the AL parameter.

The PD ratio parameter was first introduced by Issa et al.[9] who found that in nonglaucomatous patients, the higher PD ratio was followed by the greater postoperative absolute reduction in IOP and indicated it as a strong predictor of this reduction (r^2 = 73.0%). The significant predictability of 34.1% for the PD ratio in nonglaucomatous patients was also established by Dooley et al. [10]. Hsu et al. [21] confirmed its significance as a predictor of both the absolute (r^2 = 52.9%) and the percentage (r^2 = 39.0%) postoperative IOP reductions in the nonglaucomatous patients, and Coh et al. [8] in patients with POAG.

The RLP is a parameter dependent on the thickness of the natural lens, its anteposition, the depth of the anterior chamber, and the total length of the bulb. Its role as a predictor of IOP change is significant in individuals with a narrow angle, and can be potentially used in individuals with an open angle.

Hsu et al. [21] found no association between RLP and the postoperative IOP changes in the multivariate analysis in nonglaucomatous patients. Examining nonglaucomatous patients and patients with POAG, neither Coh et al.[8] found a significant association between RLP and the postoperative absolute or percentage IOP reduction in either the univariate or multivariate analyses. In contrast, DeVience et al. [31] found the significant association between RLP and the postoperative IOP reduction in nonglaucomatous patients in the univariate analysis, but without calculating the prediction for RLP.

Our results show that the RLP is of limited use when viewed separately, but when other possible predictors, such as the preoperative IOP, age and gender are added, it can be helpful to a clinician in recognizing in which direction the postoperative change in IOP in patients with PXG may be expected.

Our research has several limitations. We did not subdivide glaucomatous patients by glaucoma stage that some studies have found to be related to the postoperative IOP change [29]. Patients with Shaffer's angle grade 3 and 4 were included in the study, so we did not have the data on IOP changes in patients with PXG and with a secondary narrow or closed angle. Leaving patients postoperatively on the same medication regimen as they had had preoperatively, we obtained data on change in IOP indicating the true impact of cataract surgery on IOP, but also we are aware of the hypotensive effect of medications on the IOP measurement results. It follows that the most accurate data on the impact of cataract surgery in patients with PXG could be obtained if the trial was performed in patients who had undergone the preoperative wash-out period since the antiglaucoma therapy, which is almost inapplicable to patients with PXG. Alternatively, the newly diagnosed patients may be examined without initiating medication or laser treatment, which will be feasible in some future studies.

Conclusion

Through the prospective study, we have found that cataract surgery results in a moderate decrease in IOP in both medically controlled patients with PXG and in nonglaucomatous patients. The occurrence and extent of this reduction may be indicated by clinical variables readily available by standard ophthalmic diagnostic equipment. In the PXG group, the preoperative IOP, AL, and PD ratio proved to be significant predictors of both the absolute and the percentage changes in IOP, whereas the RLP parameter proved to be a significant predictor of only the percentage IOP change. Of all the parameters tested, the preoperative IOP and the PD ratio stood out in the control group as the only significant predictors only of the absolute change in IOP.

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Received on April 21, 2020
Accepted on August 3, 2020
Online First August, 2020

ORIGINAL ARTICLE

UDC: 617.77-089.844
DOI: https://doi.org/10.2298/VSP190824078N

Evaluation of upper blepharoplasty outcome – objective measurements and patient satisfaction

Procena rezultata hirurške korekcije gornjih kapaka – objektivno merenje i zadovoljstvo pacijenta

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Abstract

Background/Aim. Upper blepharoplasty is one of the most often performed aesthetic operations and is indicated for periorbital rejuvenation or correction of some functional problems. The aim of the study was to evaluate the outcome of this procedure and to assess patients' satisfaction and possibilities of objective measurement of operative results. **Methods.** A two-year prospective observational study was conducted among female patients who had upper blepharoplasty done by the same surgeon. Data were collected from medical documentation, questionnaire, and objective measurements conducted via standardized digital photographs taken before and two months after surgery. Tarsal platform show (TPS) and brow fat span (BFS) were measured at three points (P1, P2, P3) before and two months after the surgery. A questionnaire conducted before surgery and two months after it included general complaints considering upper eyelids (visual field narrowing, fallen eyelids, the sensation of heavy eyelids, raising head backward in order to enhance vision, headache), patients' assessment of eyelid asymmetry and an additional questionnaire after surgery included questions for the surgeon and patients concerning satisfaction with treatment outcome. **Results.** This study involved 50 female patients aged between 33 and 67 years (49.98 ± 8.6 years). There was a statistically significant difference in all points for TPS and BFS measurements before and after the operation. No significant asymmetries were noticed between eyes neither before nor after surgery. There was a statistically significant difference in operation success among 3 age categories in TPS-P1 (χ^2 = 13.089, df = 2, p = 0.001) and TPS-P2 (χ^2 = 8.386, df = 2, p = 0.015) with best results achieved in older patients (> 55 years). There was strong positive, statistically significant correlation between patient's and surgeon's satisfaction (r= 0.704, p = 0.002), as well as between patients' satisfaction and their age (r = 0.704, p = 0.002). **Conclusion.** Realistic expectations, adequate information about the surgery and possible complications, are essential to satisfied patients. Objective measurements correlate with patients' satisfaction and together with photographs can be a useful tool in communication with them.

Key words:
blepharoplasty, eyelids; patient satisfaction; treatment outcome; surgical procedures, operative; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Blefaroplastika je jedna od najčešćih estetskih hirurških procedura koja je indikovana u cilju podmlađivanja ili rešavanja nekog funkcionalnog problema. Cilj studije bio je da se procene rezultati ove procedure, zadovoljstvo pacijentkinja i mogućnost objektivnog merenja rezultata. **Metode**. U ovu prospektivnu studiju bile su uključene pacijentkinje kojima je urađena korekcija gornjih kapaka u periodu od dve godine, od strane istog hirurga. Podaci su prikupljeni iz medicinske dokumentacije, upitnika i objektivnog merenja standardizovanih digitalnih fotografija koje je hirurg napravio pre operacije i dva meseca nakon operacije. Mereni su *tarsal platform show* (TPS) i *brow fat span* (BFS) u tri tačke (P1, P2 i P3), pre operacije i dva meseca nakon operacije. Anketa sprovedena pre operacije i dva meseca posle operacije bazirala se na upitniku sa pitanjima o najčešćim simptomima u vezi sa kapcima (smanjeno vidno polje, pali kapci, osećaj težine u kapcima, zabacivanje glave unazad kako bi se olakšalo gledanje, glavobolja); dva meseca posle operacije sprovedena je i dodatna anketa u cilju procene zadovoljstva hirurga i pacijentkinja ishodom operacije. **Rezultati.** Studijom je bilo obuhvaćeno 50 pacijentkinja životne dobi od 33 do 67 godina (49,98 ± 8,6). Nađena je statistički

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značajna razlika u sve tri tačke merenja za TPS i BFS pre i posle operacije. Nije postojala značajna asimetrija između levog i desnog oka ni pre, ni posle operacije. Postojala je značajna razlika u uspešnosti operacije kod tri starosne kategorije u rezultatima merenja za TPS-P1 (χ^2= 13,089, df = 2, p = 0,001) i TPS-P2 (χ^2 = 8.386, df = 2, p = 0,015), sa najboljim rezultatom postignutim kod starijih pacijentkinja (> 55 godina). Utvrđena je jaka pozitivna, statistički značajna korelacija između zadovoljstva pacijentkinja i hirurga (r = 0,704, p = 0,002), kao i između zadovoljstva pacijentkinja i njihove životne dobi (r = 0,704, p = 0,002). **Zaključak.** Realna očekivanja, adekvatna informisanost pacijentkinja o operaciji i mogućim komplikacijama su bitni za njihovo zadovoljstvo. Objektivna merenja koreliraju sa zadovoljstvom pacijentkinja, a zajedno sa fotografijama pre i posle operacije mogu biti korisno sredstvo u komunikaciji sa njima.

Ključne reči:
blefaroplastika; kapci; zadovoljstvo pacijenta; lečenje, ishod; hirurgija, procedure, operativne; ankete i upitnici.

Introduction

Upper blepharoplasty is one of the most often performed aesthetic operations in general. It usually involves resection of excess skin of eyelid, sometimes segment of orbicular muscle, and, if needed, reduction of retroseptal fat pads. Different adjuvant procedures can be added to conventional surgery in order to achieve better, aesthetically more pleasing results [1, 2]. There are no strict guides on how to resolve the aesthetic problem; every patient has to be analyzed individually and the operation planned, keeping in mind different surgical options and desired results. On the other hand, there are no universal beauty standards and procedures for precise evaluation of specific anatomical features, possibilities, and patients' desires.

Upper blepharoplasty procedure has a high rate of patient satisfaction, but in the light of social media pressure, expectations can be unrealistic, and the patient can be unsatisfied with the result as with any other aesthetic procedure [3]. Sometimes, surgeon's satisfaction with the outcome does not correlate with the patient's satisfaction and, therefore, objective measurements could be a useful tool in explaining to a patient what we have achieved with surgery. Moreover, objective measurements and standardized scales could allow the comparison of different techniques or establishing criteria for exclusion of a patient that could not benefit much from the surgery.

When we analyze upper eyelids, tarsal platform show (TPS) and brow fat span (BFS) are the key measurements. Changes in those parameters can simply illustrate what we have achieved with surgery. Besides these features, every patient has a different orbital bone structure, sometimes prominent eyebrow ridge, sometimes fatty periorbital region prone to edema. All these factors have to be analyzed as they will influence the final result and sometimes limit the possibilities of the surgery. Preexisting asymmetries should be noticed and discussed with patients as they are sometimes unaware of them. A surgeon is trying to reach a balanced appearance that will ultimately please the patient. Usually, it is not obliterated tarsal platform nor unnaturally elongated, less fluffy eyelid appearance, or hollow old-looking eye.

The aim of this study was to evaluate the outcome of upper blepharoplasty and to assess patient's satisfaction and possibilities of objective measurement of operative results.

Methods

Study protocol

This research was designed as a prospective observational study that included 50 consecutive patients who underwent upper eyelid blepharoplasty by the same surgeon during the period of two years, between October 2016 and October 2018, at the Clinic for Plastic and Reconstructive Surgery, Clinical Center of Vojvodina, Novi Sad, Serbia. The exclusion criteria were as follows: previous eyelid surgery or trauma, brow lift operation, hyper/ hypothyroidism, eyelid ptosis, neurotoxin treatment in less than 6 months before the surgery, facial nerve paresis, male gender (as there was only one male patient in this period).

Data were collected from medical documentation, questionnaire, and objective measurement conducted via standardized digital photographs taken before surgery and 2 months after surgery, during standard follow-up procedure. All photographs were taken with the patients in an upright position, primary gaze, frontal and lateral view, with frontal muscle fully relaxed. All measurements were conducted with photo size calibrated to 11.5 mm cornea diameter. All photographs, computer calibrations, and measurements were done by the same person. We measured TPS and brow fat span BFS at three points (P1, P2, and P3). TPF was defined as the distance between the upper eyelid margin and palpebral crease, and BFS as the distance between the upper margin of the brow to the palpebral crease with the patient gazing in the primary position. TPS and BFS were measured along the vertical meridian at three points (the center of the pupil – P1, lateral corneal limbus – P2, eyelid lateral canthus – P3) before and two months after the surgery (Figure 1).

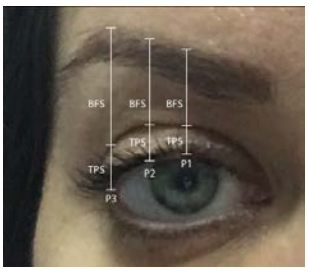

Fig.1 – Illustration of tarsal platform show (TPS) and brow fat span (BFS) measurements at three points (P1-center of the pupil, P2-lateral corneal limbus, P3-lateral canthus).

The patient survey was conducted using two questionnaires: one before operation considering eyelid asymmetry, problems and symptoms that bother patient and are connected to upper eyelids; and two months after surgery, considering patient's and surgeon's satisfaction with the overall outcome, improvement in symptoms, the perceived difference in eyelid symmetry. The overall result concerning patient's and surgeon's satisfaction was graded by Lickert's scale, where 0 was aesthetically poor/completely unsatisfied, and 5 was aesthetically excellent/completely satisfied.

Surgical technique

All blepharoplasties were done by a single surgeon in local anesthesia as a one-day surgery procedure. A marking pen was used to mark planned skin resection. After a local anaesthetic had been infiltrated in this area, redundant skin was resected. If there was redundant orbicular muscle, a narrow strip of muscle was separately resected. The orbital septum was opened in order to expose both retroseptal fat pads (nasal and central), which were evaluated and trimmed with cauterization if needed. Wound edges were closed with continuous nonabsorbable suture (nylon 6.0.) that involved skin-muscle-skin in order to better define supratarsal fold. The muscle was not sutured separately from the skin. Adhesive tapes were applied, and each patient was advised to cool upper eyelids during the first 48 hours with cold pads, protect eyes with sunglasses, clean the face with running water as usual, and use artificial tear eye drops if needed. The skin sutures were taken off after one week. After that, patients were advised to use silicone gel with SPF 50 for scar treatment and avoid exposure to the sun. They were scheduled for another control in two months.

Statistical analysis

Statistical analysis was performed with the software SPSS 20. The Kolmogorov-Smirnov test was used to examine whether the variables followed a normal distribution. Descriptive statistics were shown using mean, standard deviation, minimum and maximum values. The Wilcoxon test was used to determine the existence of statistically significant differences between two dependent samples for variables that did not follow a normal distribution. The Man-Whitney independent samples *t*-test was used to determine the existence of statistically significant differences between two independent groups for variables that did not follow a normal distribution. The Kruskal-Wallis test was used as a non-parametric tool to discover statistically significant differences in more than three independent groups. The Spearman correlation was used to measure the strength and direction of association between two ranked variables. All tests were performed on a 0.05 significance level.

Results

This study involved 50 female patients aged between 33 and 67 years (49.98 ± 8.6). Most of the patients (46%) belonged to the 45–55 age group, 22% had more than 55 years and 32% less than 45 years.

According to the results of the normality test for TPS and BFS measured before and two months after the operation, all variables displayed deviation from the normal distribution. As a consequence, non-parametric test was used in the remaining analysis. Descriptive statistics of all TPS points measured before and after the operation is shown in Table 1.

Table 1

Tarsal platform show (TPS) and brow fat span (BFS) measurements (in mm) at three points (P1, P2, P3) for upper eyelids (n = 100) before (pre op.) and two months after (post op.) upper blepharoplasty

Measurement*	Mean	SD	Minimum	Maximum	*p* (Wilcoxon test)
TPS-P1					
pre op.	1.080	1.3271	0.0	5.0	0.000
post op.	3.820	0.9307	2.0	6.0	
TPS-P2					
pre op.	0.800	1.0987	0.0	4.0	0.000
post op.	3.525	0.8858	2.0	5.0	
TPS-P3					
pre op.	0.89	1.222	0	4	0.000
post op.	3.360	0.8471	2.0	5.0	
BFS-P1			1		
pre op.	16.775	2.7810	0.0	29.0	0.000
post op.	14.98	2.482	10	25	
BFS-P2					
pre op.	18.27	2.957	12	30	0.000
post op.	16.130	2.5953	10.0	27.0	
BFS-P3					
pre op.	20.53	3.227	14	33	0.000
post op.	17.985	2.7000	12.0	30.0	

***For explanation see Figure 1; SD – standard deviation.**

The results of the testing difference in TPS and BFS for all three points between before and after the operation showed a statistically significant difference in all points before and after the operation (Table 1).

Furthermore, we assessed whether there was significant asymmetry comparing the left and right eye before and after the surgery. According to the results of the normality test for all points measured before and after operation of the left and right eye, a non-parametric test was used in the further analysis. Descriptive statistics of all TPS points measurements before and after the operation of the left and right eye are shown in Table 2.

The results of testing the statistical difference in points between the left and right eye before and after the operation are given in Table 3. According to the results, there was no difference between the left and right eye both before and after the operation.

Besides objective measurements presented in Table 3, patients were also asked about their perception of upper eyelid asymmetry before and after surgery. The distribution of patients according to answers is presented in Figures 2 and 3.

Table 2

Tarsal platform show (TPS) and brow fat span (BFS) measurements (in mm) at three points for left (L) and right (D) eyes (n = 50, each) before and after upper blepharoplasty

Measurement*	Before	After
	mean ± SD	mean ± SD
TPS-P1		
D	1.100 ± 1.3553	3.780 ± 0.9592
L	1.060 ± 1.3118	3.860 ± 0.9094
TPS-P2		
D	0.730 ± 1.0653	3.490 ± 0.9340
L	0.870 ± 1.1375	3.560 ± 0.8430
TPS-P3		
D	0.90 ± 1.329	3.300 ± 0.8806
L	0.88 ± 1.118	3.420 ± 0.8167
BFS-P1		
D	16.560 ± 2.7174	14.68 ± 2.453
L	16.990 ± 2.8544	15.28 ± 2.499
BFS-P2		
D	18.14 ± 2.983	15.910 ± 2.5906
L	18.40 ± 2.955	16.350 ± 2.6074
BFS-P3		
D	20.40 ± 3.301	17.830 ± 2.5387
L	20.66 ± 3.179	18.140 ± 2.8697

***For explanation see Fig. 1; SD – standard deviation.**

Table 3

Difference (in mm) between left and right upper eyelid before and after operation

Measurement*	Before	$p^{\dagger}$	After	$p^{\dagger}$
TPS-P1	1,227.5	0.867	1,172	0.571
TPS-P2	1,176.5	0.565	1,183	0.631
TPS-P3	1,212.5	0.77	1,174	0.583
BFS-P1	1,148.5	0.48	1,048.5	0.156
BFS-P2	1,174.5	0.60	1,110.5	0.331
BFS-P3	1,176	0.601	1,182.5	0.638

***For explanation see Figure 1.**
†Mann-Whitney *U* test (2-tailed).

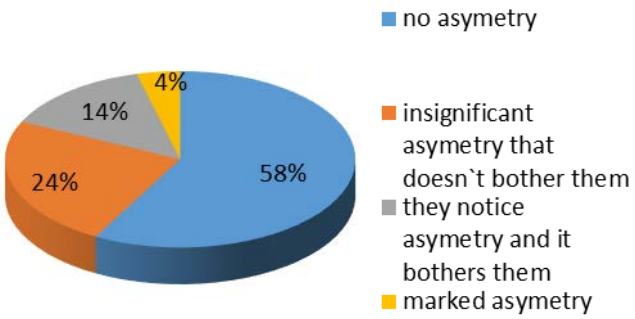

Fig. 2 – Patients' perception of upper eyelid asymmetry before surgery.

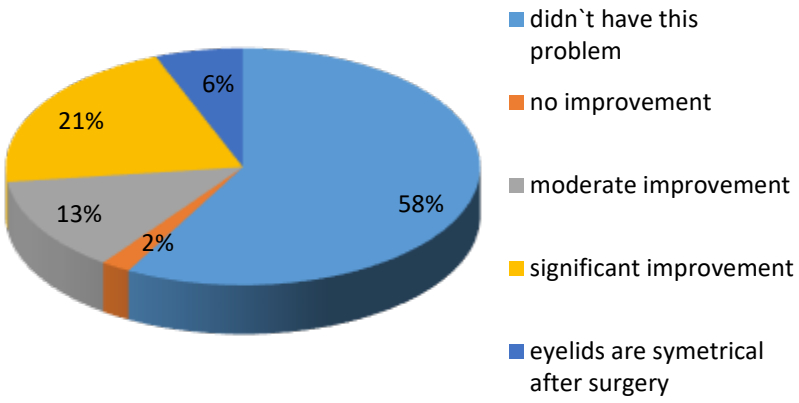

Fig. 3 – Patients' perception of achieved improvement in eyelid asymmetry after surgery.

Table 4

Difference (in mm) between points before and after operation by age groups of patients

Age (years)	Measurement*					
	TPS-P1	TPS-P2	TPS-P3	BFS-P1	BFS-P2	BFS-P3
33–45	2.5000	2.5313	2.3438	-1.8281	-2.2188	-2.5625
45–55	2.5543	2.5543	2.4022	-1.9674	-2.4130	-2.5543
> 55	3.4773	3.3636	2.7955	-1.3864	-1.4545	-2.5000

***For explanation see Fig. 1.**

Mean values of the differences before and after the operation at all points in different age groups of patients are listed in Table 4.

There was a statistically significant difference in the operation success between the 3-age categories in TPS-P1 (χ^2 = 13.089, df = 2, p = 0.001) and TPS-P2 (χ^2 = 8.386, df = 2, p = 0.015).

Major complaints considering upper eyelids before and after the surgery are presented in Figures 4 and 5.

Results of the survey presenting the patients' and surgeon's satisfaction with the overall result of blepharoplasty are presented in Figure 6. All patients as well as the surgeon were satisfied.

There is a strong positive and statistically significant correlation between the patients' and surgeon's satisfaction (r = 0.704, p = 0.002), and also between the patients' satisfaction and their age (r = 0.704, p = 0.002).

We calculated the absolute differences between preoperative and postoperative measurements of TPS at all points, as well as the mean value of these differences for each patient. With this quantity, we wanted to measure the objective achievement and compare it with the subjective satisfaction of the patients.

There was a positive and statistically significant

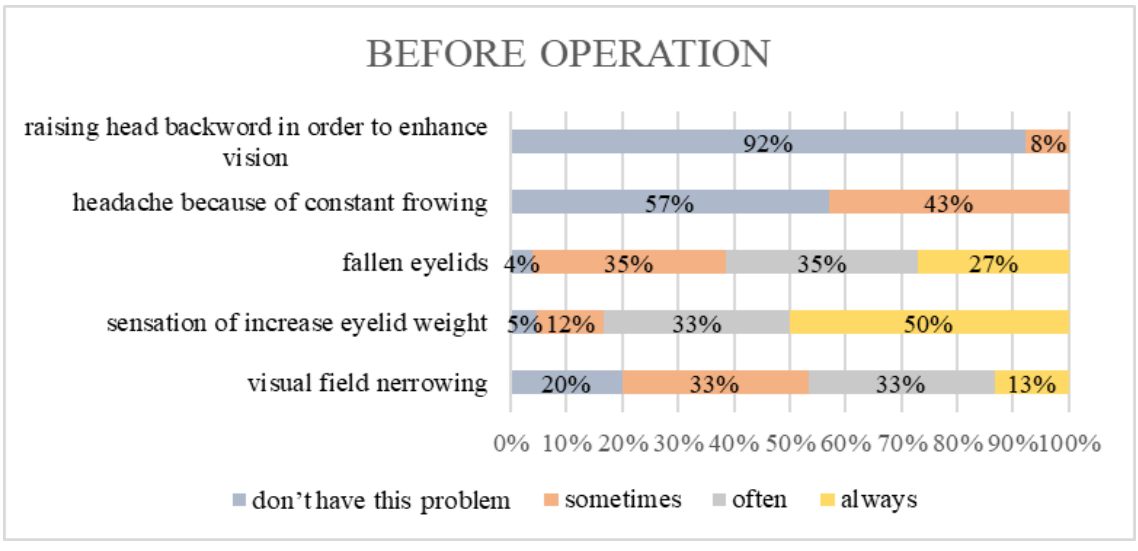

Fig. 4 – Preoperative complaints in the patient seeking upper blepharoplasty.

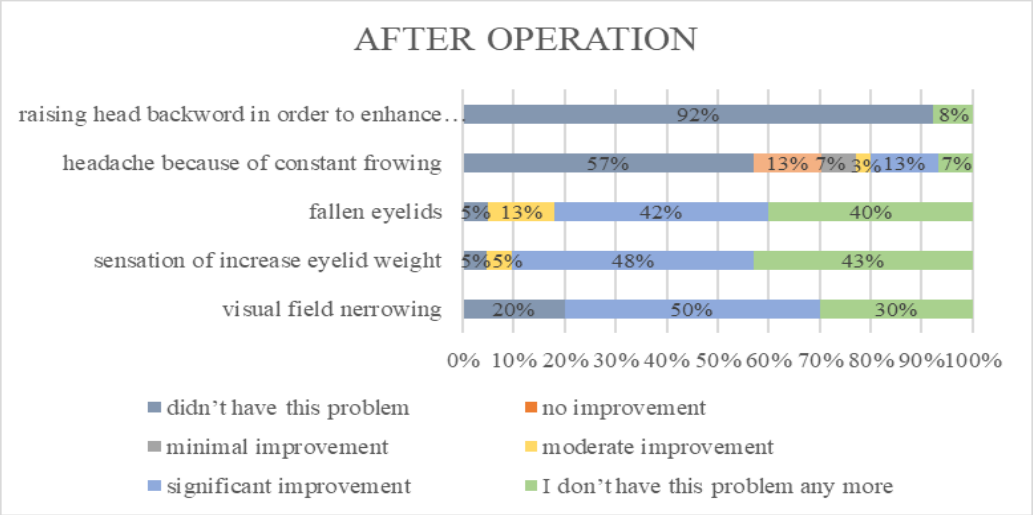

Fig. 5 – Postoperative changes in symptoms patients had before upper blepharoplasty.

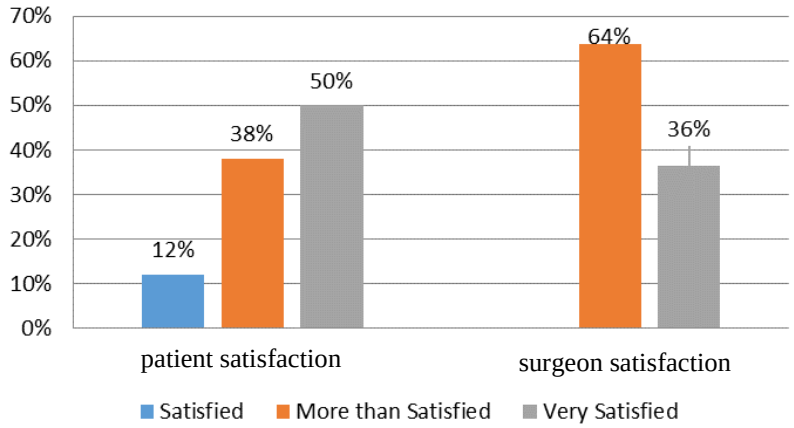

Fig. 6 – Patients' and surgeons' satisfaction with results of blepharoplasty.

correlation between the patients' subjective satisfaction and objective operation results (r = 0.498, p = 0.000). Similarly, there was a positive and statistically significant correlation between the surgeon's subjective satisfaction and objective operation results (r = 0.600, p = 0.000).

Discussion

As every face ages differently and some periorbital anatomical features can differ from patient to patient, there is no universal surgical solution for eyelid correction. Every plastic surgeon during his/her professional carrier changes technique in many ways influenced by new anatomical studies or new appealing techniques in order to find something that works well in his/her hands. After many publications, there is still a lack of consensus on the way we treat muscle in blepharoplasty – should we resect the orbicular muscle or not, if so, how much muscle should be resected, is it necessary to suture the muscle, how should we suture the skin, etc. [4, 5].

We don't believe in wide resection of the orbicular muscle and radical excision of fat pads in every patient, as the preservation of muscles and some amount of fat pads should give fullness to the eyelids and their youthful appearance. "Hollow eyes„ are one of the stigmas of blepharoplasty and sign of radical fat resection. These days, with a better understanding of the aging process and after detailed analyses of volume changes in aging face, we are more oriented to volume preservation and restoration than resection and forced tightening as it was done previously. If there is an obvious redundant muscle, we resect just a small strip of the muscle in order to avoid muscle fold formation. A similar practice is found in many other studies in literature [6–10]. If necessary, as part of primary or secondary blepharoplasty, restoring the fullness of the upper eyelid can be accomplished by different adjuvant techniques, such as fat grafting, medial fat pad transposition, imbrication of orbicular muscle, hyaluronic acid injections, etc. We have to keep in mind that the incidence of complications

(lagophthalmos, sluggish eyelid closer, dry eye syndrome) as presented by Kiang et al. [11] is expected to be lower in muscle-sparing techniques. As might be expected, not all studies agree with this finding. Before accusing muscle resection of a higher rate of complications, we have to classify patients into the same risk level groups considering nicotine consumption, exposition to ultraviolet (UV) rays, diabetes, and other factors that are expected to influence the rate of complications besides surgical technique. Saalabian et al. [12] demonstrated on a group of 387 patients that the extent of tissue resection had no statistically significant effect on the patients' satisfaction with the final result of upper eyelid surgery.

On the other hand, some authors are in favor of wide muscle resection as they consider skin and muscle as a single unit. According to them, redundancy in skin also reflects redundancy in muscle [13–15].

When arguing about aesthetic achievements related to muscle resection, we have to keep in mind the influence of muscle resection on brow position as all these components are closely connected. Widgerow [16] advocates that the resection of orbicular muscle is supposed to allow the frontal muscle advantage over antagonistic orbicular muscle and thus produce eyebrow elevation. This hypothesis gives a new perspective on the importance of muscle resection in the rejuvenation of the periorbital region. However, the authors did not give us any objective measurements of pre- and postoperative results; therefore, the real value of this undoubtedly interesting point cannot be assessed.

The upper eyelid crease is formed by the union of skin dermal component, deep aponeurosis of the *orbicularis oculi* muscle, the aponeurosis of the palpebral levator muscle, and the septum at the level of the upper tarsus. That is why we choose to close the wound by a continuous suture that involves the skin and fascial layer of the *orbicularis oculi* muscle together in order to promote scar formation that would better define the crease. Even when we did not resect the muscle, we made the same suture that attached skin edges to the underlining muscle. Different authors use different techniques, but this option gives good results, as confirmed by this study.

The measurings of TPS and BFS are usually used to compare two different techniques or evaluate the outcome of blepharoplasty. Figueiredo et al. [17] used it to evaluate brassiere sutures as an adjuvant procedure during upper eyelid surgery.

In this study, all patients had statistically significant improvement after surgery, and this improvement was verified by a change in TPS and BFS. This improvement was greatest in the older population. We expected to get such a result as skin excess is more pronounced in the older population, hence change in appearance and benefit from surgery is greatest. These patients were also most satisfied with the result. A strong positive correlation between patients' satisfaction and the age of the patients ($r = 0.704$, $p = 0.002$) was noticed after questionnaire analysis. Besides objective measurement and more evident changes after surgery in the older population, these patients are traditionally more objective and have more realistic expectations. They approach surgery with "real" problems, have many complaints that are pronounced, thus they feel significant relief after correction of eyelids. For them, skin excess is not just an aesthetical problem, but also often impairs their everyday functioning and diminishes their quality of life.

We also wanted to evaluate whether we managed to correct asymmetries in eyelids with surgery. However, the results of testing statistical difference in all points between the left and right eye before the operation did not show any significant difference; therefore, we could not expect to be able to measure improvement later on. This correlates with the results of the questionnaire, where 58% of patients did not perceive eyelid asymmetry, and 24% noticed insignificant asymmetry before the operation (Figure 2). Most of those who noticed asymmetry had moderate to significant improvement after the surgery (Figure 3).

Considering preoperative complaints, patients most often complained about the sensation of fallen eyelids, increased eyelid weight, and narrowing of the visual field. These symptoms were marked as "significantly improved" or "absent" after surgery (Figure 5). In our study, as in most studies in literature, upper blepharoplasty had a high rate of patients' satisfaction [18, 19]. This satisfaction also correlated with the surgeon's satisfaction with the final outcome (Figure 6).

As there was a positive and statistically significant correlation between patients' subjective satisfaction and objective operation results ($r = 0.498$, $p = 0.000$), our measurement proved to be a reliable and simple tool for estimating the final result. One more interesting role of objective measurements in eyelid surgery is the implementation of measurement criteria that could allow health insurance companies to limit falsification of medical necessity for upper blepharoplasty, which is often seen in practice [20]. Thus, it would be easy to separate patients who have just aesthetic problems from those entitled to health insurance coverage as they have some functional disability or important visual field narrowing caused by the change in upper eyelids.

Conclusion

When arguing about the achieved results, objective measurements (TPF and BFS), according to the before/after photos, can be a useful mean in approaching a patient after eyelid surgery. We are not seeking a single standardized solution for periorbital rejuvenation, and we are not trying to reach some imaginary goal, we are rather looking at this as a constant journey of improving ourselves as surgeons in order to be able to offer more and to have a satisfied patient in the end.

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Received on August 24, 2019
Revised on February 9, 2020
Accepted on August 3, 2020
Online First August, 2020

ORIGINAL ARTICLE

UDC: 616.3-052-056.253:613.24
DOI: https://doi.org/10.2298/VSP200605085R

Risk factors for malnutrition among hospitalized gastroenterological patients

Faktori rizika od pothranjenosti hospitalizivanih gastroenteroloških bolesnika

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Abstract

Background/Aim. Risk factors for malnutrition of patients during hospitalization have not been precisely determined. The aim of the study was to determine these factors in hospitalized gastroenterological patients. **Methods**. Nutritional status (NS) of 650 gastroenterological patients was assessed at the hospital admission and at discharge by the six parameters: unintentional weight loss, lymphocyte count, serum albumin concentration, body mass index, triceps skinfold thickness, and mid-upper arm muscle circumference. The influence on NS at discharge was tested for ten factors: gender, age, affected organ, the nature, severity, and complications of the disease, the length of hospitalization, mobility worsening during hospitalization, Karnofsky score, and NS on admission. Primary and secondary risk factors were defined among the factors significantly influencing malnutrition. **Results**. Seven factors were found to be the independent predictors for malnutrition in hospitalized gastroenterological patients. NS at admission was considered as a primary risk factor (Forward: Wald multivariate logistic regression analysis, $p < 0.001$ for five applied assessment parameters). The other six factors, obtained in the evaluation according to 1-3 assessment parameters, were considered as secondary risk factors: severe disease activity, malignancy, the existence of complications, male gender, hospitalization > 14 days, and mobility worsening during the hospitalization (Forward: Wald multivariate logistic regression analysis, p from 0.001 to 0.027). **Conclusion**. There are seven risk factors for malnutrition among gastroenterological patients during hospitalization. Timely nutritional support in these patients can prevent the development of intrahospital malnutrition and its negative influence on the clinical outcome.

Key words:
gastrointestinal diseases; hospitalization; malnutrition; nutritional status; risk factors; treatment outcome.

Apstrakt

Uvod/Cilj. Faktori rizika od pothranjenosti bolesnika tokom hospitalizacije nisu precizno definisani. Cilj studije bio je da se determinišu ovi faktori kod hospitalizovanih gastroenteroloških bolesnika. **Metode.** Nutritivni status (NS) 650 gastroentroloških bolesnika bio je procenjivan na prijemu i na otpustu pomoću šest parametara procene: nenamerni gubitak težine, broj limfocita, koncentracija albumina u serumu, indeks telesne mase, debljina kožnog nabora tricepsa i obim sredine nadlaktice. Uticaj na NS na otpustu bio je testiran za deset faktora: pol, starost, oboleli organ, prirodu, težinu i komplikacije bolesti, dužinu hopsitalizacije, pogoršanje pokretnosti tokom hospitalizacije, Karnovski indeks i NS na prijemu. Među faktorima koji su tokom hospitalizacije značajno uticali na pothranjenost, definisani su faktori rizika. **Rezultati.** Za sedam faktora je dobijeno da su nezavisni prediktori pothranjenosti. NS na prijemu je bio primarni faktor rizika (*Forward: Wald* multivarijantna logistička regresiona analiza, $p < 0,001$ za pet primenjenih parametara procene). Ostali faktori, dobijeni procenom prema 1-3 parametra, označeni su kao sekundarni faktori rizika: teška aktivnost bolesti, malignitet, postojanje komplikacija, muški pol, hospitalizacija preko 14 dana i pogoršanje pokretnosti tokom hospitalizacije (*Forward: Wald* multivarijantna logistička regresiona analiza, p od 0,001 do 0,027). **Zaključak.** Postoji sedam faktora rizika za pothranjenost gastroenteroloških bolesnika za vreme hospitalizacije. Pravovremena i adekvatna nutritivna podrška kod ovih bolesnika može da spreči nastanak intrahospitalne pothranjenosti i njen negativan uticaj na klinički ishod.

Ključne reči:
gastrointestinalne bolesti; hospitalizacija; pothranjenost; nutritivni status; faktori rizika; lečenje, ishod.

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Introduction

Malnutrition is a serious health problem that affects more than 20% of patients upon hospital admission [1–3]. It significantly contributes to the many adverse outcomes, such as cardiovascular and infective complications, increased morbidity and mortality, prolonged hospitalization, increased hospitalization costs, and increased readmission rates after discharge from the hospital [4–8]. Although these consequences of poor nutritional status (NS) are well known, malnutrition is often undiagnosed on hospital admission. Furthermore, some patients experience deterioration of their nutritional status and become malnourished during hospitalization, regardless of their initial nutritional status. However, this change often remains unrecognized by the medical staff [7, 9, 10]. If we keep in mind that malnutrition can potentially be prevented and treated, identification and definition of risk factors for malnutrition is of particular interest. Many studies investigated the risk factors for malnutrition among the patients on hospital admission [1, 2, 11, 12]. However, there is a lack of data regarding the prevalence and risk factors for malnutrition during hospitalization. Some authors highlight gender, age, malignant tumors, reduced food intake, prolonged therapeutic fasting as factors associated with malnutrition, but the significance of these factors has not been precisely determined [13, 14].

This study presents our experience with risk factors for malnutrition among hospitalized gastroenterological patients. As well, information is presented on how to recognize a risky patient who is a candidate for nutritional intervention.

Methods

Study design and patient population

A prospective study included 650 gastroenterological patients treated in our Clinic for fifteen months. The criteria on inclusion were: 18 years of age or above, Karnofsky score > 40 upon admission, and length of hospital stay for at least seven days. The study protocol was approved by the local Ethics Committee, and each patient gave written informed consent before entering the study.

Assessment of nutritional status

Nutritional status was evaluated at the hospital admission and within 24 hours prior to hospital discharge. We used six NS assessment parameters (NSAPs): unintentional weight loss (WL), lymphocyte count (LYM), serum albumin concentration (ALB), body mass index (BMI), triceps skin-fold thickness (TSF), and mid-upper arm muscle circumference (MAMC). In the patients with hypersplenism and ascites, NS was not evaluated according to LYM and weight loss, respectively.

BMI (kg/m^2) was calculated as weight (kg) divided by the square of the height (m^2). In the case of ascites, BMI was defined using the Powel-Tuck equation [15]. TSF and mid-upper arm circumference (MAC) were determined at the mid-point between the acromion and olecranon processes of the non-dominant side by calipers and tape. MAMC was estimated using the following formula: MAMC (cm) = MAC (cm) – 3.14 x TSF (cm).

According to NS at the admission and at discharge, patients were classified into two groups: non-malnourished (normally nourished, overweight and obese) and malnourished [16].

Factors influencing the NS during hospitalization

The influence on NS was tested for the following ten factors: gender, age, affected organ, the nature and severity of the disease, complications of the disease, the length of hospitalization, mobility worsening during the hospital stay, NS and Karnofsky score at the hospital admission. The Karnofsky score was used as a tool for assessing the functional capacity of the patient.

According to the affected organ, patients were divided into groups with the disease of: esophagus, stomach, and duodenum (ESD); liver and bile ducts (LBD); pancreas; intestine. The biological nature of the disease was defined as benign and malignant. Malignity was confirmed histopathologically and/or by a significant increase of tumor markers in the blood or ascites. The severity of the disease was defined according to the recommendations of the European Society for Clinical Nutrition and Metabolism (ESPEN) as severe, moderate, and mild [17]. Complications included infectious complications (pneumonia, urinary tract infections, sepsis) and organic failure (cardiac, respiratory, and renal failure). According to mobility, patients were classified as mobile (able to carry out usual activities), semi-mobile (need some help of hospital staff, particularly for activities that require leaving the hospital room), and stationary (bedridden).

Risk factors for malnutrition during hospitalization

Risk factors were determined among the factors that significantly influenced malnutrition during hospitalization. The factors obtained by estimation of NS according to the 4-6 NSAPs were qualified as the primary risk factors, while those obtained by estimation according to the 1-3 NSAPs were qualified as the secondary risk factors.

Statistical analysis

Statistical analysis was performed using SPSS 11.5 for Windows software (SPSS, Inc., Chicago, IL), and a *p*-value of < 0.05 was considered statistically significant. The Student's *t*-test for parametric data and the Mann-Whitney *U*-test for categorical data were performed to compare characteristics between two groups. Correlation between two variables was tested by binary logistic analysis. Prediction of malnutrition at discharge was determined using the Forward: Wald multivariate logistic regression analysis.

Results

Characteristics of the patients

During the study period, 989 consecutive patients were screened. We excluded 339 patients from the study: at screening, 67 patients did not qualify for participation in the study; in 186 patients, the length of hospital stay was shorter than seven days; 42 patients had a lethal outcome in the hospital; 44 patients left the study for their own reasons. The study was completed with 650 patients: 360 males and 290 females. Average values for age, Karnofsky score, and length of hospitalization were: 60.3 ± 16.1 years; 94.8 ± 8.8, and 13.5 ± 6.7 days, respectively. Malignant diseases were diagnosed in 236 (36.30%) patients. The most common were intestinal diseases (40.92%), followed by the LBD (34.46%), pancreatic (14.16%), and ESD diseases (10.46%).

Assessment of nutritional status

On hospital admission, depending on the NSAPs we used, malnutrition was diagnosed in 7.7–31.7% of patients, while 68.3–92.3% of patients were non-malnourished. At discharge, malnutrition was diagnosed in 8.0–38.2% of patients, while 61.8–92% were non-malnourished (Table 1).

Factors influencing the NS during hospitalization

Gender. Malnutrition was significantly more common in males if the NSAPs were MAMC (binary logistic analysis, $p < 0.001$) and ALB (binary logistic analysis, $p = 0.040$).

Age. Regardless of the NSAPs applied, the average age was similar for malnourished and non-malnourished patients at discharge (Student's *t*-test, $p > 0.05$).

Affected organ. Regardless of the NSAPs applied, malnutrition was more common in the patients with intestinal disease and LBD diseases than in the patients with disease of ESD and pancreas. These differences were statistically significant if the assessment parameter was ALB (Binary logistic analysis; $p = 0.035$).

The nature, severity, and complications of the disease. Regardless of the NSAPs applied, malnutrition was significantly more common in the patients with malignant disease (binary logistic analysis, p from < 0.001 to 0.028), with severe disease (binary logistic analysis, p from 0.001 to 0.006), and with complications of the disease (binary logistic analysis, p from < 0.001 to 0.013).

The length of hospitalization. The average length of hospitalization was higher in patients who were malnourished at discharge, but these differences were statistically significant if the assessment parameters were BMI (Student's *t*-test, $p = 0.005$), TSF (Student's *t*-test, $p = 0.020$), or ALB (Student's *t*-test, $p < 0.001$). Furthermore, malnutrition was significantly more common in patients with hospitalization longer than 14 days if the assessment parameters were WL (binary logistic analysis, $p = 0.022$), TSF (binary logistic analysis, $p = 0.030$), or ALB (binary logistic analysis, $p < 0.001$).

Mobility worsening during the hospital stay. Malnutrition at discharge was significantly more common in the patients with mobility worsening during the hospital stay than in the patients without mobility worsening. The differences were not statistically significant only if the assessment parameter was TSF (binary logistic analysis, $p > 0.05$).

Karnofsky score at admission. The average Karnofsky score on admission and at discharge was always significantly lower in patients who were malnourished at discharge (Mann-Whitney test, $p < 0.001$). Regardless of the NSAPs applied, malnutrition at discharge was significantly more common in the patients with admission Karnofsky score ≤ 80 than in the patients with admission Karnofsky score > 80 (binary logistic analysis, $p < 0.001$).

Nutritional status at admission. Malnutrition at discharge was more common in the patients who had been malnourished at the hospital admission than in patients who had been non-malnourished at admission. These differences were not statistically significant only if the assessment parameter was WL (binary logistic analysis, $p > 0.05$).

Risk factors for malnutrition during hospitalization

There were 9 factors that could influence NS during hospitalization (Table 2). Six of them influenced NS according to the evaluation of NS with 5 or all 6 parameters: nutritional status at admission, mobility worsening during the hospital stay, Karnofsky score at admission, the nature, severity, and complications of the disease. The remaining

Table 1

Nutritional status according to different NSAPs

NSAP	Patients, n	Admission, n (%)		Discharge, n (%)	
		non-malnourished	malnourished	non-malnourished	malnourished
WL	620[1]	496 (80.0)	124 (20.0)	486 (78.4)	134 (21.6)
BMI	650	600 (92.3)	50 (7.7)	598 (92.0)	52 (8.0)
TSF	650	578 (88.9)	72 (11.1)	568 (87.4)	82 (12.6)
MAMC	650	457 (70.3)	193 (29.7)	458 (70.5)	192 (29.5)
ALB	650	444 (68.3)	206 (31.7)	402 (61.8)	248 (38.2)
LYM	636[2]	474 (74.5)	162 (25.5)	468 (73.6)	168 (26.4)

NSAP – nutritional status assessment parameter; WL – weight loss; BMI – body mass index; TSF – triceps skinfold thickness; MAMC – mid-upper arm muscle circumference; ALB – serum albumin concentration; LYM – lymphocyte count.
[1]Thirty patients with ascites were not analyzed; [2]Fourteen patients with hypersplenism were not analyzed.

three factors influenced NS under certain conditions: gender, affected organ, and the length of hospitalization.

Seven factors were found (Forward: Wald multivariate logistic regression analysis) to be the independent predictors for malnutrition during hospitalization. Only NS at admission was obtained in the evaluation according to more than half of NSAPs. Therefore, it was considered a primary risk factor. The other six factors: severe disease activity, malignant disease, the existence of complications, male gender, the length of hospitalization > 14 days, and mobility worsening during the hospital stay, were obtained in the evaluation according to 1–3 NSAPs. Therefore, they were considered secondary risk factors (Table 3).

Table 2

Factors significantly influencing the nutritional status (NS) at discharge (binary logistic analysis)

Factors	WL	BMI	TSF	MAMC	ALB	LYM
	p	*p*	*p*	*p*	*p*	*p*
Gender	-	-	-	0.001	0.040	-
Affected organ	-	-	-	-	0.035	-
The nature of the disease	0.001	0.007	0.013	0.028	0.001	0.001
Severity of the disease	0.004	0.001	0.002	0.003	0.001	0.001
Complications of the disease	0.001	0.001	0.001	0.001	0.001	0.013
Karnofsky (admission) ≤ 80	0.001	0.001	0.001	0.001	0.001	0.001
Hospitalization >14 days	0.022	-	0.030	-	0.001	-
Mobility worsening	0.003	0.007	-	0.001	0.001	0.024
NS on admission	-	0.001	0.001	0.001	0.001	0.001

WL – weight loss at discharge; BMI – body mass index; TSF – triceps skinfold thickness; MAMC – mid-upper arm muscle circumference; ALB – serum albumin concentration; LYM – lymphocyte count.

Table 3

Risk factors for malnutrition at discharge

Factors	WL	BMI	TSF	MAMC	ALB	LYM
	p	*p*	*p*	*p*	*p*	*p*
Gender	-	-	-	0.001	-	-
The nature of the disease	0.026	-	-	-	-	-
Severity of the disease	-	-	-	0.001	0.002	0.021
Complications of the disease	0.001	-	-	0.027	0.001	-
Hospitalization >14 days	-	-	-	-	0.016	-
Mobility worsening	-	-	-	0.001	-	0.014
Nutrition status on admission	-	0.001	0.001	0.001	0.001	0.001

WL – weight loss at discharge; BMI – body mass index; TSF – triceps skinfold thickness; MAMC – mid-upper arm muscle circumference; ALB – serum albumin concentration; LYM – lymphocyte count.

Forward: Wald multivariate logistic regression analysis: relative risks (RR) and 95% confidence intervals (CI) are: for Gender (RR = 0.335; 95% CI = 0.177–0.634); for The nature of the disease (RR = 1.594; 95% CI = 1.058–2.402); for Severity of the disease (RR = 1.717–2.249; 95% CI = 1.085–1.369/ 2.550–3.696); for Complications of the disease (RR = 3.887–4.830; 95% CI = 1.752-2.722 /8.572–8.621); for Hospitalization > 14 days (RR = 1.778; 95% CI = 1.111–2.844); for Mobility worsening (RR = 0.103–0,245; 95% CI= 0.028–0.079/ 0.372–0.756); for Nutrition status at admission (RR = 35.976–139.059; 95% CI = 21.404–69.462/ 60.471–278.385).

Discussion

Although interest and awareness of the clinical significance of NS have existed for over forty years, malnutrition is a problem that is still present, even in large hospitals. Previous studies have shown that the prevalence of malnutrition on hospital admission is in the range from 22% to 73% [1–3]. In our study, depending on the NSAPs we used, malnutrition on hospital admission was diagnosed in 7.7–31.7% of patients, which is partly in accordance with the results of the other authors. Probably, a lower percentage of malnutrition could be explained by the specificity of our series, which only included gastroenterological patients. The percentage of malnourished patients at discharge was higher by 0.7–6.5%, and malnutrition existed in 8.0–38.2% of our patients. Compared to values at admission, 0.616% of patients had a deterioration in NS at discharge. Similar results have been published by some other authors who reported a significant decrease in MAMC, fat-free mass, albumin level, weight, and BMI during hospitalization [7, 9, 10]. In our study, there was a decrease in the values of all NSAPs, except LYM.

Gender and age. According to our results, the male gender is a secondary risk factor for malnutrition during hospitalization. This is in accordance with the results obtained by the other authors [13, 18, 19]. The result of our study could be explained by the higher prevalence of malignancies among men and greater weight loss in men than in women during hospitalization. Although malnutrition often accompanies older age and older patients are at increased

risk for malnutrition at both admission and discharge [8, 20–22], the average age for malnourished and non-malnourished patients at discharge in our patients was similar. In the study of Kang et al. [2], malnutrition was higher in the patients over 70 years, while Zhu et al. [3] showed that malnutrition at discharge was significantly higher at the age of 65 and above. The difference in results between our and the other studies is probably related to the specificity of our series, which consisted of gastroenterological patients only, while most other studies included patients with various internal and neurological diseases.

Affected organ. Generally, in the patients with gastroenterological diseases, the prevalence and the risk of hospital malnutrition is higher than in patients with other diseases due to impaired digestion and absorption, loss of appetite, prolonged therapeutic fasting, and increased nutritional requirements [23–26]. Following the changes in the NS of their patients, Cui et al. [14] found a significant reduction in body weight and calf circumference in the patients with benign digestive tract disease at the discharge from the hospital. In our study, malnutrition was more common in the patients with the intestinal disease and LBD disease, probably due to the higher prevalence of malignant diseases in these patients, but they were not found to be independent predictors for malnutrition during hospitalization. This result is similar to the results of some other studies [27–29].

Severe disease activity and malignant disease. In our series, severe disease activity and malignancy were secondary risk factors for malnutrition among hospitalized patients. Severe disease activity is thought to cause increased nutritional requirements due to stress metabolism [30, 31]. Therefore, many authors agree that the risk of intrahospital malnutrition correlates with the severity of the disease and that malnutrition is more pronounced in advanced stages of the disease [12, 28, 32, 33]. There is no doubt regarding the association between malignant disease and NS. It is known that numerous metabolic disorders and negative energy balance in malignancies lead to malnutrition and cachexia [34, 35]. According to the results of other studies, the prevalence of hospital malnutrition is high in oncology patients [21, 36, 37]. Pirlich et al. [38] pointed to malignancy as one of three independent predictors of malnutrition at hospital admission. However, in the current literature, the association between malignant disease and malnutrition during hospitalization has been less studied. We found that malignancy is a risk factor for malnutrition during hospitalization. Similar results were published by some other authors [14, 39]. An interesting result of Pañella et al. [40] is that NS is not associated with the stage of malignancy.

Complications of the disease. In our patients, the presence of complications was a secondary risk factor for malnutrition during hospitalization. There is evidence in the literature that most of these conditions are characterized by hypermetabolism due to the action of proinflammatory cytokines [41, 42]. Unlike many clinical and epidemiological studies that define malnutrition as a risk factor for infection and poor outcome [43–45], studies that define clinical complications as a risk factor for malnutrition are rare. Pinchcofsky and Kaminski [46] and Kinyoki et al. [47] considered persistent fever a risk factor for deteriorating NS of adult hospitalized patients and children under 5 years of age, respectively. The results of some studies indicate that the presence of infection adversely affects NS in surgical and nonsurgical patients [13, 36, 48].

The length of hospitalization. Most authors agree that prolonging hospitalization increases the risk of malnutrition [48–50]. In their study, Pinchcofsky and Kaminski [46] found significant decreases in nutritional parameters after three weeks of hospitalization. Weinsier et al. [51] demonstrated that hospitalization longer than 14 days was critical for the onset of malnutrition. This is the result obtained in our series as well. The authors consider that patients during hospitalization have higher nutrient needs and lower appetite due to inflammatory processes associated with the disease, as reported by Correia [52].

Mobility worsening during the hospital stay. Although recommended in various tests for initial NS screening, mobility worsening has been less discussed in studies to date. According to our results, mobility worsening was a risk factor for hospital malnutrition. This result is in line with the results of some other studies [24, 36, 53]. It is not surprising since mobility is a component of a patient's functional ability, substantial for performing daily activities independently.

Karnofsky score at admission. In our study, malnutrition at discharge was significantly more common in the patients with admission Karnofsky score ≤ 80, but we did not find that it was an independent predictor for malnutrition. Although the results of some other studies show that malnutrition is associated with a lower Karnofsky score, none of them pointed to this score as a risk factor for malnutrition [54–57]. In these studies, a low Karnofsky score could be a consequence rather than a cause of malnutrition.

Nutritional status at admission. According to our results, NS at admission was the only primary risk factor for malnutrition at hospital discharge. Doctors must be aware that patients may already be malnourished at admission and that 35–70% of hospitalized patients do not consume enough calories to meet their nutritional needs. In the study by McWhirter and Pennington [58], patients who were severely malnourished at admission had the greatest weight loss at discharge. Similar results have been published by some other authors [5, 14, 16, 59].

Limitation of the study

This paper has limitations that we want to mention. First of all, only patients from one gastroenterology clinic were included, therefore, the results might be different if the study had been conducted in multiple centers, including gastroenterology departments outside university clinics. Second, according to the inclusion criteria, patients who participated in the study had Karnofsky score over 40. As the Karnofsky score indicates the functional capacity of the patients, the inclusion of patients with a lower score might increase the percentage of malnourished patients on admission. Considering these limitations, the next study

should certainly be designed as a multicenter one and include a wider patient population.

Conclusion

There are seven risk factors for malnutrition of gastroenterological patients during hospitalization, one primary and six secondary. The primary risk factor is NS at the hospital admission, while severe disease activity, malignancy, the existence of complications, male gender, hospitalization > 14 days, and mobility worsening during the hospitalization are secondary risk factors. Clinicians should pay more attention to the identification and continuous monitoring of these factors and patients' NS during hospitalization. Only in this way it will be possible to provide timely and adequate nutritional support and prevent/treat malnutrition and its negative influence on clinical outcomes.

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Received on June 5, 2020
Revised on July 22, 2020
Accepted on August 3, 2020
Online First August, 2020

ORIGINAL ARTICLE

UDC: 618.2-055.26:613.72]:618.4
DOI: https://doi.org/10.2298/VSP200311080B

Effect of aerobic exercise on frequency of vaginal birth – A meta-analysis

Uticaj aerobnog fizičkog vežbanja na učestalost vaginalnog porođaja – meta analiza

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Abstract

Background/Aim. Pregnancy is a state where different morphological and physiological changes occur in a pregnant woman's body. There are many factors that have an effect on maintaining a healthy pregnancy. Physical activity is one of the possible factors that can have an impact on the quality of life during pregnancy. Healthy pregnant women, without medical or obstetric complications, are advised to exercise in a moderate and proper manner. The aim of this meta-analysis was to evaluate the impact of continuous aerobic exercise on the frequency of vaginal birth in healthy pregnant women with normal body mass index (BMI) values. **Methods.** This meta-analysis was based on a systematic review and selection of randomized clinical trials. The affect of physical exercise was assessed using the "*RStudio*" programming language and environment. Heterogeneity of studies was assessed by Q statistics. Possible publication bias of studies was identified. The primary outcome analysis was related to the frequency of vaginal birth, while the secondary outcome analysis was related to BMI in the first measurement and total weight gain after the second measurement. The suitability of 10 individual studies is shown by graphical and statistical analysis of the extracted data. **Results.** Physically active pregnant women who exercised with light to moderate intensity 3 times a week (35–60 minutes), had a more frequent vaginal birth than sedentary pregnant women (67.4% vs. 60.5; relative risk (RR) 1.11, 95% confidence interval (CI), 1.04–1.18). RR was statistically significantly different from unit ($p = 0.002$). All pregnant women had optimal BMI values in the first measurement before intervention. Also, all pregnant women gained the recommended number of kilograms during pregnancy. **Conclusion.** Analysis of selected individual studies showed that continuous aerobic physical exercise during the second and third trimesters does not have a negative effect on measured outcome of pregnancy. Physically active pregnant women were more likely to give birth vaginally than sedentary pregnant women.

Key words:
delivery, obstetric; exercise; meta-analysis; pregnancy; pregnancy outcome.

Apstrakt

Uvod/Cilj. Trudnoća je stanje kada dolazi do različitih morfoloških i fizioloških promena u organizmu trudnice. Mnogi su faktori koji imaju uticaja, na održavanje zdrave trudnoće. Fizička aktivnost je jedan od mogućih faktora koji može imati uticaja na kvalitet života tokom trudnoće. Zdravim trudnicama, bez medicinskih ili akušerskih komplikacija, preporučuje se umereno i pravilno fizičko vežbanje. Cilj ove meta-analize je bio da proceni uticaj kontinuiranog aerobnog fizičkog vežbanja na učestalost vaginalnog porođaja kod zdravih trudnica sa normalnim vrednostima indeks telesne mase (ITM). **Metode.** Ova meta-analiza je bila zasnovana na sistematskom pregledu i selekciji randomizovanih kliničkih studija. Uticaj fizičkog vežbanja je procenjen pomoću "RStudio" programskog jezika i okruženja. Heterogenost studija je procenjena Q statistikom. Utvrđena je moguća publikaciona pristrasnost studija. Analiza primarnih ishoda odnosila se na učestalost vaginalnog porođaja, dok se analiza sekundarnih ishoda odnosila na ITM u prvom merenju i ukupnom dobitku telesne mase nakon drugog merenja. Podobnost 10 individualnih studija prikazana je grafičkom i statistčkom analizom ekstrahovanih podataka. **Rezultati.** Fizički aktivne trudnice, koje su vežbale lakim do umerenim intenzitetom 3 puta nedeljno (35–60 minuta), imale su češće vaginalni porođaj u odnosu na sedentarne trudnice [67,4% prema 60.5%, relativni rizik (RR) 1.11, 95% indeks poverenja:

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1.04–1.18]. RR je bio statistički značajno različit od jedinice ($p = 0{,}002$). Sve trudnice su imale optimalne vrednosti ITM u prvom merenju pre intervencije. Takođe, sve trudnice su dobile preporučen broj kilograma tokom trudnoće. **Zaključak.** Analiza odabranih individualnih studija pokazala je da kontinuirano aerobno fizičko vežbanje, tokom drugog i trećeg trimestra, nema negativan uticaj na mereni ishod trudnoće. Fizički aktivne trudnice su se češće imale vaginalni porođaj u odnosu na sedentarne trudnice.

Ključne reči:
porođaj; vežbanje; meta-analiza; trudnoća; trudnoća, ishod.

Introduction

Pregnancy is a period of life when a woman's body undergoes various morphological and physiological changes. There are various factors that can affect the course and outcome of the pregnancy. It is believed that weight gain can have both positive and negative impact [1]. Maintaining optimal weight, in addition to affecting the prevention of pregnancy problems and the most common diseases, also affects the sense of self-esteem and, consequently, a better quality of life during pregnancy [2]. Also, weight gain can have an impact on method of giving birth. Studies show that more obese women are more likely to give birth by caesarean section [3.] One way to reduce obesity and maintain optimal body weight is through regular physical activity, both before and during pregnancy. Controlled aerobic physical exercise is recommended for healthy pregnant women, while increased physical activity is not recommended for pregnant women who have any medical complications [4].

A positive pregnancy outcome comes down to having a healthy newborn. Childbirth can be carried out in many ways and it may depend on the health of the pregnant woman, the growth and development of the fetus. It is expected that every pregnant woman will expect "natural" and as painless childbirth as possible, so fear of potential delivery by caesarean section may occur. In this meta-analysis, an estimate of the incidence of childbirth occuring exclusively vaginally was performed. It is believed that certain factors, such as daily physical activity, can have an impact on the outcome of pregnancy itself as it relates to the way of delivery.

Many healthy pregnant women do not meet the recommended minimum for physical exercise (at least 3 times a week for at least 20–30 minutes a day) [4]. Due to different research results, it remains unclear how continuous aerobic exercise can have an impact on childbirth. This meta-analysis investigated the impact of identical exercise way on the frequency of vaginal birth in healthy and pre-sedentary pregnant women with normal body weight.

Methods

Search method

Searching the literature and selecting the right references was implemented in accordance with the Preferred Reporting Items for Systematic reviews and meta-Analyses (PRISMA) rules and recommendations [5]. The research protocol included a systematic review of the scientific literature, selection of individual studies according to set criteria, as well as extraction and analysis of primary and secondary outcomes. Selection of scientific studies was performed using two bibliographic databases ("PubMed" and "Scopus"). The search was enabled with the help of selected keywords: "Aerobic exercise", "Pregnancy", defined type of publication ("Randomized control or Clinical trial"), without time limit and with concluding research period until the end of 2018.

Literature selection

A detailed selection of scientific studies is presented in the “Flow diagram” (Figure 1). Duplicate studies, studies with inappropriate design, exercise and measured outcomes were excluded from the study, as well as studies indicating that pregnant women had a risky or a complicated pregnancy. For further research, high quality published scientific studies that meet the set criteria were singled out. Only randomized clinical studies (RCTs) were selected for this meta-analysis.

Participants

The criterion for the selection of participants included exclusively healthy pregnant women with a single fetus pregnancy (27.6–33.8 years of age). Pregnant women were randomized into two research groups. The experimental group consisted of physically active pregnant women (PAPW) who exercised during the second and third trimesters (at the earliest from the 12th and at the latest until the 39th gestational week), while the control group consisted of sedentary pregnant women (SPW) who did not exercise during pregnancy. All pregnant women received regular prenatal care. Pregnant women were previously physically inactive or sedentary and had normal body mass index (BMI) values (18.5–24.9 kg/m^2).

Intervention

Meta-analysis included RCTs that in detail described the phases of physical exercise (introductory part – warm-up, main part – development of endurance and strength, final part – relaxation and stretching) and exercise load (frequency, volume, intensity, manner and type of physical exercise). The intensity of the aerobic exercise method was controlled by a pulsometer or a Borg scale for self-assessment. All studies provide recommendations and guidelines for proper exercise during pregnancy [4, 6, 7] The studies described aerobic exercises (types of aerobics, water

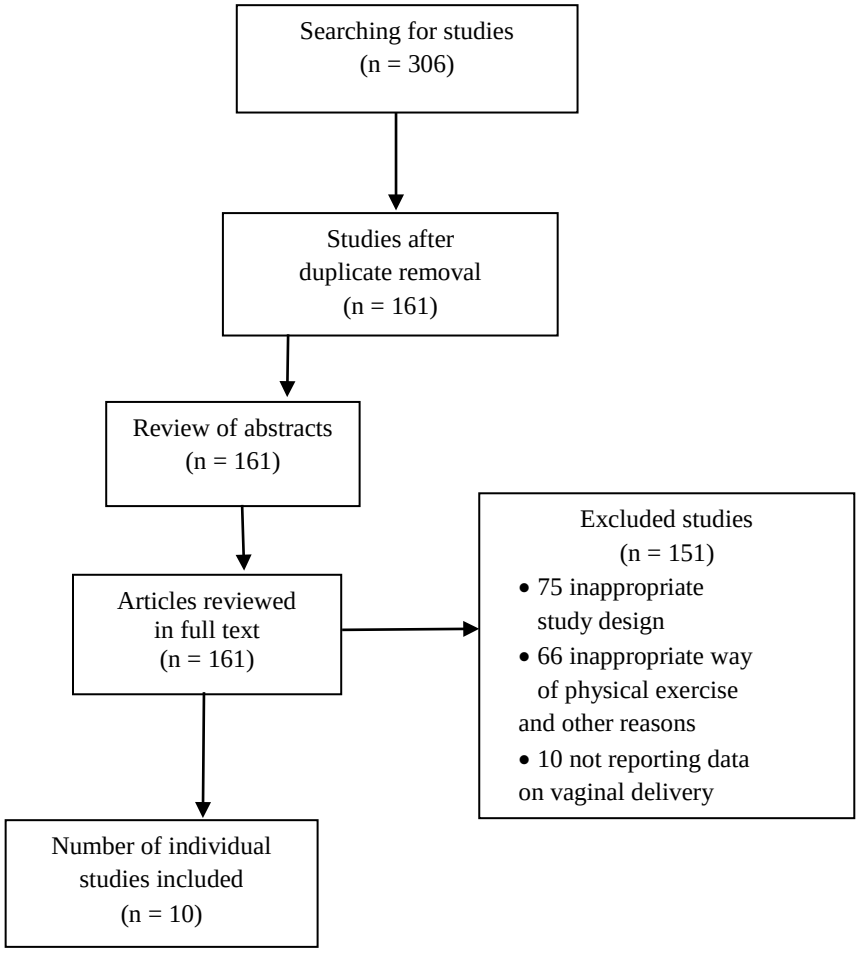

Fig. 1 – Flow diagram of studies selection.

aerobics, dancing, swimming, brisk walking and riding a stationary bike).

Outcome measurement

Primary (vaginal birth) and secondary (BMI and total body weight gain) measurements of pregnancy outcomes were measured. Secondary outcomes were measured before (about the 12th gestational week) and after the intervention (around the 37th gestational week), that is, continuous aerobic physical exercise. BMI was calculated using the following formula (kg/m^2), while the total body weight gained during pregnancy was calculated as the difference in kilograms between prenatal (first) and last measurement. A second weight measurement was usually done at the end of the third trimester. Workout sessions were regularly recorded in the diary.

Data extraction and risk of bias

Assessing the risk of bias in selected individual studies is essential to assessing the reliability of the meta-analysis itself [8]. The assessment of data quality (data inclusion, risk of bias, data extraction, and final analysis) was performed by two independent authors (AB and AJ). If there was disagreement when analyzing the extracted data, the final decision was resolved by discussion between the reviewer and the third author (KB). Table 1 shows the extracted data included into the analysis.

Data analysis

The meta-analysis unit was an individual scientific study. The unification of the results that determine the frequency of the primary outcome was done by determining the Relative risk (RR), while determining the secondary outcomes was done by determining the standardized differences in the means of numeric outcomes. The heterogeneity of the studies was determined graphically using the "Forest" and "L'Abbels" plots, and heterogeneity analysis was performed by calculating Q statistics. A fixed-effect model or a random-effect model was applied depending on the homogeneity of the studies, while adjustments of methods were made according to the DerSimonian and Laird [9] method. Publication bias was evaluated based on the funnel diagram, while testing was performed using the Egger test [10]. The obtained results of the analysis were tested at statistical significance level (alpha level) of 0.05. Data analysis was performed in R programming language and environment using "metafor" [11] and "meta" [12] packages for R.

Table 1

Outline of extracted data from individual studies for meta-analysis

Individual studies	Started analysis		Age (years)		BMI – Baseline (kg/m^2)		MWG (kg)	
	PAPW (n)	SPW (n)	PAPW (mean ± SD)	SPW (mean ± SD)	PAPW (mean ± SD)	SPW (mean ± SD)	PAPW (mean ± SD)	SPW (mean ± SD)
Barakat et al. [9]	138	152	31.4 ± 3.2	31.7 ± 4.5	24.0 ± 4.3	23.6 ± 4.0	11.9±3.7	13.7±4.1
Barakat et al. [10]	40	43	32.0 ± 4.0	31.0 ± 3.0			12.5±3.2	13.8±3.1
Barakat et al. [11]	382	383	31.6 ± 4.2	31.8 ± 4.5	23.6 ± 3.8	23.4 ± 4.2	12.1±3.7	12.9±4.5
Barakat et al. [12]	227	202	31.8 ± 4.6	31.3 ± 3.4	23.4 ± 3.7	23.7 ± 3.8	12.3±3.6	13.3±4.1
Barakat et al. [13]	33	32	33.1 ± 3.0	33.8 ± 2.0	24.1 ± 3.9	24.4 ± 6.0	10.9±2.7	11.8±4.8
Perales et al. [14]	90	77	31.1 ± 3.4	31.7 ± 3.9	23.5 ± 3.5	24.3 ± 4.4	11.9±4.2	13.9±10.2
Perales et al. [15]	52	54	31.0 ± 3.7	33.4 ± 4.0	27.9 ± 3.1	28.0 ± 2.6		
Perales et al. [16]	38	25	32.0 ± 3.5	31.8 ± 2.8	23.4 ± 4.2	23.1 ± 3.1	11.4±3.6	15.4±4.4
Perales et al. [17]	120	121	31.0 ± 4.0	31.0 ± 4.0				
Price et al. [18]	31	31	30.5 ± 5.0	27.6 ± 7.3	26.6 ± 3.1	27.8 ± 5.4	12.4±3.9	10.5±4.9

BMI – body mass index; PAPW – physically active pregnant women; SPW – sedentary pregnant women; MWG – maternal weight gain; SD – standard deviation.

Results

Out of total number of female participants from selected individual studies (n = 3,747), 69% of pregnant women were randomized, of whom 88% (n = 2,271) began the analysis and 80% (n = 2,067) had completed the analysis. A total of 92% of physically active and 90% of sedentary pregnant women completed the analysis. Weighted values indicated the similar age of all pregnant women who started the analysis (PAPW 31.6 ± 4.0 years; SPW: 31.6 ± 4.2 years).

Analysis of the extracted data describing the manner of exercise in individual studies highlighted the following common values. PAPW exercised about 3 times a week for 35–60 minutes. The training phases were described in eight studies: the introductory phase or warm-up lasted 5–12 minutes, the main part of the workout or the development phase of endurance and strength lasted 20–30 minutes, while the last phase of exercise or relaxation lasted 5–12 minutes. The main part of the workout included aerobic exercise in the form of various types of aerobics (dance or step). Exercise intensity was monitored by heart rate control with the help of pulsometer (55–60 beats per minute) and a Borg scale for self-assessment (10–14 or "medium difficult"). Table 2 shows the data from individual studies describing characteristics of exercise [13–22].

Out of total of 10 studies, the obtained weighted values showed that all pregnant women had optimal BMI values in the first measurement (PAPW: 23.9 ± 3.8 kg/m^2; SPW: 24.0 ± 4.1 kg/m^2), with no statistically significant difference between PAPW and SPW ($p = 0.650$). However, there was a statistically significant difference between the groups ($p < 0.001$) after the second measurement. Out of total of 10 studies, the weighted values of the extracted data indicated that physically active pregnant women gained slightly less weight during pregnancy (PAPW: 12 ± 3.7 kg/m^2; SPW: 13.2 ± 5.1 kg/m^2).

Selected individual studies did not have a high risk of bias (Figure 2), so they were not excluded from further analysis. Accordingly, a total of 10 individual studies were included in the meta-analysis.

Standardized mean of the incidence of vaginal birth was calculated from 10 individual studies, accounting for 3.3% of the total number of scientific studies reviewed (n = 306). The

Table 2

Description of continuous aerobic physical exercise in individual studies by frequency, duration, intensity of exercise and workout stages

Individual studies	Duration of aerobic exercise		Intensity of aerobic exercise		Duration of training phases in minutes (min–max)			
	number of training sessions (weekly)	training time (minutes)	heart rate (%)	Borg scale (9–20)	warm up	aerobic endurance	power	relaxation
Barakat et al. [13]	3	40–45	< 70		7–8	25		7–8
Barakat et al. [14]	3	35–45	< 70		7	25		7–8
Barakat et al. [15]	3	50–55	< 70	12–14	10–12	25–30		10–12
Barakat et al. [16]	3	55–60	< 70	12–14				
Barakat et al. [17]	3	55–60	55–60	10–12	10–12	20–25		10–12
Perales et al. [18]	3	55–60	55–60		5–8	25	20	5–8
Perales et al. [19]	3	55–60	55–60	10–12	5	20	10	5–10
Perales et al. [20]	3	55–60	55–60		7–8	25–30	10	7–8
Perales et al. [21]	3	55–60	55–60		5–7	25–30		5–10
Price et al. [22]	4	45–60		12–14				5–10

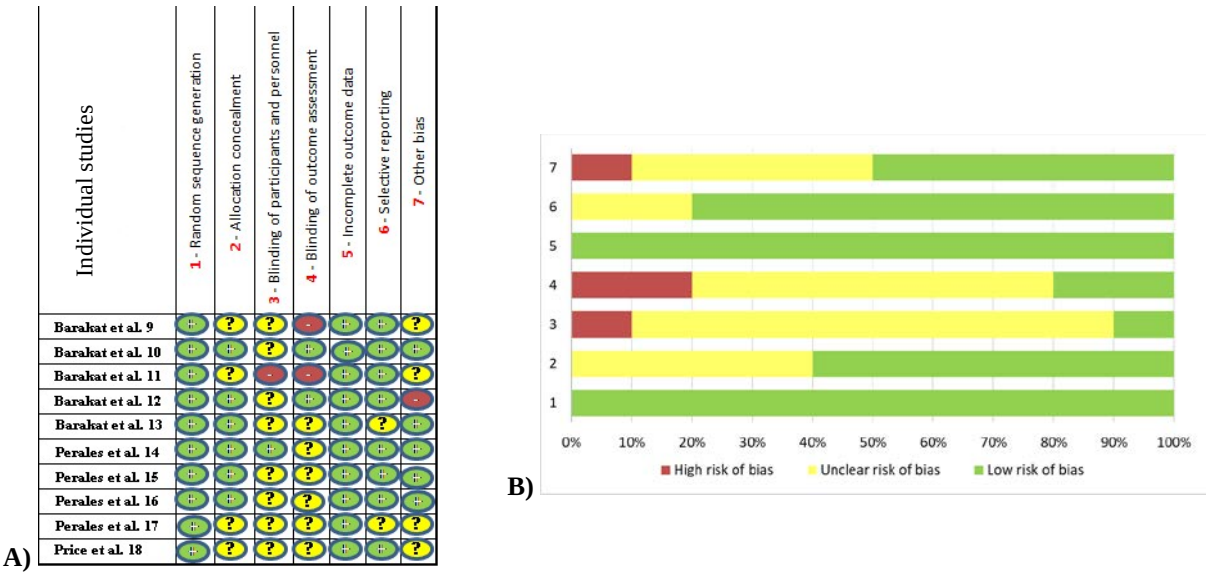

Fig. 2 – Assessment of risk of bias: A) Summary of risk of bias for each trial; B) Risk of bias graph about each risk of bias item presented as percentages across all included studies.

results of the meta-analysis showed that the incidence of vaginal birth was higher in pregnant women who were physically active during the second and third trimesters (PAPW: 67.4%; SPW: 60.5%) (Figure 3).

The combined RR value from the fixed effects model was 1.11 [95% confidence interval (CI) 1.04; 1.18]. Relative risk was statistically significantly different from unit ($p = 0.002$). The combined value of the results of individual studies showed that physically active pregnant women had a higher incidence of vaginal birth than sedentary pregnant women. The data were not statistically significantly heterogeneous ($p = 0.243$; I^2=22%; $\tau^2 = 0.003$), as can be seen in the L'Abbe plot. No publication bias was detected, as can be seen on the funnel graph that is symmetrical (0.69) (Figure 4).

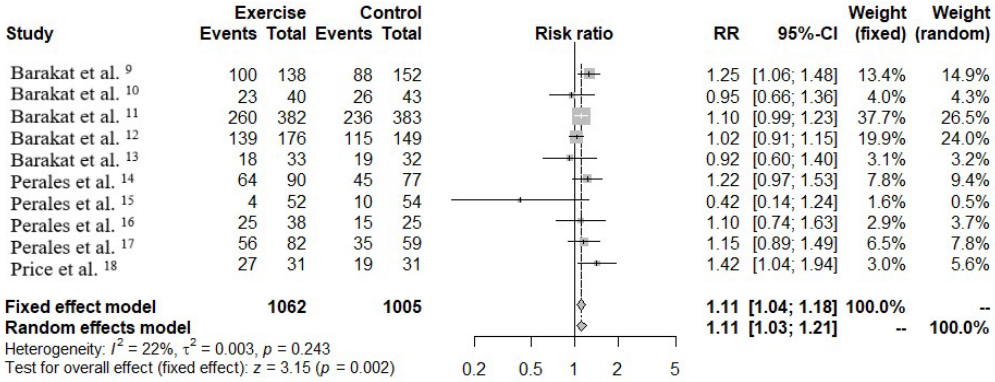

Fig. 3 – Outline of individual results and combined value of relative risk (RR) of vaginal birth efficiency. Data are presented as number in the experimental group (physically active pregnant women) vs. number in the control group (sedentary pregnant women).

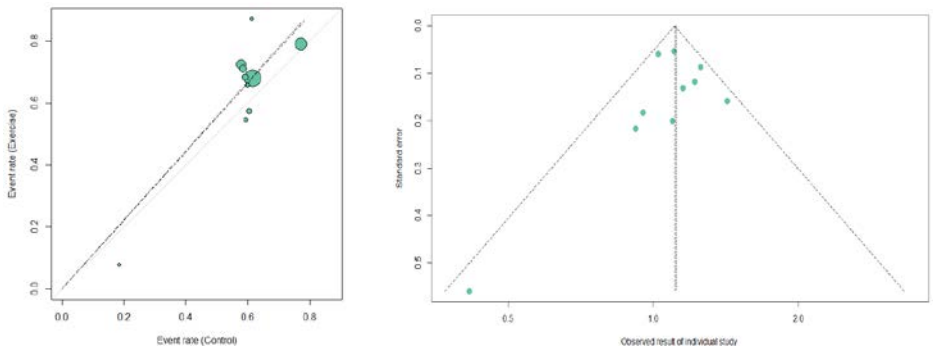

Fig. 4 – Demonstration of heterogeneity (left) and publication bias (right) of relative risk efficiency of vaginal delivery.

Discussion

The positive effects of daily physical activity during pregnancy are being mentioned more often nowadays. Continuous aerobic physical exercise is recommended if the pregnant woman is healthy [23, 24], whereas increased physical activity is not recommended if there is a medical or obstetric problem [4, 6]. Although more and more authors are addressing this problem today, the impact of different exercise intensities remains to be fully investigated [25]. It also raises the question of whether sufficient information is provided to pregnant women about the possible negative and positive effects of proper exercise during pregnancy [26–28]. Physically active pregnant women have been shown to have a better quality of life [2] less likely to suffer from common pregnancy problems [29], and less likely to suffer from certain pregnancy diseases [3].

Obesity is thought to be increasing among pregnant women. Being overweight has an effect on metabolism, and therefore can have an effect on pregnancy outcome [30]. Obese pregnant women are more likely to suffer from gestational diabetes mellitus. Also, obese pregnant women are more likely to give birth prematurely or by cesarean section [3]. Starting BMI values dictate how many kilograms a pregnant woman needs to gain in order to maintain a healthy pregnancy [31]. Therefore, it is assumed that the total body weight a pregnant woman should gain (10–12 kg) largely depends on the initial morphological values. In this meta-analysis, pregnant women who started the analysis had similar BMI values in the first measurement before intervention (PAPW: 23.9 ± 3.8 kg/m^2; SPW: 24.0 ± 4.1 kg/m^2). It is expected that physically active pregnant women will ingest more nutrients since increased physical activity and pregnancy development lead to increased caloric consumption in the body. The data were statistically significant ($p < 0.001$) even though physically active pregnant women gained about 1 kg less than sedentary pregnant women during pregnancy.

It is believed that age can also have an impact on the positive pregnancy outcome in addition to body weight [32]. In this study, all pregnant women were of similar age (PAPW: 31.6 ± 4.0 years; SPW: 31.6 ± 4.2 years). The homogeneity of the study groups was observed, which had a positive effect on the reached conclusions regarding the described continuous aerobic physical exercise during the second and third trimesters of pregnancy.

In meta-analysis where pregnant women underwent a change in diet and increased physical activity, the results of the study showed a positive effect of the intervention on weight regulation, as well as a decrease in the incidence of birth by cesarean section [18]. Some studies have come up with reports that physically active pregnant women had an easier birth than sedentary ones, and that the most common birth was "natural" or vaginal [9, 33]. Water is thought to have a beneficial effect, so swimming and exercising in water can have a positive effect during childbirth [34]. It is often stated that pregnant women who exercised during pregnancy had a more frequent vaginal delivery (PAPW: 73.6%; SPW: 67.5%; RR = 1.09, 95% CI = 1.04–1.15), as well as less frequent cesarean delivery [12]. In this meta-analytic study, the result confirmed the positive impact of daily exercise, that is, stated that physically active pregnant women had a more frequent vaginal delivery than sedentary pregnant women (67.4% vs. 60.5%; RR = 1.11, 95% CI = 1.04–1.18).

Full term birth, especially “natural” or vaginal, can certainly be considered a positive pregnancy outcome. Therefore, it is necessary to continue to monitor and investigate the various possible factors that could affect mother’s health and the birth of a healthy newborn.

Conclusion

The result of this meta-analysis, showed that physically active pregnant women had a higher frequency of vaginal birth than sedentary ones. On the other words, in previously physically inactive or sedentary pregnant women who were physically active during the second and third trimesters (different types of aerobics, 3 times a week for 35–60 minutes), no negative impact of continuous aerobic physical exercise was observed.

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Received on March 11, 2020
Revised on August 5, 2020
Accepted on August 5, 2020
Online First August, 2020

ORIGINAL ARTICLE

UDC: 615.322:615.28
DOI: https://doi.org/10.2298/VSP200508083S

Composition and antimicrobial activity of essential oils of *Salvia fruticosa* and *Salvia ringens* (Lamiaceae)

Sastav i antimikrobna aktivnost etarskih ulja *Salvia fruticosa* i *Salvia ringens* (Lamiaceae)

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Abstract

Background/Aim. Plant essential oils (EOs) can have a significant antibacterial effect especially through additive or synergistic action as antibiotic adjuvants. We investigated the composition and activity of EOs of two species of genus *Salvia (S)* from Greece with the aim to determine their antimicrobial activity as well as the activity in combination with selected antibiotics. **Methods.** The aerial parts of wild-growing *S. fruticosa* and *S. ringens* were subjected to a steam distillation and the obtained EOs were analyzed by gas chromatography and gas chromatography/mass spectrometry. The broth-microdilution method was used in order to determine the minimum inhibitory concentrations (MICs) of EOs on seven strains of bacteria and one yeast. Antimicrobial activity of the combination of EO and antibiotics was investigated by checkerboard method and estimated by calculating fractional inhibitory concentration (FIC) of each component and fractional inhibitory concentration index (FICI). **Results.** Dominant component of *S. fruticosa* EO was *trans*-thujone (54.2%) and for *S. ringens* EO it was α-pinene (28.1%). The MICs of EOs of both species were in the range from 200 μg/mL to > 500 μg/mL. The strongest antimicrobial effect was achieved against *Bacillus subtilis* and *Candida albicans*. According to FICI values, EO of *S. fruticosa* had additive effect with ciprofloxacin against most of bacterial strains but not with amikacin. **Conclusion**. The essential oils of *S. ringens* and *S. fruticosa* showed modest antimicrobial activity. However, the essential oil of *S. fruticosa* showed a promising additive effect in combination with ciprofloxacin.

Key words:
anti-infective agents; lamiaceae; oils, volatile; plants, medicinal; salvia.

Apstrakt

Uvod/Cilj. Etarska ulja različitih biljaka mogu imati značajna antibakterijska svojstva, posebno kao adjuvanti antibiotika sa kojima ostvaruju aditivno ili sinergistično dejstvo. Ispitivali smo sastav i aktivnost etarskih ulja dve vrste roda *Salvia (S)* iz Grčke sa ciljem da odredimo njihovu antimkrobnu aktivnost, kao i dejstvo u kombinaciji sa odabranim antibioticima. **Metode.** Nadzemni delovi samoniklih *S. fruticosa* i *S. ringens* su destilovani vodenom parom i dobijena etarska ulja su analizirana gasnom hromatografijom i gasnom hromatografijom sa masenom spektrometrijom. Radi određivanja minimalnih inhibitornih koncentracija (MICs) etarskog ulja na sedam sojeva bakterija i na jednoj patogenoj gljivici korišćena je mikrodiluciona metoda. Antimikrobna aktivnost kombinacije etarskog ulja i antibiotika ispitana je *checkerboard* metodom i procenjena je na osnovu frakcione inhibitorne koncentracije (FIC) svake komponente i indeksa frakcione inhibitorne koncentracije (FICI). **Resultati.** Dominantna komponenta etarskog ulja *S. fruticosa* je bio *trans*-tujon (54,2%), a etarskog ulja *S. ringens* α-pinen (28,1%). MICs etarskog ulja obe vrste su bile u opsegu od 200 μg/mL do > 500 μg/mL. Najsnažnija antimikrobna aktivnost ostvarena je protiv *Bacillus subtilis* i *Candida albicans*. Na osnovu FICI vrednosti, etarsko ulje *S. fruticosa* je sa ciprofloksacinom, ali ne i sa amikacinom imalo aditivni efekat protiv većine bakterijskih sojeva. **Zaključak.** Etarska ulja *S. ringens* i *S. fruticosa* su pokazala skromnu antimikrobnu aktivnost, ali je etarsko ulje *S. fruticosa* u kombinaciji sa ciprofloksacinom ispoljilo značajan aditivni efekat.

Ključne reči:
antiinfektivni agensi; lamiaceae; ulja, etarska; biljke, lekovite; salvia.

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Introduction

Excessive and inappropriate use of antibiotics in treating various infectious diseases has led to the resistance of many pathogens. This is a global problem in healthcare practice today, which is also recorded in Serbia [1]. In order to find new antimicrobial agents that would help to decrease the use of antibiotics, research into traditionally used medicinal plants and herbal products, as well as related species of known medicinal plants, has been intensified.

Earlier studies have shown that essential oils as mixtures of different plant secondary metabolites can have a significant antimicrobial effect, which is based on a different mechanism of action than the one of antibiotics [2]. One possible approach to the use of essential oils in therapy is their use as an antibiotic adjuvant, with the aim of achieving a multitarget effect on pathogens through additive or synergistic actions [3].

Many studies on the anti-multidrug-resistant bacteria activity of aromatic members of Lamiaceae family have been performed, primarily on the essential oils of lavender, mint and thyme [2]. Recently, it has been proved that the essential oil isolated from young leaves of Dalmatian sage (*S. officinalis* L.) also has an antimicrobial effect on certain human pathogens, and that the use of the essential oil potentiated the inhibitory effect of antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA) [4].

Within genus *Salvia*, comprised of about 1,000 worldwide distributed species, beside *S. officinalis*, only several species with medicinal properties are aromatic plants with a significant amount of essential oils [5]. The essential oil of *S. tomentosa*, with β-pinene, α-pinene and camphor as the main components, showed antimicrobial activity against a panel of microorganisms [6]. A study of composition and antimicrobial effects of essential oil and extracts of *S. ringens* from North Macedonia, rich in 1,8-cineole, camphene, and borneol has shown that Gram-positive strains were more sensitive to the essential oil [7]. The essential oil of *S. amplexicaulis* characterized by a high amount of sesquiterpenes, with germacrene D, viridiflorol, caryophyllene oxide and β-caryophyllene being the main components, showed inhibitory properties against Gram-positive bacteria and a yeast *Candida (C) albicans* [8]. The Greek sage, *S. fruticosa*, as the most widespread sage species in the Mediterranean, has been used for its healing properties since ancient times [5]. The oil of this species with high contents of 1,8-cineole, α- and β-thujone, and camphor, was tested for antimicrobial activity against eight common bacterial strains [9], as well as against human pathogenic yeast [10]. The combined effect of tetracycline and essential oils of *S. officinalis*, *S. sclarea* and *S. fruticosa* against clinical isolates of methicillin-resistant *Staphylococcus epidermidis* showed synergistic or additive effects [11, 12]. However, there are no consistent results of the antimicrobial activity of the combination of the essential oil of *S. fruticosa* and antibiotics with a different mechanism of action and different standard bacterial strains.

Knowing that the composition of essential oil of *Salvia* species differs throughout different developmental stages [13] and that it is highly influenced by climatic conditions, we investigated the composition and activity of the essential oil of two species of genus *Salvia* from Greece (*S. fruticosa* and *S. ringens*), collected during the late flowering stage with the aim to evaluate their antimicrobial activity as well as the activity of essential oil of *S. fruticosa* in combination with antibiotics [amikacin (AMI) and ciprofloxacin (CIP)].

S. fruticosa Miller (Syn.: *S. triloba* L.) is a shrub up to 120 cm high, widespread in Central and Eastern Mediterranean region. Its leaves are valued and utilized in the region similar to *S. officinalis* L. The plant is recognized for its white tomentose stem with simple or pinnate leaves with a pair of small lobes at the base and a large oblong elliptical rugose terminal segment. Verticillasters contain 2 to 6 flowers, with pink or lilac two-lipped flowers [14, 15].

S. ringens Sibth. & Sm. is a perennial herb, woody at the base, up to 60 cm high, endemic to Southern and Eastern parts of the Balkan Peninsula. This melliferous drought-tolerant plant grows in scrub habitat and open coniferous woodland. It is recognized on rosette of pinnate or lobed rugose leaves with 1–3 pairs of small lobes at the base. Flowering stems are with verticillasters consisting of attractive violet-blue two-lipped flowers [14, 15].

Methods

The plant material was sampled in Greece in June 2017. The aerial parts of wild-growing *Salvia fruticosa* Miller were collected on Mount Dhirfis on Evia island, and the aerial parts of *S. ringens* Sibth. & Sm. were collected on Mount Kyllini near Trikala, both during the late flowering stage. Voucher specimens are kept in the Herbarium of Belgrade University, Serbia. Sample details are given in Table 1.

For the isolation and analysis of the essential oil, air-dried plant material was subjected to a 2-hour steam distillation in a Clevenger-type apparatus according to Ph. Eur. 8.0 [16].

A qualitative analysis of the essential oils was performed using analytical gas chromatography (GC/FID) and gas chromatography/mass spectrometry (GC/MS). GC/FID and GC/MS analyses were carried out using an Agilent 6890N GC system equipped with FID and an Agilent 5975 MSD. The capillary column used was a HP-5 MS (30 m ×

Table 1

Plant material sample details

Species	Latitude	Longitude	Altitude	Voucher
S. fruticosa	N 38.632981°	E 23.786606°	520 m	BEOU 46566
S. ringens	N 37.984287°	E 22.458935°	1,250 m	BEOU 46586

BEOU – Herbarium of University of Belgrade.

0.25 mm i.d., film thickness 0.25 μm). The thermal program was 60ºC to 280ºC at a 3ºC/min rate. Injector temperature: 200ºC. FID temperature: 300ºC. Transfer-line temperature: 250ºC. Carrier gas, He (1.0 mL/min); injection volume, 1 μL; split ratio, 10:1. EI Mass spectra (70 eV) were acquired over the *m/z* range of 35–550. Identification of the compounds was based on the comparison of their retention times (RT), retention indices (RI), and mass spectra with those obtained from authentic samples and/or the NIST, Wiley libraries and literature [17]. The linear retention indices (RI) were determined in relation to a homologous series of *n*-alkanes (C_9–C_{24}) under the same operating conditions. Relative area percentages obtained by FID were used for quantification.

The antimicrobial activity of the essential oils was determined by broth-microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. This method was used in order to determine minimum inhibitory concentrations (MICs) of the essential oil that inhibit the growth of microorganisms. For the experiment, we used seven standard strains of bacteria (Gram-positive: *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633 and Gram-negative: *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* NCIMB-9111, *Salmonella* Abony NCTC 6017, *Pseudomonas aeruginosa* ATCC 9027 as well as *Acinetobacter baumannii* ATCC 19606) and one standard strain of yeast – *Candida albicans* ATCC 10231.

Each strain was inoculated into Müller-Hinton broth (MHB) for 24h incubation at 35°C prior to the experiment. The cultures were then diluted with MHB to the final concentration in each plate well, adjusted to 2×10^6 CFU/mL. For *C. albicans* yeast, Sabouraud dextrose broth was used. The essential oils were dissolved in 1% dimethylsulfoxide for the stock and diluted to the desired concentrations with MHB. The essential oils were tested in the concentration range 31.25–500 μg/mL. Two standard antibiotics were used for comparison: an aminoglycoside antibiotic amikacin and a fluoroquinolone antibiotic ciprofloxacin and the range of antibiotic concentrations was 0.01–8.0 μg/mL.

After the incubation for 24h at 35°C in aerobic conditions, MICs were determined in the following way: lack of broth turbidity in a well of a microtiter plate indicates an inhibition of growth or death of an inoculated microorganism. The well with the lack of turbidity at the minimum concentration of an essential oil represents MIC. Each broth-microdilution test was repeated three times and the mean values are presented.

Antimicrobial activity of the combination of essential oil and antibiotics was investigated by checkerboard method according to Langeveld et al. [3]. The determination was performed with the aerial parts' essential oil of *S. fruticosa* because there were small quantities of essential oil in aerial parts of *S. ringens*. This method is based on the application of decreasing concentrations of essential oil horizontally and decreasing concentrations of antibiotics vertically to the 96-well microtiter plate (AMI or CIP). Beside the microorganism strains used in broth-microdilution method, standard strain of *Acinetobacter baumannii* ATCC 19606 was added to the experiment.

The essential oil and antibiotic mixture was prepared by adding 50 μL of each to the same well of microtiter plate, and inoculated with 100 μL of previously prepared bacterial suspensions (10^6 CFU/mL). The last two vertical rows of microtiter plate were positive control wells of bacterial growth suspensions. After the incubation for 24h at 35°C in aerobic conditions, MIC of the combination was determined. The interaction between the essential oil and antibiotic was estimated by calculating fractional inhibitory concentration (FIC) of each component and fractional inhibitory concentration index (FICI).

The FIC of each compound was calculated by dividing the concentration of the compound in effective MIC of the combination with the MIC of the drug alone (e.g. $FIC_{essential\ oil} = MIC_{essential\ oil\text{-}antibiotic\ combination}/MIC_{essential\ oil}$). FICI values were calculated as the sum of $FIC_{essential\ oil}$ and $FIC_{antibiotic}$. They were interpreted as following: $FICI \leq 0.5$ synergy; $0.5 < FICI \leq 1$ additivity; $1 < FICI \leq 2$ indifference (no effect) and $FICI \geq 2$ antagonism [19, 20].

Results

The aerial parts of *S. fruticosa* were characterized by the high content of essential oil (0.9%) with dominant oxygenated monoterpenes (56.6%) followed by sesquiterpene hydrocarbones (25.3%). The main constituents were *trans*-thujone (54.2%), γ-cadinene (9.2%), (*E*)-caryophyllene (5.1%), β-pinene (4.7%) and myrcene (4.3%). *S. ringens* arial parts yielded 0.4% essential oil characterized by monoterpene hydrocarbons (57.4%), and oxygenated monoterpenes (33.5%) with α-pinene (28.1%), 1,8-cineole (13%) and β-pinene (12.2%) as its main constituents. The detailed chemical composition of the analyzed essential oils is shown in Table 2.

The MICs of *S. fruticosa* and *S. ringens* essential oils were in the range from 200 μg/mL to > 500 μg/mL (Table 3). Antimicrobial activity of the investigated oils was modest against most of the selected bacteria when compared to antibiotics. The greatest antibacterial activity was noted against *B. subtilis*, with MICs of 200 and 250 μg/mL of *S. ringens* oil and *S. fruticosa* oil respectively. Also, *C. albicans* growth was inhibited by 300 μg/mL and 200 μg/mL of essential oils of the listed plant species.

Due to the small quantities of *S. ringens* aerial parts essential oil, further investigation of combined oil/antibiotics antimicrobial activity was performed with more abundant essential oil of *S. fruticosa*.

Antimicrobial activity of the combination of the essential oil and antibiotics is presented in Table 4. According to FICI values, there was a significant difference in contribution of *S. fruticosa* essential oil to antimicrobial activity of antibiotics with different mechanisms of action (AMI and CIP). With CIP, the additive effect was observed for all bacterial strains except for standard strain of *Acinetobacter baumannii*, against which the combination was found to be indifferent. The combination of essential oil and AMI yield

Table 2

The content (in percentages) and chemical composition of essential oils of studied *Salvia* species

Number	RI	Constituent	*S. fruticosa*	*S. ringens*
			0.9 (oil content, % v/w)	0.4 (oil content, % v/w)
1	851	3-(*Z*)-Hexenol	0.2	–
2	926	α-Thujene	0.4	0.4
3	934	α-Pinene	0.8	28.1
4	949	Camphene	0.4	6.9
5	973	Sabinene	0.7	t
6	978	β-Pinene	4.7	12.2
7	991	Myrcene	4.3	1.0
8	1024	ρ-Cymene	t	1.1
9	1029	Limonene	0.6	3.7
10	1029	β-Phellandrene	0.5	3.7
11	1032	1,8-Cineole	0.2	13.0
12	1036	(*Z*)-β-Ocimene	0.3	–
13	1059	γ-Terpinene	0.2	t
14	1100	Linalool	t	0.9
15	1109	*cis*-Thujone	0.7	t
16	1118	*trans*-Thujone	54.2	0.7
17	1123	*cis*-*p*-Menth-2-en-1-ol	–	2.7
18	1140	*trans*-*p*-Menth-2-en-1-ol	t	2.0
19	1147	Camphor	0.3	3.7
20	1168	Borneol	0.6	6.6
21	1179	Terpinen-4-ol	0.3	1.0
22	1209	*trans*-Piperitol	–	1.0
23	1287	Bornyl acetate	0.3	1.8
24	1352	α-Cubebene	3.2	t
25	1378	α-Copaene	1.3	t
26	1391	β-Cubebene	0.4	–
27	1423	(*E*)-Caryophyllene	5.1	1.6
28	1456	α-Humulene	1.1	2.9
29	1479	γ-Muurolene	0.3	–
30	1482	α-Amorphene	0.5	–
31	1495	*trans*-Muurola-4(14)-5-diene	0.2	–
32	1496	γ-Amorphene	2.2	–
33	1521	γ-Cadinene	9.2	t
34	1526	δ-Cadinene	1.8	t
35	1544	γ-Cuprenene	-	1.2
36	1587	Caryophyllene oxide	1.7	0.8
37	1591	$C_{15}H_{26}O$	0.3	–
38	1612	Humulene epoxide II	0.3	1.8
39	1624	1,10,-di-*epi*-Cubenol	1.2	–
40	1631	1-*epi*-Cubenol	0.8	–
41	1645	Cubenol	0.4	–
42	1672	*epi*-β-Bisabolol	–	0.1
43	1815	(*Z*)-α-*trans*-Beragmotol acetate	–	0.7
Monoterpene hydrocarbones			13.2	57.4
Oxygenated monoterpenes			56.6	33.5
Sesquiterpene hydrocarbones			25.3	5.7
Oxygenated sesquiterpenes			4.4	3.4
Other			0.2	0
Total identified			99.7	100

RI – linear retention indices determined in relation to a homologous series of *n*-alkanes (C_9-C_{24}); t – trace.

Table 3

Minimum inhibitory concentrations (MICs) of essential oils and antibiotics

Microorganisms	MIC (μg/mL)			
	S. fruticosa	*S. ringens*	Amikacin	Ciprofloxacin
Staphylococcus aureus ATCC 6538	> 500	> 500	2	0.12
Bacillus subtilis ATCC 6633	250	200	0.5	0.06
Escherichia coli ATCC 8739	> 500	> 500	2	0.06
Klebsiella pneumoniae NCIMB 9111	> 500	> 500	0.5	0.06
Salmonella enterica subsp. *enterica* serovar S. abony NCTC 6017	> 500	> 500	2	0.06
Pseudomonas aeruginosa ATCC 9027	> 500	> 500	1	0.25
Acinetobacter baumannii ATCC 19606	> 500	> 500	8	0.25
Candida albicans ATCC 10231	200	300	–	–

Table 4

The activity of *Salvia fruticosa* essential oil in combination with antibiotics

Bacterial strain	MIC (FIC)		FICI	Effect	MIC (FIC)		FICI	Effect
	Amikacin	*S. fruticosa*			Ciprofloxacin	*S. fruticosa*		
Staphylococcus aureus ATCC 6538	2 (1)	62.5 (0.0625)	1.0625	IN	0.125 (0.25)	500 (0.5)	0.75	AD
Bacillus subtilis ATCC 6633	0.125 (0.25)	250 (1)	1.25	IN	0.031 (0.5)	125 (0.5)	1	AD
Escherichia coli ATCC 8739	2 (1)	125 (0.125)	1.125	IN	0.031 (0.5)	125 (0.125)	0.625	AD
Klebsiella pneumoniae NCIMB 9111	1 (2)	250 (0.25)	2.25	ANT	0.031 (0.5)	62.5 (0.0625)	0.5625	AD
Salmonella enterica subsp. *enterica* serovar *S. abony* NCTC 6017	2 (1)	62.5 (0.0625)	1.0625	IN	0.031 (0.5)	125 (0.125)	0.625	AD
Pseudomonas aeruginosa ATCC 9027	1 (1)	125 (0.125)	1.125	IN	0.125 (0.5)	62.5 (0.0625)	0.5625	AD
Acinetobacter baumannii ATCC 19606	8 (1)	62.5 (0.0625)	1.0625	IN	0.25 (1)	62.5 (0.0625)	1.0625	IN

MIC – minimal inhibitory concentration; FIC – fractional inhibitory concentration; FICI – fractional inhibitory concentration index; FICI ≤ 0.5 synergy; 0.5 < FICI ≤ 1 additivity; 1 < FICI ≤ 2 indifference (no effect); FICI ≥ 2 antagonism IN – indifference; ANT – antagonism; AD – additivity.

ed no significant results, as it was indifferent against all strains except *Klebsiella pneumoniae* where the antagonistic effect was manifested.

Discussion

Previous research has shown a highly variable composition of essential oil of *S. fruticosa*, even when it comes to samples from similar habitats. In an early study [9] on composition and antimicrobial activity of *S. fruticosa* oil, the samples had 1,8-cineole (47.48%), thujone (11.93%) and camphor (9.04%) as the main components, while our sample had *trans*-thujone (54.2%) as a dominant compound and a low amount of 1,8-cineole (0.2%). In the same study, investigation of antimicrobial activity of the essential oil and its main compounds by disk diffusion assay showed relatively low levels of antimicrobial activity against the bacteria tested [9]. Taking into account the differences between antimicrobial assessment methods, similar results were presented by Khoury et al. [21] for *S. fruticosa* essential oil from Lebanon. The research was conducted using broth-microdilution method and obtained MICs were greater than 500 μg/mL for *S. aureus*, *E. coli* and *C. albicans*, which is consistent with our findings.

The overall composition of *S. ringens* essential oil is also variable, according to the results given by Alimpić et al. [7] when compared with our results. While the main constituents of *S. ringens* oil from Greece in our study were α-pinene (28.1%), β-pinene (12.2%) and 1,8-cineole (13%), the oil obtained from areal parts of the plant from North Macedonia was rich in 1.8-cineole (32.0%), camphene (17.1%) and borneol (11.9%) [7]. In the same study, moder-

ate antibacterial activity of the essential oil was shown against Gram-positive bacteria, especially *S. aureus* and it was attributed to the high content of 1,8-cineole. Considering the lower content of this monoterpene in our sample, the results of its antibacterial activity in our study were expected.

Given that the combined antibacterial activity of *S. fruticosa* oil with AMI or CIP was not tested before, we used these antibiotics in our study [3]. On the other hand, Chovanová at al. [12] have recently showed that among *Salvia* species tested, the essential oil of *S. fruticosa* was the best in reducing the MIC of tetracycline due to decreasing antibiotic efflux and decreasing the expression of *tet*(K) gene in tetracycline resistant clinical isolates of *Staphylococcus epidermidis* [12]. Although tetracycline and AMI belong to different classes of antibiotics, we expected some similar synergistic effect because their mode of action is based on the inhibition of protein synthesis by targeting 30S ribosomes, but the anticipated results did not occur even though the antagonistic effect was observed with *K. pneumoniae*. However, according to FICI, the additive effect of *S. fruticosa* essential oil with CIP was evident against almost all tested bacterial strains, thus suggesting that the essential oil of the examined species could be used as a potential adjuvant to fluoroquinolone class of antibiotics. Considering that these investigations have not been performed so far, our results provide a good basis for future testing on different bacterial strains.

Conclusion

The essential oils of Greek samples of *S. ringens* characterized by high concentration of α-pinene and *S. fruticosa* characterized by high concentration of *trans*-thujone, showed modest antimicrobial activity according to MIC values obtained by broth-microdilution method on standard bacterial strains and a moderate antifungal activity on *C. albicans*. In combination with antibiotics, according to FICI values, the contribution of *S. fruticosa* essential oil to antimicrobial activity of amikacin was not evident, but with ciprofloxacin, a promising additive effect was achieved.

Acknowledgment

The results of this study are a part of the national project OI 173021 supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia. The authors are grateful to Miloš Zbiljić from the University of Belgrade, Faculty of Pharmacy, Department of Botany, for the collection of plant material.

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Received on May 8, 2020
Accepted on August 19, 2020
Online First August, 2020

ORIGINAL ARTICLE

UDC: 355/359:61]:617.7
DOI: https://doi.org/10.2298/VSP200607067R

Pupillary response in air force and air defence pilots when exposed to +Gz acceleration

Reakcija zenice oka pilota ratnog vazduhoplovstva i protivvazduhoplovne odbrane tokom izlaganja +Gz ubrzanju

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Abstract

Background/Aim. In aviation, visual functions are important for the simultaneous monitoring the instrument panel and signs in the environment. From the very beginning of the development of aviation, visual function has been deemed particularly important. The effect of +Gz acceleration on the organ of vision is very significant for investigations in aviation medicine. Visual functions are the most important of all sensory functions where flight safety and quality of flight performance are concerned. High acceleration onset rates may cause changes in pupil diameter of a pilot with significant changes in visual function. However, it is important to maintain visual functions due to rapid pilot's orientation in the space. The aim of this study was to establish whether there was any pupillary response in Air Force and Air Defence pilots or changes in pupil diameter when exposed to +Gz acceleration in the human centrifuge. **Methods.** The study was conducted on 65 Air Force and Air Defence pilots aged from 28 to 45 years of age. The pilots were exposed to an acceleration of +5.5Gz to +7Gz. We examined the obtained differences in pupil diameter according to a rate of acceleration in the period of three consecutive days. **Results.** Changes in pupil diameter during the pilot's exposure to different high values of acceleration in the course of three days, measured before, during and after the exposure, generated statistically significant results. No statistically significant differences in pupil diameter were noticed when the pilots were exposed to the same values of acceleration before the testing on the first, second or third day. During the test, pupil diameter was statistically significantly larger than before the test. **Conclusion.** Transient changes in pupil diameter occurred in pilots exposed to a +7Gz acceleration. Pilots were able to withstand exposure to a +5.5Gz acceleration, without any major changes in the pupil diameter. Physiological training of pilots in the human centrifuge mimicking conditions of real G acceleration, improves tolerance to acceleration, which is important for flight safety.

Key words:
acceleration; aerospace medicine; centrifugation; pilots; reflex, pupillary; vision, ocular; vision tests.

Apstrakt

Uvod/Cilj. U avijaciji su vidne funkcije značajne zbog praćenja signala na instrument tablama, kao i znakova okoline. Od samog početka razvoja vazduhoplovstva, funkciji vida se pridaje izuzetan značaj. Uticaj +Gz ubrzanja na organ vida je veoma važan za istraživanja u vazduhoplovnoj medicini. Vidne funkcije su najvažnije od svih čulnih funkcija kako u pogledu bezbednosti letenja, tako i za kvalitet izvršavanja letačkih zadataka. Visok početni stepen ubrzanja može izazvati promene u dijametru zenice oka pilota i izazvati značajne promene u funkciji vida. Međutim, važno je održati vidne funkcije zbog brze orijentacije pilota u prostoru. Cilj istraživanja je bio da se utvrdi da li postoji reakcija zenice oka kod pilota ratnog vazduhoplovstva (RV) i protivvazduhoplovne odbrane (PVO), kao i promene u njenom dijametru tokom izlaganja +Gz ubrzanju u humanoj centrifugi. **Metode.** Ispitivanje je izvršeno kod 65 pilota RV i PVO starosti od 28 do 45 godina. Piloti su izlagani ubrzanju od +5,5Gz do +7Gz. Posmatrali smo dobijene razlike u širini zenice u zavisnosti od stepena ubrzanja, u periodu od tri uzastopna dana. **Rezultati.** Promene dijametra zenice tokom izlaganja različitim visokim vrednostima ubrzanja tokom tri dana testiranja, merene pre, tokom i nakon izlaganja različitim vrednostima ubrzanja, bile su statistički značajno različite. Nije uočena statistički značajna razlika u vrednostima dijametra zenica izme

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renih pre testa prvog, drugog i trećeg dana kada su piloti izlagani istim vrednostima ubrzanja. U toku testa, dijametar zenica bio je statistički značajno veći nego pre testa. **Zaključak.** Došlo je do prolaznih promena u dijametru zenice pilota podvrgnutih +7Gz ubrzanju. Izlaganje vrednostima od +5,5Gz ubrzanja piloti mogu da podnesu bez većih promena u dijametru zenice. Fiziološka trenaža pilota u humanoj centrifugi, gde su prisutni uslovi realnog G ubrzanja, poboljšava njihovu toleranciju na ubrzanje što je važno za bezbednost letenja.

Ključne reči:
ubrzanje; medicina, vazduhoplovna; centrifugovanje; piloti; refleks, pupilarni; vid; vid, testovi.

Introduction

The development of modern aviation has placed loads on the human body that are ten and more times greater than the force of gravity. Such loads cause changes that are brought about by forces of inertia occurring due to changes in acceleration. In aviation, the applied acceleration is commonly called G load, and it represents the ratio of acceleration to the gravitational force. From the aspect of aviation physiology, the important forces are inertial forces that act on the human body and to which a pilot is exposed during acceleration. In +Gz acceleration, inertial forces act on the pilot's body along its longitudinal axis, causing the pilot to be pressed back into the seat. In any flight maneuver, forces act on the body along its longitudinal axis (z) and they are referred to as +Gz loads. The sign + shows that the force acts in the direction of the head, and the resultant force acts from the head towards the feet, while G is used for comparing the magnitude of the acting force with the gravitational force of 1G. The force of +9 Gz acts in the direction of the head and it is nine times greater than the gravitational force. The force acting on the pilot is the inertial (centrifugal) force, acting from the head towards the feet, which originates as a result of the force keeping the plane on a curved path (centripetal force). Because of its considerable practical importance in air combat, this load is very important for research [1].

From the very beginning of the development of aviation, visual function has been attached great importance. Of all sensory functions that a man is endowed with, vision is the most important one for both flight safety and quality of performance of flight tasks. Owing to its great practical importance in air combat, the effect of +Gz acceleration on the organ of vision is very significant for research. Information we receive by means of our sense of sight is the most important for maintaining orientation on the ground and in the air during flight. In conditions of poor visibility, spatial orientation may be compromised [2].

Maneuverability of modern aircraft enable considerable acceleration which affects the organ of sight. If acceleration exceeds +3.5 Gz and its duration is longer than 6–12 sec, visual functions become impaired. Spatial disorientation and loss of consciousness in flight occurring as a result of +Gz acceleration, represents the greatest danger to flight safety and ranks as number one cause of accidents [3]. That is the reason why visual acuity of Air Force and Air Defence pilots of the Serbian Armed Forces was tested in the human centrifuge before and after exposure to +Gz acceleration, with aim being to increase their individual capacity to tolerate +Gz acceleration. Namely, the main sensory functions, primarily the organ of vision, may be affected when exposed to high +Gz acceleration.

Over a short period time, new generations of aircraft that are capable of extremely high increase in acceleration and extraordinary maneuverability have already posed a challenge to aviation physiology, which deals with a selected healthy population and investigates the influence of ecophysiological factors of flight (altitude, dynamic flight factors, noise, vibrations, sudden changes in temperature, and the like), to determine the ultimate physiological limits of endurance for every pilot, for each type of flight stress, thus selecting pilots with the best physiological defense mechanism against flight stress [4]. The pilot profession, as a highly demanding and responsible occupation, requires that the best possible pilots be selected.

The pupil is a circular opening in the centre of the iris. Through this opening, light comes into the inside of the eye and transforms into visual energy in the retina. The amount of light going into the eye is regulated by the pupil itself expanding or constricting. The pupillary light reflex is controlled by the parasympathetic system when constricting and the sympathetic system when expanding. Changes in pupil diameter are conditioned by two muscles, the one that helps the pupil constrict [*musculus* (*m) sphincter pupillae*] and the other that expands it (*m. dilator pupillae*). *M. sphincter pupillae* is a round muscle in the pupillary portion of the iris that is innervated by *nervus oculomotorius*, and apart from the parasympathetic innervation, it is also innervated by the sympathetic system. In response to the sympathetic stimulus, it relaxes immediately. *M. dilator pupillae* is, for the most part, located in the ciliary portion of the iris and is innervated by the sympathetic system. Peripheral parts of the sympathetic and parasympathetic segments of the pupillomotor pathway are controlled by the top layers of the cerebral cortex and hypothalamus. Pupil size differs from one person to another. In any person, pupil diameter depends on different factors. According to diameter, there is the dilated pupil (mydriatic) whose diameter is 4 mm and more, mid-dilated, and constricted (myotic) pupil, up to 2 mm in diameter. The average pupil diameter is 3 to 5 mm. Even in complete absence of external and internal influences on the organism, the pupil is never absolutely immovable as it changes its diameter by 0.5 mm while constricting, the number of constrictions being 30 to 120 per min [5]. There are two normal pupillary responses: a constriction response which is quick and intensive, and a dilation response, which is milder in intensity and slower than the former. The dilation response includes: the pupillary

light reflex, the trigeminal pupillary reflex, the accommodation-convergence reflex, eyelid closure reflex and the Galvanic skin response – Bumke. The dilation response also consists of the following reflexes: pupil dilation response to sensory stimuli (pain, touch, physical exertion), acoustic and otogene response, vestibular ocular reflex, pupil dilation response caused by mental stress, and pupil response to horizontal eye movement. The dilation response may occur as a result of two factors: reducing the tone of *m. sphincter pupillae* when dilation is a passive phenomenon, or active contraction of *m. dilator pupillae*, where it is an active phenomenon. This means that the dilation response of the pupil is basically either an inhibition of *nuclei nervi oculomotorius* or a stimulus by the sympathetic system travelling from the cervical spine to the eye [6]. Besides contrast and the sharpness of the edge of the observed object, identification of details is also affected by the adaptation of the eye to different levels of brightness in our field of vision. Furthermore, visual acuity is also affected by the size of the pupil opening [7]. Higher intensity of light reduces pupil diameter and increases visual acuity, thus reducing the refractive error of the eye, if it exists [8]. The size of the pupil opening is much more affected by the portion of the retina responsible for central visual acuity than the peripheral retina [9]. The connection between intensity of light and the size of the pupil opening is important for visual acuity only when the intensity of light is low [10–12].

The aim of this study was to establish whether there was any pupillary response in Air Force and Air Defence pilots or changes in pupil diameter when exposed to +Gz acceleration in the human centrifuge.

Methods

The investigation was carried out on a defined population of 65 respondents (pilots) from 28 to 45 years of age. All the respondents were males. We examined the prevalence of exposure to positive acceleration of +5.5Gz to +7Gz and investigated the effect of acceleration on pupil diameter and its response before, in the course of and after the exposure to acceleration. The investigation was performed in the gravity-altitude laboratory (human centrifuge). All of the respondents were highly selective, with no history of ocular or system diseases. The pilots had to have good distance and near visual acuity, good stereo and color vision, normal pupil diameter, absence of any changes in the fundus, absence of ocular diseases such as glaucoma, cataract, or uveitis, and no hypertension or any heart rhythm disorder. Pupil diameter was not measured in the pilots with poor tolerance to +Gz acceleration, those who had had interruption in the continuity of consciousness when exposed to acceleration, or in those who had exhibited two consecutive delayed responses to photostimulation of peripheral vision. The pilots were tested over the period of three consecutive days, when they were exposed to different values of acceleration. The size, diameter, shape and evenness of the pupil before, in the course of and after exposure to +Gz acceleration were measured by the pupilometer, a ruler for measuring pupil diameter, and the values were expressed in millimeters. The measuring was performed upon entering the human centrifuge and on exiting it, as well as in the course of exposure to +Gz acceleration on the screen, as there was a camera inside the centrifuge cabin enabling the monitoring of the pilots' responses. The testing took place over the course of three days. On the first day, the test of linear increase in acceleration was applied, the linear increase of acceleration being +5.5Gz without the anti-G suit, with acceleration gain of 0.1 G/s. On the second day the test of tolerance to acceleration was performed, with acceleration increasing linearly to +7Gz, the acceleration gain being 0.1 G/s, without the anti-G suit, with the decelleration of also 0.1 G/s. On the third day, the test of intermittent increase in acceleration was performed in the same way as on the second day, except that now the pilots had a 2-min recess after being exposed to a +5.5 Gz acceleration before the acceleration was upped to +7 Gz for 10 s, when the pilots were requested to use a stick and respond to light signals, simultaneously activating the anti-G suit. The investigation was performed in groups of 5 to 7 respondents each day.

Results

No statistically significant differences were noticed between pupil diameter values measured prior to testing on the first, second or third day (p = 0.559) (Table 1). Considering the measuring periods, neither were any statistically significant differences noticed in the pupil diameter values measured in the course of the test (p = 0.262) or after the test (p = 0.412). Measurements taken on

Table 1

Changes in pupil diameter before, in course and after exposure the pilots to acceleration of +5.5Gz to +7Gz in human centrifuge

Time of testing	mean ± SD (median; minimum–maximum), mm			p (Fridman's test)
	first day	second day	third day	
Before the test	4.02 ± 0.65 (4.0; 3.0–5.0)	4.01 ± 0.51 (4.0; 3.0–4.8)	3.97 ± 0.57 (4.0; 3.0–5.0)	0.559
During the test	4.46 ± 0.64 (4.5; 3.5–5.5)	4.49 ± 0.48 (4.5; 3.5–5.2)	4.37 ± 0.57 (4.2; 3.5–5.5)	0.262
After the test	4.14 ± 0.61 (4.0; 3.0–5.5)	4.21 ± 0.45 (4.0; 3.5–5.0)	4.12 ± 0.54 (4.0; 3.5–5.5)	0.412
p (Fridman's test)	0.000	0.000	0.000	

SD – standard deviation.

all three days produced a statistically significant difference in pupil diameter before the test (p = 0.000), in the course of the test (p = 0.000) and after the test (p = 0.000) (Table 1). Intergroup comparisons showed a statistically significant difference in pupil diameter in all the measuring periods: before the test, in the course of the test and after the test (Table 2). In the course of the test, pupil diameter was statistically considerably larger than before the test. Even though the diameter of the pupil decreased after the test, the pupils were still statistically larger than before the test (Figure 1).

Table 2

Intergroup comparisons of pupil diameter before, during and after the acceleration test in human centrifuge, over a period of three days of monitoring

Time of testing	Before the test (p)*	During the test (p)*
First day		
during the test	0.000	
after the test	0.002	0.002
Second day		
during the test	0.000	
after the test	0.000	0.000
Third day		
during the test	0.000	
after the test	0.000	0.000

***Wilcoxon's – test.**

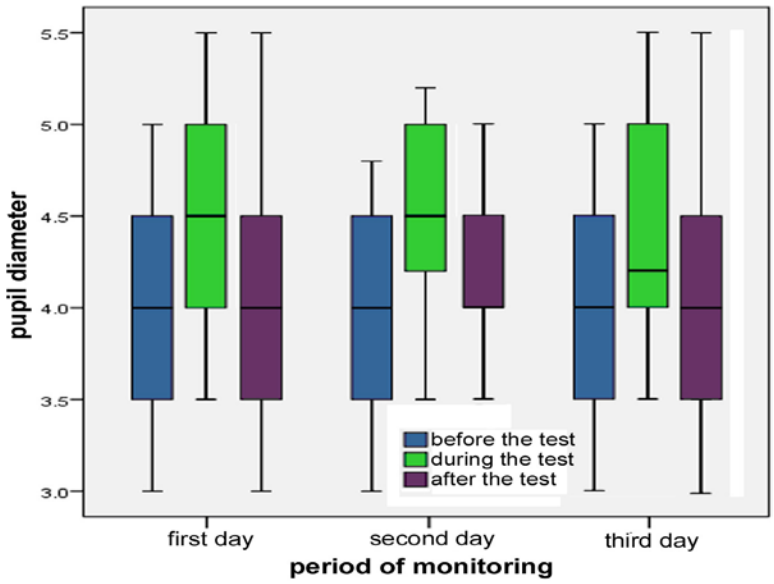

Fig. 1 – Pupil diameter (mm) before, during and after the acceleration test in human centrifuge, measured over a period of three days.

Discussion

The pilot profession is a job involving optimum mental and physical fitness, full personal integrity and excellent health. In aviation medicine, the journey from theory to practice is somewhat longer since any research calls for complex tehnical innovations and high level of security for their verification [13]. Aviation medicine includes a wide range of tasks, the end goal of which is continuous creation of measures and procedures which would best equip the pilot for flight and performance of duties he/her has been entrusted with, at the same time reducing risks to life, health and material resources to a minimum. In the field of physiology of acceleration, notable variations in tolerance to G acceleration have been noticed. The real response of a human organism to acceleration differs from the response that can be anticipated in theory. Within 20 sec from the onset of acceleration, pulse rate increases, a response also noticed in the air force pilots who were exposed to an acceleration of +7 Gz without the anti-G suit, while at the same rate of acceleration and with the anti-G suit, the increase in the pulse rate was slightly lower. With high onset rate of acceleration, significant changes in pupillary response and its diameter may occur, which then may affect the maintenance of visual acuity, colour vision and stereo vision. This is important because a pilot has to be able to orientate himself in space rapidly, visually scan the configuration of

terrain, positions of enemy aircarft and also monitor the instrument panel in the cockpit [14]. Tolerance to +Gz acceleration, and the ensuing changes in visual functions, including pupillary response, may be compromised if pilots do not fly in the conditions of high +Gz acceleration over a longer period of time [15]. This is why there is a question of how much air combat training a pilot needs in order to maintain good tolerance. It is a known fact that a pilot who flies in the conditions of air combat at least once a week tolerates +Gz acceleration better than pilots who do this once in two weeks or once a month [16]. When exposed to an acceleration of +5.5 Gz, the air force pilots showed greater tolerance to acceleration and fewer changes in pupil diameter compared to when they were exposed to an acceleration of +7 Gz.

The effect of positive acceleration on pupil diameter shows that pupils dilate throughout the pilot's exposure to acceleration [17]. The study by Cheung and Hofer [17] states that pupils automatically dilate when exposed to acceleration as a response of the sympathetic nervous system to an onslought of a powerful stimulus, that is +Gz acceleration of high values. The increased width of the pupil represents a response of the sympathetic nervous system to increased acceleration [18]. The response time of the pupil to changes in +Gz acceleration differs. The changes were observed on the monitor as there was a camera inside the centrifuge cabin recording what was going on in the cabin and the pilots' responses to acceleration. In the group of Air Force and Air Defence pilots, there was a statistically significant change in pupil diameter (p = 0.022) that did not occur until the pilots were exposed to acceleration values of +5 Gz to +7 Gz. Changes were also statistically significant after being exposed to a +7 Gz acceleration, without the anti-G suit. Darkening of vision or a blackout due to pupil dilation when exposed to acceleration, which pilots may experience while turning or pulling the plane out of a dive, may prove dangerous in combat. In a paper by Johnston et al. [19] that dealt with this problem, it was noticed that pilots exposed to positive acceleration in the human centrifuge experienced a subjective feeling of loss of peripheral vision and that pupil diameter reached its maximum. In our investigation, when the pilots were exposed to an acceleration of +5.5 Gz, they did not experience any statistically significant reduction in vision as the noticed difference in pupil diameter was not statistically significant. Pupil size is dependent on numerous external, physiological and psychological factors, such as light, fatigue, eye movement, eye pressure, hearing, smell [20]. We did not notice any impact of external factors on our respondents' pupil diameter. Fear, pain, and the level of difficulty of combat task may also affect pupil diameter. Tsai et al. [20] have claimed that an increase in pupil diameter when a pilot was exposed to a +6 Gz acceleration lasted 15 minutes after the exposure and pupil diameter ranged from 3.56 ± 0.72 mm to 5.65 ± 0.56 mm. Exposure to a +9 Gz acceleration caused the dilation ranging from 3.54 ± 0.73 mm to 5.56 ± 0.67 mm and lasted 30 minutes after the acceleration ceased. Pupil dilatation also causes decrease in distance of visual acuity when exposed to positive acceleration and it is a useful quantitative parameter for assessing response to a positive acceleration [21].

The results we obtained expand the knowledge base necessary for quality selection of pilots, the most expensive population in any army. It is important to know the limits of tolerance to positive acceleration and find a way to tolerate acceleration successfully, with fewest possible consequences for visual functions and pupillary response of pilots flying modern high-performance combat aircraft.

The objective of further investigations and research in the field of aviation physiology is to consolidate data obtained by sensory means and measuring instruments and classify them using information technology, have them represented on graphs and projected onto the pilot's visor in three dimensions, which will make flying safer, more secure and efficient. It is necessary to carry out further research that will generate more precise information on how +Gz acceleration affects visual functions and what kinds of responses of human organism this impact induces.

Conclusion

One of the most sensitive physiological indicators is the response and change in pupil diameter when exposed to high values of acceleration. In the Air Force and Air Defence pilots, a change in pupil diameter occurs when they are exposed to a +7Gz acceleration. Pilots tolerate exposure to a +5.5Gz acceleration without any major changes in pupil response or its diameter. By receiving physiological training in the human centrifuge, which simulates real G acceleration conditions, pilots will improve their tolerance to acceleration and become familiar with their body's response to excessive acceleration, which is important for flight safety.

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Received on June 7, 2020
Accepted on July 6, 2020
Online First July, 2020

SHORT COMMUNICATION

UDC: 616.62-089
DOI: https://doi.org/10.2298/VSP200416052A

Orthotopic ileal neobladder "Belgrade pouch" in females

Ortotopska ilealna neobešika „*Belgrade pouch*" kod žena

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Abstract

Background/Aim. Orthotopic continent neobladder provides adequate capacity, low pressure reservoir, acceptable rate of continence and satisfactory voiding frequency. Standard surgical techniques require the usage of an ileal segment in the length of 50–65 cm for neobladder creation with favorable results. However, the usage of a long intestinal segment is associated with high neobladder capacity, voiding problems, and metabolic complications. The aim of this study was to analyze clinical outcomes of the "Belgrade pouch" orthotopic bladder replacement in female patients and to promote the usage of shorter ileal segment for neobladder creation. **Methods.** A prospective study conducted in our institution from 2009 to 2019 included 37 female patients who underwent orthotopic bladder replacement according to "Belgrade pouch" technique with the usage of shorter ileal segment whose average length for neobladder creation was 28 cm. Inclusion criteria were: female continent patients older than 18, organ-confined muscle-invasive bladder carcinoma and, the American Society of Anestesiologists (ASA) score 1 or 2. Exclusion criteria were: diabetes mellitus, obstructive pulmonary diseases, systemic illnesses and metabolic diseases which may have some influence on results interpretation, renal deterioration and preoperative incontinence. We analyzed operative time, blood loss, histopathological findings, continence rate, metabolic disorders, immediate and delayed complications and survival rate in two-year periods of follow-up. **Results.** Average age of patients was 58 (32–67) years. Average time of surgical procedures was 199 (155–320) min. Blood transfusion was intraoperatively applied in 32.43% of the patients in average volume of 385 (300–640) mL. A total of 29.47% patients had anemia preoperatively. In the early postoperative period we reported one patient with paralytic ileus which was resolved conservatively and one patient with urinary fistula appearance; 56.75% of the patients were in pT2 stage. Two years following the surgery, daytime continence was achieved in 91.89% of the patients, neobladder capacity was 459 (345–592) mL, post-void residual urine volume was 27 (0–40) mL, 24 h voiding frequency 6, metabolic acidosis appeared in 2.7% of the patients. Survival rate in 2-year period was 86.48%. **Conclusion.** Orthotropic ileal neobladder created from the shorter ileal segment ("Belgrade pouch") in females provides a high level of continence without a significant increase of voiding frequency, with adequate capacity, without urinary tract retention and with decrease of metabolic complications.

Key words:
colonic pouches; female; postoperative complications; serbia; treatment outcome; urinary bladder neoplasms; urological surgical procedures.

Apstrakt

Uvod/Cilj. Ortotopska kontinentna neobešika omogućava adekvatan kapacitet, nizak pritisak, visok nivo kontinencije i zadovoljavajuću frekvenciju mokrenja. Standardne hirurške tehnike preporučuju upotrebu segmenta tankog creva u dužini od 50 do 65 cm za kreiranje neobešike, uz zadovoljavajuće rezultate. Upotreba dužeg crevnog segmenta za kreiranje neobešike može da dovede do nepotrebnog povećanja kapaciteta neobešike, problema sa mokrenjem i metaboličkih poremećaja. Cilj ove studije je bio da prikaže rezultate kreiranje neobešike po metodi „*Belgrade pouch*" i da promoviše upotrebu kraćeg segmenta tankog creva za kreiranje neobešike. **Metode.** Prospektivna studija, sprovedena u periodu od 2009. do 2019. godine u našoj ustanovi, je uključila 37 bolesnika, operisanih po metodi „*Belgrade pouch*" uz upotrebu kraćeg segmenta tankog creva prosečne dužine 28 cm. Kriterijumi za uključenje u studiju su bili: kontinentne bolesnice starije od 18 godina sa ograničenim karcinomom prelaznog epitela mokraćne bešike i *American Society of Anesthesiologists* (ASA) skorom 1 ili 2. Kriterijumi za isključenje iz studije su bili: *diabetes mellitus*, opstruktivne bolesti pluća, metaboličke i sistemske bolesti koje bi imale uticaja na interpretaciju rezultata, bubrežna

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slabost i preoperativna inkontinencija. Analizirali smo vreme operacije, potrebu za transfuzijom krvi, patohistološki nalaz, kontinenciju, metaboličke poremećaje, neposredne i udaljene komplikacije, kao i stopu preživljavanja u dvogodišnjem periodu praćenja. **Rezultati.** Prosečna životna dob pacijentkinja bila je 58 (32–67) godina. Prosečno vreme operacije iznosilo je 199 (155–320) minuta. Transfuzija krvi primenjena je intraoperativno kod 32.43% bolesnica, u prosečnoj zapremini od 385 (300–640) mL. Ukupno 29.47% bolesnica je preoperativno imalo anemiju. U ranom postoperativnom periodu kod jedne bolesnice je došlo do razvoja paralitičkog ileusa koji je rešen konzervativno. Jedna bolesnica je imala urinarnu fistulu. Patohistološka analiza je pokazala da je najviše bolesnica (56.75%) bilo u pT2 stadijumu. Dve godine nakon operacije, dnevna kontinencija je postignuta kod 91.89% operisanih. Kapacitet neobešike je iznosio 459 (345–592) mL, zapremina rezidualnog urina 27 (0–40) mL, 24-časovna frekvencija mokrenja je bila 6, a metabolička acidoza je zabeležena kod 2.70% bolesnica. Stopa preživljavanja u dvogodišnjem periodu iznosila je 86.48%. **Zaključak.** Ortotopska ilealna neobešika kreirana upotrebom kraćeg segmenta tankog creva po metodi „*Belgrade pouch"* obezbeđuje visok nivo kontinencije, bez značajnog povećanja frekvencije pražnjenja. Adekvatnog je kapaciteta, bez značajnijeg rezidualnog urina, uz minimalan broj slučajeva ureterohidronefroze i uz značajno manje metaboličkih komplikacija.

Ključne reči:
creva, rezervoari; žene; postoperativne komplikacije; srbija; lečenje, ishod; mokraćna bešika, neoplazme; hirurgija, urološka, procedure.

Introduction

Bladder tumor is the sixth most common malignancy in human population, and it appears 3.5–4 times more rarely in female population [1]. Despite the fact that this malignancy is not so frequent in females, bladder cancer in this subgroup of population is presented with more advanced disease forms and has worse survival rate [2]. In 25% of patients, bladder cancer appears in its muscle-invasive form [3]. Radical cystectomy (RC) is the gold standard management for muscle-invasive bladder cancer (MIBC). RC in females includes removal of the bladder, anterior vagina wall, uterus, ovaries, fallopian tubes and regional lymph nodes. After RC, urinary diversion may be resolved through ureterocutaneostomy, ileal conduit or neobladder substitution.

The aim of this study was to present our very first results of the "Belgrade pouch" technique in females and to promote the usage of a shorter ileal segment for neobladder creation in females.

Methods

In the prospective clinical study, we included 37 female patients surgically treated in the 2009–2019 period at the Urology Clinic of Military Medical Academy (MMA) in Belgrade, Serbia. The "Belgrade pouch" technique was approved by the Ethics Committee of the MMA and all the patients signed informed consent for this procedure. Inclusion criteria were: female continent patients older than 18 years, organ-confined muscle-invasive bladder carcinoma (transitional cell carcinoma – TCC) and the American Society of Anestesiologists (ASA) score 1 or 2. Exclusion criteria were: diabetes mellitus, obstructive pulmonary diseases, systemic illnesses and metabolic diseases which could have certain influence on results interpretation, renal deterioration and preoperative incontinence. Ileal orthotopic neobladder was constructed according to the original "Belgrade pouch" technique with the usage of a shorter ileal segment for neobladder construction. Terms "neobladder" and "pouch" are used as synonyms. After ovariectomy and hysterectomy, cystectomy with removal of anterior vaginal wall was performed (Figure 1). Ileal segment in the average length of 28 cm was resected with raw preservation of mesenterial root. Detubularisation of ileal segment was performed and a U-shaped plate was formed. The next step was spherical neobladder formation. Previously, ureters were implanted in the neobladder roof directly, from both sides separately with ureteral stents conducted to the anterior

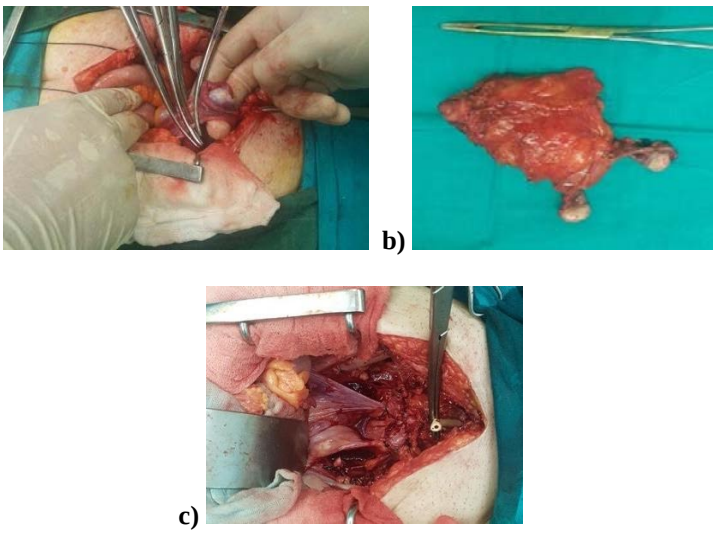

Fig. 1 – a) ovariectomy; b) hysterectomy; c) cystectomy with resection of anterior vaginal wall.

a)  b)  c) 

Fig. 2 – a, b) creation of U-shaped "Belgrade pouch" from a shorter ileal segment; c) neobladder-urethral anastomosis.

abdominal wall through a small incision. Neobledder-urethal anastomosis was formed with 5–7 stitches (Figure 2). Three days following the surgery, we started to flush neobladder through the catheter with 20–30 mL of saline liquid twice a day. Urethral stents were removed, in average 12 (11–14) days following the surgery. We considered those with the use of 0–1 pad/24 hrs as continence patients. A detailed description of the "Belgrade pouch" technique was previously published by Bančević et al. [4].

Results

Average age of patients was 58 (32–67) years. Twenty-one (56.76%) and 16 (43.24%) of the patients were classified as the ASA 1 and ASA 2 score, respectively.

Average operation time was 199 (155–320) minutes. Average intestinal length used for neobladder creation was 28 (25–35) cm.

Eleven (29.74%) patients had anemia preoperatively. Blood transfusion was applied intraoperatively in 12 (32.43%) of the patients in average volume of 385 (300–640) mL.

We did not notice neither wound dehiscence nor high body temperature during hospitalization.

In the early postoperative period we observed one (2.7%) patient with paralytic ileus which was resolved conservatively within 8 days postoperatively with prokinetic drugs, correction of serum potassium concentration and prolonged nasogastric tube placement.

One (2.7%) patient had a urinary fistula that appeared two months following the surgery. This patient did not receive chemotherapy. After 4 weeks of conservative treatment and catheter placement, that fistula did not heal spontaneously. Endoscopy showed a 5-milimeter neobladder-vaginal fistula. Transvaginal fistula repair using the Martius flap technique was performed with satisfying results.

Our histopathological examination confirmed TCC in all patients, in 3 (8.11%) with squamocellular component, in 4 (10.81%) with sarcomatoid component beside TCC. G3 pT1 stage was reported in 2 (5.41%) patients, and both of them underwent multiple transurethral resections for recurrent, *Bacillus Calmettequerin* (BCG) refractory TCC. Twenty-one (56.75%) patients were in pT2 stage, 10 (27.02%) patients were in pT3a stage, and 4 (10.82%) patients in pT3b stage. In 2 (5.41%) of the patients N1 stage was reported.

In the youngest patient, aged 32, the right ovary was preserved, and in other two patients, aged 37 and 42, internal genital organs were preserved.

Two years following the surgery, two patients had hydronephrosis, and 35 (94.59%) of the patients were with normal finding on upper urinary tract (Figure 3). One patient during the second year of the follow-up underwent bilateral reimplantation of ureter in neobladder with JJ stent insertion due to bilateral hydronephrosis. In the same period of the follow-up, we reported just one patient with mild acidosis, but all other patients were with normal pH value without the need for any kind of alkalizing agents. Delayed characteristics and complications in our patients are shown in Table 1.

Survival rate in the 2-years periods was 86.48%.

Fig. 3 – Multislice computed tomography (MSCT) of kidneys, ureters and "Belgrade pouch" two years following the surgery shows adequate pouch capacity and absence of hydronephrosis in a female patient.

Table 1

Characteristics and delayed complications of "Belgrade pouch" in female patients

Characteristics complications	3 months	6 months	1 years	2 years
Day continence, n (%)	20 (54.05)	28 (75.66)	31 (83.78)	34 (91.89)
Night continence, n (%)	18 (48.65)	27 (72.97)	30 (81.08)	33 (89.20)
Neobladder capacity (mL), mean (range)	287 (220–348)	371 (285–449)	436 (330–504)	459 (345–592)
PVR urine volume (mL), mean (range)	10 (0–18)	16 (0–21)	19 (0–39)	27 (0–40)
24-voiding frequency, n	9	8	7	6
Acidosis, n (%)	0	0	3	1 (2.7)
Vitamin B12 deficiency, n (%)	0 (0)	0 (0)	0 (0)	0 (0)
Pouch calculosis, n (%)	0 (0)	0 (0)	0 (0)	1 (2.7)
Hydronephrosis, n bilateral unilateral	 4 (grade I) 0	 2 (grade I)	 1 (grade II) 1 (grade I)	 1 (grade III) Re-anastomosis 1 (grade III) PCN

PVR – post-voided residual; PNC – percutaneous nephrostomy.

Discussion

Standard orthotopic ileal neoblader techniques require the usage of an intestinal segment in the length of 55–60 cm [5, 6]. The "Belgrade pouch" surgical technique for orthotopic neobladder construction was aimed to create a urinary reservoir that may obtain adequate capacity and continence with reasonable frequency and reduction of metabolic disorders. Standard surgical techniques such as Studer's and Hautmann's neobladder finally gain unnecessary large capacity with increased residual urine [5]. Moreover, these neobladders have been associated with significant metabolic problems. Therefore, the usage of a shorter intestinal segment for the creation of neobladder appeared challenging to minimize the above mentioned problems. Our team reported promising results using this technique in a group consisted of mainly male patients [4]. During the time, there is a constant enlargement of neobladder capacity, which may be the reason for voiding problems and metabolic disorders. The key point of the presented technique is to use a shorter ileal segment for neobladder orthotopic construction, to preserve favorable characteristics of the other types of ileal substitutes and to attempt the reduction of metabolic and other complications. In our experience, the "Belgrade pouch" is a surgical technique which requires the usage of the shortest ileal segment for neobladder construction. Our idea was to take an ileal segment in the length of 30 cm, but the vessels distribution in intestinal mesenterium and the attempt to provide maximal vascular preservation after transluminiscence of mesenterium resulted in the average length of the ileal segment in our study of 28 cm.

The patients sheduled for orthotopic urinary derivation are often younger, usually under the age of 65 [7]. In this study cohort, the average age of the patients was 58, and the youngest one was at the age of just 32. Although age is not a contraindication for orthotopic diversion, the majority of authors do not perform this kind of surgery in octogenarians [8, 9].

The most common early complications of such kind of surgical procedure in other studies were ileus (up to 15%) and wound infection (up to 12%) [10]. In this study, there was only one patient with paralytic ileus and there was no report of any wound infection and dehiscence, but our results are probably influenced by a smaller number of patients.

Referent studies reported that one-quarter to one-third of patients received blood transfusion intraoperatively [11] and similar results have been reported in this study. Some higher results in our study are in correlation with a higher percentage of preoperative anemia in the patients.

Urinary fistula is an uncommon, but possible complication. Carmel et al. [12] described their experience with this kind of complications and suggested that Martius or omental flap should be interposed between vagina and (neo)bladder, after the excision of fistulous channel and closure of the defect. The procedure using Martius flap, through transvaginal approach was done in our one patient and with excellent results.

Kretschmer et al. [13] reported 22.4% of patients with tumor *in situ* (TIS), 23.0% of patients in pT1 stage, 27.6% in pT2 stage, 18.4% in pT3 and 4.6% in pT4 stage with missing data in 4% of patients. The majority of patients in our study were in pT2 and pT3 stage. Some of them had unfavorable histopathological features of squamocellular or sarcomatoid components which might influence the treatment outcome as well as nodal involvement. Although we used the "Belgrade pouch" technique only in patients with malignancy, it is suitable also for patients with benign conditions requiring cystectomy.

In the youngest patient, aged 32, the right ovary was preserved. In other two patients aged 37 and 42, uterus and ovaries were preserved, on their own request. For selected patients, sparing of internal genitalia has proven to be oncologically safe, but still there are no strict recommendations for such cases [14].

Oncological outcome and continence probably are the most important facts for improved life quality after a radical cystectomy with orthotopic diversion. Different factors have the influence on the quality of micturition and continence:

strength of urethral sphincter, neobladdeer capacity and shape, position of neobladder in pelvis and angle with urethra, diameter of urethra-neobladder anastomotic channel, body mass index and intraabdominal pressure, patients level of training for micturition in new wave, post-voiding residual urine volume and mucus production [15]. The experience of surgeons (> 100 previous cystectomies) has been demonstrated to be a prognostic predictor for continence [13]. During the time, there is a constant enlargement of pouch capacity which improves continence level during the first period. In our previous publication, the neobladder constructed from a shorter ileal segment 12 months following the surgery achieved the average capacity of 440 (290–710) mL, compared to the standard-dimensioned pouch which achieved some unnecessarily large capacity of average 840 (480–2050) mL [16].

The pouch may became unnecessarily large and become a reason for voiding problems and metabolic complications. Diverse studies reported daytime continence in range of 88–99% and during the night 10–20% lower continence [17, 18] In 11 studies reported, daytime and night-time continence rates were 58–100% and 42–100%, respectively, and self-catheterisation rate was 9.5–78% [19]. Our study showed that even with a shorter length of the intestinal segment, it was possible to create a neobladder with adequate capacity and reasonable voiding frequency, with a high level of continence one year following the surgery. During the first months after the surgery, we observed the higher level of incontinence, because a small pouch needs time to enlarge its capacity which is an important factor for continence achievement. Smith et al. [20] reported hypercontinence in 10–20% of patients as one of the most frequent problems in females following the surgery. In our study, we had no patients with hypercontinence problem, so there were no patients for intermittent catheterization. We suppose that the smaller pouch, adequate capacity, spherical shape and position in pelvis contribute to avoiding hypercontinence.

The absorption of potassium, hydrogen and chloride ions results in a hyperchloraemic metabolic acidosis. A prolongated metabolic acidosis may include calcium resorption and decreased circulating phosphate levels, leading to hyperparatyreoidism, osteomalacia and osteoporosis [21]. Kim et al. [22] reported metabolic acidosis rate of 52%, 19.5%, and 7.3% in 1 month, 1 year and 2 years after the surgery, respectively. One of the goals of this novel "Belgrade pouch" technique is to prevent acidosis and our data showed just one patient in the second year of the follow-up with mild acidosis. Studer [5] recommends the intake of sodium bicarbonate (2–6 g/day) to all patients, but in our study alkalizing agents were not used.

The appearance of neobladder calculi can be found in 10–20% of patients [21]. Mucus production, infections, urethral stenosis may be risk factors for stone formation in neobladder. Also, a higher concentration of urinary calcium caused by metabolic acidosis may predispose patients to a higher risk of urinary calculi formation. Just one of our patients, at the end of the follow-up period, had pouch calculus and this result is encouraging.

The reasons for ureterohydronephrosis appearance in patients after the neobladder construction may be stricture on uretero-pouch anastomosis, urine reflux, calculosis of the renal pelvis or ureter, tumor in ureter or extraluminal compression on ureter. An early, postoperative transitional hydronephrosis may be caused by edema at anastomosis and reduced compliance and capacity of the neobladder [23]. Ureterohydronephrosis rate in referent studies appears in 5–19% of patients [23]. In our study, initially higher number of low grade bilateral hydronephrosis was caused by urine reflux during the period while neobladder was still with a smaller capacity. This kind of hydronephrosis is transitional, and usually is not connected with renal deterioration and infection rate. On the other hand, in the two-year period of follow-up we reported one patient with hydronephrosis grade III, which was resolved by placing a percutaneous nephrostomic tube. The other patient was resolved through a very complicated surgical procedure of reimplantation of both ureters in the neobladder.

Vitamin B12 deficiency is more frequent in neobladders constructed of more than 60 cm of terminal ileum. Fujisawa et al. [24] reported 13.6% of patients vitamin B12 deficiency, detected between 9 months and 3 years after surgery. In our study we did not observe any vitamin B12 deficiency.

Conclusion

The orthotropic ileal neobladder created from a shorter ileal segment, the so-called "Belgrade pouch", provides a high level of continence with adequate capacity, insignificant residual urine without urinary tract retention and without significant increasing of voiding frequency. We reported a small percentage of pouch calculosis without significant acidosis and without vitamin B12 deficiency in two years of the follow-up. The appearance of refluxive ureterohydronephrosis is transient, low-grade and caused by smaller neobladder capacity in the first months after the operation.

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Received on April 16, 2020.
Revised on May 7, 2020.
Accepted on May 12, 2020.
Online First May, 2020.

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UDC: 616.727-08:617.571
DOI: https://doi.org/10.2298/VSP200609065M

Acromioclavicular joint injuries treatment

Lečenje povreda akromioklavikularnog zgloba

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Key words:
acromioclavicular joint; athletic injuries; joint dislocations; ligaments, articular; orthopedic procedures; reconstructive surgical procedures; shoulder injuries.

Ključne reči:
zglob, akromioklavikularni; povrede, atletske; zglob, iščašenja; ligamenti zgloba; ortopedske procedure; hirurgija, rekonstruktivna, procedure; rame, povrede.

Introduction

The acromioclavicular (AC) joint is one of the most frequently injured shoulder joints [1]. This injury is a typical result of falling directly onto the superolateral side of the shoulder. Acromioclavicular dislocation is a common injury especially in young people and athletes [2]. The AC joint injuries account for approximately 12% of shoulder dislocations and 10% of all shoulder injuries [3, 4]. Depending on the mechanism, acromioclavicular joint injuries represent a wide range of soft tissue lesions, ranging from mild, transient pain to significant dislocations, chronic pain, and changes in shoulder biomechanics. Multiple factors are involved in the treatment algorithm, including clinical and imaging findings, as well as patient-specific factors [5]. An incorrect treatment of an AC joint injury may predispose complications and that is why it is critical to understand the indications for operative and non-operative management [5]. Indications for the operating treatment of AC joint injuries are not clearly defined and differ depending on bibliographic sources. Accordingly, there is a need to understand complete anatomy, biomechanics and pathophysiology of AC joints [5]. In this paper we attempted to cover all operative and non-operative methods, as well as those possible complications of such treatments.

Anatomy

The AC joint is a diarthrodial joint that alternates between the lateral end of the clavicle and the medial part of the acromion [6]. Joint stability is provided by both static and dynamic stabilizers. Static stabilizers are the AC ligaments, coracoclavicular (CC) igaments and coracoacromial ligament. Together, they account for about 90% of joint stability in the anteroposterior diameter [6]. The CC ligament complex, composed of two ligaments (*trapezium* and *conoideum*), connects the inferior surface of the clavicle with the coracoid and it is the major suspensory shoulder ligament [7]. These ligaments prevent superior-inferior clavicle dislocation, that is, they are responsible for vertical stability [8, 9]. The conoid ligament, which is of conical shape and average length of 11 mm, has its attachments on the calvicle and on the medial aspect of the coracoid extension [10]. These two ligaments close to each other at a 60-degree angle with a clearly limited bursa between them [11] as shown in Figure 1.

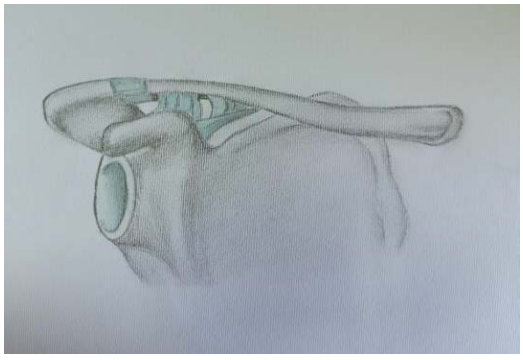

Fig. 1 – Anatomy of acromioclavicular joint.

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Biomechanics

The clavicle, AC and sternoclavicular joints all play a role in connecting the upper extremity to the axial skeleton. The biomechanics of the AC joint includes static and dynamic stabilizers and movements of the joint itself [12, 13]. AC ligaments act as primary stabilizers in the horizontal plane, in the anteroposterior direction, while CC ligaments act as the main stabilizers in the vertical plane [14, 15]. During adduction and flexion of the shoulder at up to 180 degrees, the AC joint is engaged in about 5–8 degrees of the movement while scapula and clavicle rotate through about 45 degrees [16]. Isolated movements of the AC joint are present in antero-posterior, superior-inferior and rotational planes [17]. A normal antero-posterior translation of an intact AC joint is approximately 6 mm, superior-inferior 3 mm and 3–5 degrees of axial rotation.

Classification

AC joint injuries are best classified based on the degree of damage caused by the acting force. There are 6 degrees of AC joint injuries [18–20] (Figure 2):

Type 1 – In this type of injury, the RTG finding is neat, there is only is a slight swelling of those soft tissue structures of the injured shoulder in relation to the uninjured.

Type 2 – In this type, we have a slight elevation of the lateral limb of the clavicle relative to the other shoulder. A rupture of AC ligaments occurs, while CC ligaments remain intact.

Type 3 – We have a complete joint dislocation here. In addition to the rupture of AC ligaments in this type, there is also some rupture of CC ligaments.

Type 4 – In this type, in addition to the ruptures of AC and CC ligaments, we also have a rupture of *m. trapezius* with a posterior dislocation of the calvicle into muscle.

Type 5 – The type is characterized by a significantly greater dislocation of the clavicle than in previous two types, and along with ligament rupture, we also have a rupture of the deltoid and trapezius fascia.

Type 6 – It represents the rarest type of AC joint injuries. In this type, an inferior dislocation occurs below the acromion and behind the joint tendon of the biceps and step *brachialis* [21].

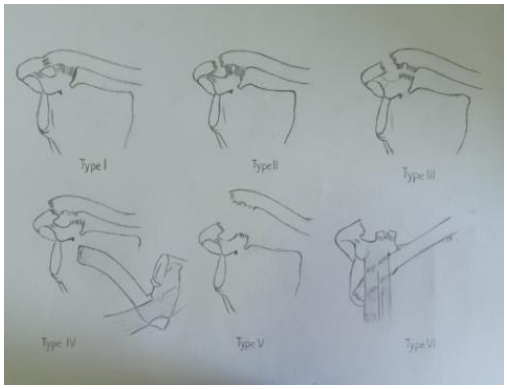

Fig. 2 – Types of acromioclavicular joint injuries (see chapter Classification for further explanations).

Clinical signs and symptoms

A detailed history that includes the mechanism of injury, duration, and localization of pain are key to the diagnosis of AC joint injuries. The information about the fall onto the lateral aspect of the shoulder, often during contact sports or a fall onto the outstretched arm, may indicate a possible injury to the AC joint [11]. Palpation of the distal clavicle end can provide valuable information on the severity of that injury and the degree of pain. Problems are most often exacerbated by active and passive shoulder movements [22]. A clinical examination followed by an intra-articular application of topical anesthetic can be quite useful in diagnosing such issues. When it comes to specific tests for the detection of acromioclavicular pathology, two tests have special clinical significance. These two are the Cross-arm adducton test and O'Brien's compression test [23]. In the O'Brien's test, the patient stands with their arm elevated at 90 degrees from the shoulder, the elbow being in its full extension with the adduction of the arm at about 10–15 degrees and the internal rotation so that the thumb is down. The doctor then applies a constant downward pressure to the arm [23]. A positive O'Brien's test can also speak in favor of labral pathology, that is, the presence of the superior labral anterior and posterior (SLAP) lesion [23]. Radiographic evaluation also plays a prominent role in the diagnosis of AC joint injuries.

The mechanism of injuries

The subcutaneous position of the joint itself and poor soft tissue coverage make it susceptible to a direct mechanism of injury [24].

One of the most common mechanisms involves a fall onto the shoulder with an arm in adduction. This force causes the displacement of the acromion medially and inferiorly leading to a standard pattern of injury – a rupture of the AC ligaments is followed by a rupture of the CC ligaments and finally a rupture of the deltoid trapezius fascia [25] (Figure 3).

Fig. 3 – The most common cause of acromioclavicular joint injury is a fall onto the shoulder with an arm in adduction.

Treatments of AC joint injuries

Non-operative treatment

In the case of type 1 and type 2 injuries, there is a general consensus that a non-operative treatment produces satisfactory results and can be treated with a specific period of immobilization in the form of arm sling and Sayer immobilization for 10–14 days. Certain studies have shown that these injuries can be symptomatic for up to 6 years [26-28]. When it comes to the type 3 injuries, there are still many controversies and disagreements today regarding what the best treatment may be. With the type 3 injuries, the AC and CC ligaments are completely ruptured and there is a dislocation with respect to the contralateral side, while in the type 5 with ruptured ligaments, we also have a rupture of the the deltoid trapezius fascia and a significant dislocation in regard to the contralateral side. In a prospective study of patients with acute 3 and 5 luxation, Smith et al. [28] compared non-operative immobilization treatment with operative reposition and fixation. Conservatively treated patients regained the full range of motion much earlier than those operated on. The authors' conclusion is that non-operative treatment is suitable for acute type 3 dislocations and that younger patients with a significant dislocation respond better to surgical treatments.

Indications for surgical treatments

The main goal of treating AC joint injuries is to achieve painless shoulder movements without any significant restriction of the range of motion [29].

Types 1 and 2 lesions are generally treated inoperatively with quite satisfactory functional results [30]. A surgical treatment is generally advised in young and active people with lesions of types 4, 5 and 6 because of the significant morbidity associated with the mechanism and the degree of injury that can lead to persistent joint dislocations, instability with changes in scapula kinetics and shoulder function [30].

Surgical treatment of AC joint injuries

When it comes to the surgical treatment of the AC joint, it is important to note that there is no method representing the gold standard. Since the beginning to our present day, there have been several treatment methods and methods of fixation in use, each of which have had more or fewer complications.

Hook plate

This method was initially made for fractures of the acromial end of the clavicle, but over time it has found its use in the treatment of AC joint injuries. The disadvantage of this technique is that it is compounded of a number of complications such as acromion fractures, plate bending, and AC joint arthritis that is, according to some studies [31] , present in every other patient. Due to all the complications mentioned above, this method is rarely used (Figure 4).

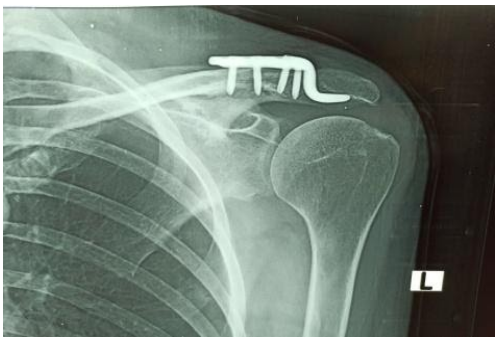

Fig. 4 – Surgical treatment of acromioclavicular joint injury by the Hook plate method.

Bosworth method

This method represents the AC joint stabilization by a single screw between the base of the coracoid and the clavicle, thus providing a rigid fixation. In this method it is crucial to achieve bicortical fixation on the coracoid (Figure 5).

Fig. 5 – Surgical treatment of acromioclavicular injury by the Bosworth method.

Endobutton method

Various techniques have been devised to achieve the anatomical reconstruction of the CC ligaments [32–34] (Figure 6).

Fig. 6 – Anatomical reconstruction of coracoclavicular ligaments by the Endobutton method.

Repair is performed using a suture between the endobuttons on lateral clavicle and coracoid [33].

Weaver-Dunn procedure

The idea behind this procedure is to replace the step-clavicular ligament with a CC ligament.This method involves removing the ligament from its insertion to the acromion, resection of the distal clavicular end and transfer of the ligament to the lateral end of the calvicle as closely as possible to the junction of the CC ligament [35]. The disadvantage of this method is that the CA ligament is not nearly as strong as the native ligament.

Tension band

It involves the placement of two K wires through the acromion and the lateral end of the clavicle and thus the anatomical reposition of the joint is established. The disadvantage of this technique is that frequent needle migration occurs as well as the appearance of the AC joint arthrosis.

CC ligament reconstruction

The method includes an anatomical reconstruction of the ligament and its insertion at the coracoid base and the lateral end of the clavicle. A modification of this method is the reconstruction of the ligaments using biological grafts that run through one or two previously made holes on the clavicle [36].

Complications

Each method has shown its disadvantages and none is without complications. The most common and significant complication is reluxation, which, judging by some works, occurs in as many as 40% of cases. Another, less common complication is the onset of infection [37]. Brachialis plexus injuries are also a potential complication, especially due to the proximity of the coracoid. Any method involving coracoid drilling has this injury as a possible complication [38]. Also, with the type 3 injury, plexus injury can appear owing to scapular dyskinesis and plexus involvement.

Conclusion

Despite the development and better understanding of this type of pathology, there is still no method that can be said to be standard and the best choice for the AC joint injuries. Each method, to a greater or lesser extent, carries with it a number of complications. When it comes to treating these injuries, it should be adjusted to every patient individually, taking into account the patient's age, physical activity and quality of life. Keeping all this in mind, the surgeon should choose the method with which the surgeon is most familiar with and which gives the best opportunity for the patient to fully recover and return to normal life activities.

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Received on June 9, 2020
Accepted on June 17, 2020
Online First June, 2020

CURRENT TOPIC

UDC: 616.314
DOI: https://doi.org/10.2298/VSP210524078Z

A single-file endodontic treatment – A promising endodontic concept

Endodontsko lečenje jednim instrumentom – perspektivni endodontski koncept

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Key words:
endodontics; nickel; root canal preparation; titanium; tooth.

Ključne reči:
endodoncija; nikl; zub, korenski kanal, priprema; titanijum; zub.

Introduction

Effective cleaning of the tooth canal system and adequate canal shaping are the basic preconditions and key requirements for the success of endodontic treatment [1]. Chemomechanical canal treatment implies complete debridement of the internal endodontic space (pulp tissue remains, bacteria), i.e. the canal formation in the form of an elongated cone and the maximum preservation of its original morphology [1–3]. Research has confirmed that proper instrumentation with adequate irrigation and medication cannot completely eliminate, but only reduce bacteria from the canal and its inaccessible parts [1, 3, 4]. A special issue in cleaning is the apical third, both due to anatomical specifics and due to inadequate diameter of the apical preparation, which further complicates the debridement of this part of the canal [5–7].

Canal preparation can be realized by manual and machine rotary files made of stainless steel and files made of nickel titanium (NiTi) alloy. The introduction of NiTi alloy into the manufacturing of endodontic files is one of the greatest evolutionary advances in endodontic dental treatment, and their application is a major step and an important standard in the chemomechanical procedure [2, 7]. Primarily due to good mechanical features (biocompatibility, low modulus of elasticity, corrosion resistance) and the feature of "smart materials" to be able to return to their original shape after deformation, rotary NiTi files opened a new perspective in canal preparation [7, 8]. They preserve the original canal morphology, reduce the possibility of transportation, significantly speed up the instrumentation and make it quite efficient and safe thanks to the outstanding flexibility [8–11]. However, despite numerous advantages, fractures of NiTi files during their clinical application are still a major issue and a significant frustration for many dentists [12, 13].

The aim of this paper was to present a new and promising concept of a single-file treatment, and to show the development and strategies of single NiTi files and the possibility of their application in different clinical situations.

The development of NiTi rotary files

Today, modern endodontic treatment cannot be imagined without the techniques of machine instrumentation and the use of NiTi files [2, 7, 9]. Endodontists currently have more than 160 different NiTi systems at their disposal that use full rotation, reciprocal movements, and eccentric or transaxial movements during canal treatment. NiTi files can differ in the specific design of the working part, conicity, cross section and length of the working part, specific tip, tilt angle of the cutting edge, special thermal preparation of the alloy, etc. [7, 9].

Although NiTi alloy was first used in orthodontics, its use in endodontics is certainly one of the most significant steps towards a more successful canal treatment [14, 15]. The main features of NiTi alloy (56% Ni, 44% Ti) are superplasticity and shape memory effect, which allow the file to return to its original shape after relief (or heating). It has been confirmed that a wire made of this alloy can deform 7–8%, or 40 times more than a stainless steel wire [14–16].

Direct and strong bonds between electrons, which after elastic or pseudoelastic deformation enable the return to the original shape, are responsible for the high flexibility of the alloy. Stress-induced thermoelastic transformation (stress, temperature) implies a martensitic transformation into the austenitic phase, where the elasticity of the alloy increases and the file returns to its original shape after the cessation of stress [7, 14, 16, 17].

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The first NiTi rotary file was introduced by John Mc Spaden in 1992 (conicity 02), and only two years later the NiTi ProFile system (conicity 04 and 06) with a specific design of the working part was introduced into endodontic practice. In the mid-1990s, the Light Speed system was introduced with a file that had a long flexible axis and a short working part (0.25–2 mm), which was used to process the canal apical segment [2, 7, 9].

New design concepts of NiTi files have shifted the focus of the dental industry towards increasing cutting efficiency and reducing possible complications (especially fracture) during canal preparation. This includes a specific design of the working part that provides efficient cutting and elimination of dentin, with a significant reduction of contacts between the file and the walls, and thus stress during the canal preparation [7, 17–19].

The first generation of 1993 NiTi files included sets of fixed conicity files along the entire length of the working part (04, 06), where it was necessary to use all the files in the set for efficient cleaning and shaping [2, 20].

The second generation of NiTi files (appeared in 2001) had active cutting edges and variable conicity on the working part of the file (progressive multiconicity), which significantly limited their cutting effect and reduced the possibility of screwing [2, 17, 19, 20].

Since 2007 (the third generation), dental technology has been focused on the production of NiTi alloy files with improved features (special thermal treatment), which has significantly increased flexibility and resistance to cyclic fatigue, i.e. reduced the possibility of fracture [2, 16, 20].

The fourth generation of NiTi files appeared on the market in 2011 and included a change in the dynamics of movement in the canal. In addition to reciprocal movements, this concept included only one file for complete canal cleaning and shaping [2, 3, 9, 18, 20].

The fifth generation (2015) has been designed so that the center of gravity and the center of rotation of NiTi files are not in the same axis, which significantly reduces the contact between the file and the canal walls, and increases the flexibility and efficiency of cutting dentin [2, 7, 10, 12].

A research has confirmed that the efficiency and safety of files can be increased by applying new NiTi alloys with superior mechanical features, i.e. improvements in the process of their production [7, 10, 12, 20]. The mechanical features of NiTi alloy (superelasticity, memory effect) can be improved by changing the chemical composition [8], finishing surface treatment of the alloy during manufacturing [7, 17] or electrochemical treatment (electropolishing) [17, 21, 22].

The introduction of new alloys into the file manufacturing (Nitinol 508; Ni 55.8%, Ti 44.2%) and their exposure to thermal treatment (alternating heating and cooling) confirmed their superior mechanical features, i.e. greater flexibility and increased resistance to cyclic fatigue [18, 20, 23–25]. In order to improve fracture resistance, dental technology today uses methods of implantation of an alloy with argon, boron or nitrogen ions, coating of files with a layer of nitrite, plasma immersion and electropolishing, or deep cryogenic treatment [7–18, 21, 22, 26–28].

A single-file endodontic treatment

Continuous development of endodontics includes numerous ideas, techniques and strategies for more efficient canal preparation, but clinical reality confirms that it is still difficult to completely and predictably clean and shape the canal system [1, 2, 7, 9]. Cleaning efficiency is particularly limited in the apical part of the canal [9, 29, 30].

Single-file canal preparation is a completely new concept that has fundamentally changed a lot in endodontics (conceptually, procedurally and economically) [2, 9, 31, 32]. Single-file canal instrumentation (regardless of diameter, length and bending degree) is a strategy based on the concept of "less is more". This significantly shortens the duration of an endodontic intervention (3–4 times), eliminates the possibility of cross-contamination (occurs in sets with multiple files) and significantly saves time and financial costs for both the patient and the endodontist [2, 9, 31–34]. Facilitated and simplified intervention, in addition to time, significantly reduces the fatigue of the file and thus "minimizes" the possibility of its fracture [2, 7, 9, 31, 32].

The concept of a single-file treatment includes different systems that use full (continuous) rotation, reciprocal movements, or a combination of full rotation and reciprocal movements. In recent years, systems with eccentric (asymmetric) or transaxial (longitudinal) movement of files have been introduced in order to reduce cyclic fatigue and increase the cutting efficiency of files [2, 7, 28, 35]. Some newer NiTi systems combine full rotation and reciprocal movements (Genius system, Ultradent, USA) using an endomotor (Elements) where there is an automatic switch from rotary to reciprocal movement (90° clockwise and 30° counterclockwise) if the resistance increases during the file rotation in the canal [7, 31–33].

Systems with eccentric (asymmetric) movement are recommended for canals with irregular morphology because they enable contact between the file and hard-to-reach parts of the canal. These movements are used by the systems such as Pro Taper Next, XP Endo Shaper and TruShape [7, 31].

Transaxial file movement is represented by a new self-adjusting file (SAF). In fact, it is a specially designed hollow and flexible file (which adapts to the shape of the canal in three dimensions) and is connected to a continuous irrigation source via a special silicone tube [8, 33, 34].

Single files with reciprocal movements

The curvature of the canal is an important issue of preparation, but also a cause for frustration and fear of dentists that this morphological specificity does not lead to fracture of the file. Deformations and frequent fractures of NiTi files, as a consequence of torsional stress and cyclic fatigue, influenced the introduction of new preparation concepts based primarily on changes in movement dynamics and reduction of the number of files for chemomechanical processing of canals [2, 31–37].

The file preparation technique with reciprocal movements is mainly represented by a single NiTi file. Reciprocal

movements in the canal are based on the technique of balanced forces and involve the rotation of the files counterclockwise (cutting direction) and a much shorter movement in a clockwise direction (file relaxation). These movements reduce the contact surface with the canal wall, and thus torsional stress and cyclic fatigue, which significantly prolongs the life of the file [35, 36, 38–40].

For the first time, a study by Yared [41] reports on the application of the Pro Taper F2 NiTi file (intended for full rotation) in the technique with reciprocal movements. The study has shown a significant reduction in cyclic fatigue (compared to full rotation) and a number of benefits related to shorter processing times, reduction of possible cross-contamination and alleviation of fear of possible deformations and fractures because only one file is used [27, 33, 41].

Several systems that use single disposable files and special motors for reciprocal movements have been introduced on the dental market since 2008. NiTi files are mainly made of special NiTi alloy with special thermal treatment (increased flexibility and resistance to cyclic fatigue), specific working part and cross section (S-shape) and progressive conicity (Wave One, Wave One Gold-Dentsply, Sirona, Ballaigues, Switzerland; Reciproc, Reciproc Blue-Vdw, Munich, Germany; Unicone- Medin, Nove Mesto, Chech Republic; Pro Design R- Eazy Equipamentos Odontologicos, Belo Horizonte, Brazil; X1 Blue File-Mk Life, Porto Alegre, Brazil) [27, 33, 35, 38, 42, 43].

Reciprocating file movements counterclockwise provides apical movement and cutting of the dentin, and a movement in a clockwise direction relaxes or reduces torsional stress and prevents "clamping" in the canal [35, 38, 43]. The preparation technique with reciprocal movements, in addition to speed and safety, allows that 80% of canals with moderate bending can be processed without prior check of passability by hand files [2, 7, 35, 39]. Fracture of NiTi files most often occurs in bent canals due to torsional stress or cyclic fatigue when a part of the file is "blocked" in the canal (curve), and the other continues to move. Cyclic fatigue fracture occurs due to the cumulative effect of bending forces at the place of maximum bending (curve) [7, 10, 18, 27, 35]. Torsional fatigue is significantly lower in reciprocal movements because the file alternately shifts from the active cutting movement to the non-cutting movement, which increases the resistance to cyclic fatigue and prolongs the clinical life of the file [31].

The results of numerous studies show that reciprocal movements of the file in the canal lead to less cyclic fatigue and significantly rarer fractures [7, 18, 31, 34, 35, 39, 42].

A study by De-Deus et al. [44] has indicated greater resistance to cyclic fatigue of the Pro Taper F2 file using the technique of reciprocal turns (630 cycles) compared to full rotation (160 cycles) and confirmed that higher speed increases the possibility of fracture. Gambarini et al. [45] and Castelló-Escrivá et al. [46] have also confirmed that reciprocal movements extend the life of the NiTi file and indicated that greater resistance is affected by the speed and cutting angles [45], i.e. the type of NiTi alloy and the diameter of the canal curvature. A study by Neelakantan et al. [47] has tested the cyclic fatigue resistance of single files in double-bent "S" canals and found that files with reciprocal movements (Reciproc) showed significantly higher resistance compared to files with full rotation (One Shape).

The advantages of files with reciprocal movements have been confirmed in a research related to canal cleaning and shaping, cutting efficiency and retreatment [7, 9, 31, 32, 35, 36, 43].

Compared to those with full rotation, files with reciprocal movements showed higher resistance to cyclic fatigue, better cutting efficiency, shorter preparation time and slightly higher accumulation of debris in the canal [48]. In an *in vitro* study by Yilmaz et al. [49] (with an intracanal temperature of 35°C), the full-rotation file (Hy Flex Edm) has shown greater resistance to cyclic fatigue compared to files with reciprocal movements (Wave One, Reciproc Blue). Azim et al. [50] have compared several systems with single files and different dynamics of movement and pointed to high resistance to cyclic fatigue of XP Endo Shaper. In a study by Alcade et al. [38], it has been confirmed that the cyclic fatigue of the file with reciprocal movements is also affected by the type of NiTi alloy the file is made of.

Al Sudani et al. [51] have examined the effect of cutting angle on the efficiency of reciprocal NiTi files and pointed out that Wave One removes the least dentin, that files with a larger cone have a weaker cutting effect and poorer centering, that all files lead to transportation, and that files with a cutting angle of less than 30° have greater resistance to cyclic fatigue.

Files with a smaller cone are more efficient in shaping the "S" canal [52], and reciprocal files (Reciproc, Wave One) are less efficient in preserving the curve [53]. Saber et al. [54] confirmed that files with reciprocal movements better keep the curve, and files with full rotation provide faster instrumentation. A study by Elashiri et al. [55] has confirmed the effective canal shaping with full-rotation files, as well as with files with reciprocal movements, with slightly better centering and greater apical extrusion in both.

The results of the research of files with reciprocal movements showed that complete cleaning of the canal is an unachievable goal and that it mostly depends on: the design of the working part, the design of the tip, alloy and its thermal treatment or movement dynamics during the preparation [2, 7, 35, 36, 56–59].

The spectral segment of the canal is a special issue in cleaning [7, 56, 59–62]. Data on canal transportation (formation of a new path deviating from the original morphology) and extrusion (transfer of cut dentin, bacteria) during the preparation generally indicate similar findings of reciprocal and full-rotation files [7, 35, 63, 64]. In addition to efficient cutting, better centering is also an important benefit of reciprocal files because the file follows the path of less resistance and can be used without creating patency with hand files [35, 41]. Studies by Dhingra et al. [63] and Zan et al. [64] have shown that reciprocal movements show better centering, fewer dentin fissures and less transportation , or less extrusion of bacteria into periapical structures.

A meta-analysis of the influence of single reciprocal and two sets of rotary files have confirmed that all files lead

to apical extrusion and that the inflammatory response in the periapex does not depend on the number but on the design and kinematics of file movement [65]. In the preparation of bent canals, single NiTi files with full rotation and reciprocal are equally safe and efficient because they do not change the curvature and do not lead to the canal transportation [66, 67].

When preparing the mesiobuccal canal of the first upper molar, full-rotation files provide better centering and less transportation than files with reciprocal movements [68]. The research has also confirmed that files with reciprocal movements provide shorter treatment times and less debris compared to multi-file and full-rotation sets [69].

Several studies have confirmed the efficacy of files with reciprocal movements in the retreatment of inadequate endodontic healing and confirmed the possibility of their use in gutta-percha removal in both straight and curved canals [70–72]. Although data on the clinical use of single files with reciprocal movements are very scarce, available studies confirm their efficiency in biomechanical preparation, which lasts shorter [73], by reducing postoperative pain after endodontic intervention [73, 74], efficient apical preparation and reducing the number of bacteria [75, 76], i.e. efficient canal obturation with single gutta-percha point after the preparation with a single file [77].

A recent research has confirmed that the use of NiTi files with reciprocal movements is a safe and simple concept of canal preparation [78, 79]. In a retrospective clinical study, postgraduate students have prepared 2,056 canals without problems and with a small percentage of file fractures [78], and in another study files with reciprocal movements were a very safe and acceptable method for practical endodontic education [79].

Single files with full rotation

The use of NiTi files with full rotation is standard in endodontics despite frequent fractures due to possible screwing in the canal [2, 7, 9, 31, 35]. The fear of possible breakage is further increased in sets with more files, so dental technology has tried new concepts of preparation with a smaller number of files in a set (three) or with only one file [7, 9, 31–33].

It has also been confirmed that NiTi files make it difficult to reach all parts of the canal (30–50% of the wall surface remains intact), so using only one file significantly reduces the efficiency of chemomechanical procedures and makes it difficult to clean inaccessible parts (isthmus, ramification, additional canals, diverticula) [1–4, 7, 32]. Cleaning and shaping canals with a single file in full rotation significantly speeds up instrumentation, but also reduces the risk of fracture (one file instead of a set) [2, 9, 31, 32, 35]. Systems with single files, made of special NiTi alloy with special thermal treatment, have increased flexibility and resistance to cyclic fatigue and provided efficient cleaning and shaping of very complex canal systems (narrow, bent) with shorter processing time [31, 32, 35, 56, 57, 80–83]. Single files with full rotation are a completely new generation of files made of Max Wire alloy with a unique geometry of the working part (asymmetric cross section) and often with a shifted center of rotation that allows “wavy” movement and more efficient cutting with reduced possibility of screwing [9, 31, 32, 37]. The combination of flexibility and fracture resistance ensures efficient preparation of bent canals while preserving the original anatomy, with a reduced risk of transportation and perforation [32, 84–86].

Since 2011, dental technology has introduced a number of single files that use full rotation for canal preparation: Hy Flex EDM (Coltene, Whaledent, Alstatten, Switzerland),One File EDM (Coltene, Whaledent, Alstatten, Switzerland), Neoniti (Neolix, Sas, Chatres-La-Foret), One Shape (Micro Mega, Besancon, France), One Curve (Micro Mega, Besancon, France), F 360 (Komet, Brasseler, Lemgo, Germany), T File (India), Edge File (Edge Endo, Albuquerque, Nm), F6 Sky Taper (Komet, Milan, Italia), Pro Taper Next (Dentsply, Maillefer, Ballaigues, Switzerland), Revo S (Micromega).

The analysis of the functionality and efficiency of single files with full rotation has shown that in bent canals, the file in the area of curvature is exposed to repeated and alternating cycles of flexion and compression, which usually leads to fracture. It has been confirmed that NiTi rotary files can withstand several hundred of these cycles, but can break suddenly after a short rotation in the canal [32, 35, 87].

The data from the available literature confirm that the cyclic fatigue of the full rotation file is influenced by numerous factors, primarily: the angle and diameter of the canal curvature, file size and conicity, kinematics of movement, rotation speed, sterilization effects, or skills and experience of the endodontist [87–92].

In a study by Topçuoğlu et al. [87] on the resistance to cyclic fatigue of single files with full rotation, the One Curve (C Wire alloy) and Edge File (Fire-Wire alloy) systems have shown higher resistance than Hy Flex (CM alloy) in double-bent “S” canals and at different temperatures (20°C, 35°C).

The resistance of files with previously heat-treated alloy (Hy Flex EDM, Vortex Blue, Pro Taper Gold, One Curve) to cyclic fatigue has been tested in a similar study and it has confirmed that Hy Flex EDM files have the highest resistance to cyclic fatigue, followed by One Curve, and that the Pro Taper Gold and Vortex Blue files are less resistant [10, 93].

The influence of temperature [94, 95], thermal treatment and design on the cyclic fatigue of three generations of single files with full rotation indicated superior features of the One Curve system compared to One Shape New Generation at all tested temperatures [96]. In a study by La Rosa et al. [95], it has been shown that single files with full rotation have a decrease in resistance to cyclic fatigue at body temperature (35°C) while lower irrigation temperature (20°C) has no effect.

The examination of the efficacy of the preparation in the first lower molars showed that there were no significant differences in the occurrence of canal transportation between different rotational systems in the apical and middle third of the canal. The file with full rotation (Neoniti) led to transportation in the coronal third due to the pronounced conicity of this file of 8% [96].

The studies on the clinical application of single files with full rotation, although there have not been many, have indicated a significant simplification of instrumentation protocols, reduced risk of cross-contamination, increased exposure to high torsional stress and cyclic fatigue, and the need for mandatory file rejection after the treatment [97, 98].

The enviable clinical performance of the F6 Sky Taper file with full rotation has been confirmed in the effective endodontic therapy of the upper first and second molars. Biomechanical instrumentation is realized with the 6% conicity file, which preserves the original curvature of the canal even at very pronounced curves [99].

Single files with eccentric rotational motion

Some NiTi files rotate asymmetrically and eccentrically in the canal, which enables more efficient cleaning and shaping of canal systems with irregular morphology, because the file can reach inaccessible parts of the canal [7, 32].

XP Endo Shaper is a unique file of the new generation of NiTi files with a "snake-like" shape and unique working part geometry, which, thanks to its exceptional flexibility, contracts and expands during rotation and thus effectively adapts to canal system irregularities. It is made of the special Max Wire alloy (FKG) which provides greater flexibility and increased resistance to cyclic fatigue, and thus more efficient cleaning with a significantly accelerated canal processing procedure. Increased speed and "snake-like" shape provide increased "turbulence" of the irrigation solution, which keeps the dentinal debris in a liquid state and removes it more efficiently from the canal walls [7, 32, 100–103].

A research has confirmed that, after the preparation of the canal with XP Endo Shaper, less smear layer remains on the walls, primarily due to adequate dimensions of the apical preparation (30.04) and better effects of irrigation solution [100, 101]. There was no difference in cleaning efficiency of oval canals using the Tru Shape, Xp Endo Shaper and SAF files [100], while XPS showed exceptional resistance to cyclic fatigue [102, 103].

Compared to Reciproc Blue and HY Flex Edm files, this single file (XPS) caused significantly less apical extrusion of dentinal debris [103].

Single files with transaxial movement

In order to find a file that could meet all the requirements of optimal canal preparation, dental technology provided practitioners with a self-adjusting NiTi file with a completely different design and kinematics of movement in the canal.

The self-adjusting file (SAF) is hollow and flexible, and it adapts to the shape of the canal in three dimensions, while it moves transaxially during chemomechanical processing. This unusual file design allows both continuous and highly efficient canal irrigation because the hollow file is connected to a source for continuous irrigation via a silicone tube. Vibration and movement of the file during instrumentation lead to "turbulence" and additional activation of the solution, which significantly increases its solvent effect and the cleaning efficiency of the canal system [31, 104, 105]. SAF has high fracture resistance, preserves the original canal morphology and provides fast and efficient cleaning of all parts of the canal (4 minutes), thanks to continuous irrigation and always fresh solution [105].

A scanning electron microscope (SEM) analysis of the canal cleaning efficiency has shown that SAF, in combination with continuous irrigation, provides efficient cleaning in the apical zone of the canal [104]. The fact that the therapist has the ability to simultaneously and alternately use irrigants (NaOCl, EDTA) and choose the strength of the solution, gives this NiTi file high performance in cleaning even very complex (highly bent) canal systems [105].

Conclusion

Great progress in endodontic treatment has been made with the introduction of NiTi rotary files. The special treatment of NiTi alloy and the specific design of the working part provide exceptional flexibility and efficiency of the files in different canal systems, but the frustrations of practitioners due to possible fractures are still present.

Canal preparation with a single file is a promising concept where a single file is used for complete cleaning and shaping of the canal. This significantly shortens the instrumentation (3–4 times), reduces file fatigue and possible cross-contamination, saves time for the patient and the therapist, and reduces the risk of fracture (only single file is used, not a set of files).

The concept of canal therapy with a single file includes files with full rotation, reciprocal movements, a combination of rotational and reciprocal, i.e. eccentric rotational and transaxial movements.

Files with reciprocal movements enable fast, simple, safe and less stressful instrumentation of most canals, with a significantly lower risk of fracture.

Full rotation of single files is a good choice for fast and safe canal treatment with respect to clinical protocol and with the use of a traditional endodontic motor.

Files with eccentric rotational movement achieve fast and efficient instrumentation, good adaptation of the file to canal irregularities, and provide greater resistance to cyclic fatigue by balanced contact with the walls.

Transaxial movement of the self-adjusting file, with always fresh and continuous irrigation, ensures fast and efficient cleaning of all parts of the canal system.

There is still no file that fully meets all the requirements of optimal canal preparation, regardless of the fact that canal therapy with a single file is more cost-effective and lasts shorter. Additional studies and strategies for improving new materials and techniques are needed, in order to ensure the efficiency and safety of endodontic files during canal treatment.

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Received on May 24, 2021
Revised on August 3, 2021
Accepted on August 4, 2021
Online First August, 2021

CASE REPORT

UDC: 617.571:616-006-089
DOI: https://doi.org/10.2298/VSP200530073A

Large hemangiopericytoma of the shoulder: A case report

Veliki hemangiopericitom ramenog pojasa

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Abstract

Introduction. Hemangiopericytoma is a rare tumour derived from the pericytes, contractile spindle cells that surround the capillaries and postcapillary venules. The tumour is found equally among males and females. **Case report.** We reported a case of a 63-year-old female who presented a giant painful mass on her right shoulder that occurred one year before admission. Limited range of motion and a sense of tingling along the affected arm was present also. An irregular, oval-shaped mass, dark red in colour, with signs of necrosis, was 14 cm in maximum diameter. Routine laboratory analysis showed results within the range of referenced values. Lung X-ray and ultrasonographic examination of the abdomen showed no signs of secondary tumour deposits, and no osteomuscular lesions in the affected region. Ultrasonographic examination of the neck and right axillary region showed no signs of regional metastases. The surgical excision of the entire tumour was performed, with the associated subcutaneous tissue and a part of fascia underneath. Results of the histopathological analysis confirmed the diagnosis of hemangiopericytoma. The specimen showed no signs or elements of the neoplastic tissue on the edges of the resection lines. Three years after the operation, there were no signs of tumour relapses, regional or systemic metastases. **Conclusion.** Considering that there are no official clinical guides and protocols for hemangiopericytoma management, as well as the occurrence of cutaneous and subcutaneous hemangiopericytomas is exceptionally rare, more extensive research in this field and more described cases are needed to gain a better understanding of the issue.

Key words:
diagnosis; hemangiopericytoma; histological techniques; reconstructive surgical procedures; shoulder; ultrasonography.

Apstrakt

Uvod. Hemangiopericitom je redak tumor porekla pericita, kontraktilnih vretenastih ćelija koje okružuju kapilare i postkapilarne venule. Tumor se javlja podjednako u muškoj i ženskoj populaciji. **Prikaz bolesnika.** Prikazana je 63-godišnja bolesnica sa džinovskom bolnom promenom na desnom ramenu, primećenu godinu dana pre prijema. Žalila se na ograničen opseg pokreta kao i osećaj trnjenja u zahvaćenoj ruci. Promena je bila nepravilno ovalnog oblika, tamnocrvene boje, sa znakovima nekroze, maksimalnog prečnika 14 cm. Rutinske laboratorijske analize pokazale su rezultate u granicama referentnih vrednosti. Rendgenski snimak pluća i ultrasonografski pregled abdomena nisu pokazali znakove sekundarnih depozita tumora, kao ni osteomišićne lezije u zahvaćenom regionu. Ultrazvučni pregled vrata i desne aksilarne regije nije pokazao znakove regionalnih metastaza. Izvršena je hirurška ekscizija celog tumora sa pripadajućim potkožnim tkivom i delom fascije ispod. Rezultati histopatološke analize potvrdili su dijagnozu hemangiopericitoma. Uzorak nije pokazao znakove ili elemente neoplastičnog tkiva na ivicama resekcionih linija, a 3 godine nakon operacije nisu bilo znakova relapsa tumora, regionalnih ili sistemskih metastaza. **Zaključak.** S obzirom na činjenicu da ne postoje zvanični klinički vodiči i protokoli za lečenje hemangiopericitoma, kao i da je pojava kožnih i potkožnih hemangiopericitoma izuzetno retka, potrebno je opsežnije istraživanje na ovu temu i više prikaza slučajeva da bi se steklo bolje razumevanje problema.

Ključne reči:
dijagnoza; hemangiopericitom; histološke tehnike; hirurgija, rekonstruktivna, procedure; rame; ultrasonografija.

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Introduction

First description of a hemangiopericytoma (HP) in literature appeared in a paper published by Stout and Murray [1] in 1942. The authors stated that HP (peritelioma – an older name) is an unusual mesenchymal neoplasm. It is believed that HP arises from the pericytes, contractile spindle cells that surround the capillaries and postcapillary venules [2]. Pericytes are described as modified smooth muscle cells or resting stem cells which are capable of differentiating in myoid, fibrohistiocytic and endothelial cells. The tumour is found equally among males and females [3]. HP is found to appear at any age but is most common in the sixth and seventh decade [4]. HP is primary an adult neoplasm, but occurrence in children is possible also. Considering that, adult and infantile forms of HP are described in the literature [5]. Some types of HP (such as glomangiopericytoma) are associated with previous trauma, hypertension, pregnancy or steroid usage but definitive aetiology is still insufficiently clarified [6]. The probability of a tumour forming is greater wherever the tissue abounds in capillaries. It has been stated that HP may occur at any anatomic site and tends to develop from subcutaneous tissue or skeletal muscle. However, in cases reported so far there are areas more affected such as the lower extremities (above the knee) and retroperitoneum [7], soft tissue of trunk and upper extremities [8], head and neck [9], thoracic cavity [10]. In the head and neck region, they are usually seen in the nasal cavity, orbit, jaw, parotid gland, and oral cavity [11]. HP also could arise intracranially within the central nervous system (CNS) and account for approximately 0.4% of all CNS tumours [12]. The majority of HPs have a profound localization, inside the muscle tissue or viscera, with rare cases where the tumour infiltrates only cutaneous and subcutaneous tissue, without deeper propagation [5]. HP of the upper extremity is rare, but in 2012 authors have reported a case of a HP in the dorsal region of the hand [13], with only several other cases described on the upper extremity. Rare occurrence of the cutaneous HP, giant size reached in our patient, and the specific localization, previously not reported in the literature, prompted us to describe our case of this unusual neoplasm in the shoulder region.

Case report

We present a case of a 63-year-old female patient who was referred to the Department of Plastic Surgery with a giant painful mass on her right shoulder. The patient was presented with pain, limited range of motion and a sense of tingling along the affected arm, on the initial examination. The tumour was present one year prior to the initial manifestation of the symptoms, when the patient noticed a growth of the neoplasm with occasional bleeding. The neoplasm was described as an irregular, oval shaped mass, coloured dark red, with signs of necrosis on different regions of the tumour and occasional bleeding and secretion (Figure 1). It was localized in the right supraclavicular region, with maximal diameter of 14 cm, on a wide base with a vague demarcation line toward the surrounding skin. The surface of the tumour was uneven, striated with scabs and fields of secondary necrosis. Surrounding skin showed signs of reactive hyperaemia. Routine laboratory analysis and coagulation time, which were performed as a part of preoperative diagnostics, showed results within the range of referenced values. Thorough history and clinical examination of the patient did not reveal any problems concerning other organ systems. Lung X-ray and ultrasonographic examination of the abdomen showed no signs of secondary tumour deposits, and no osteomuscular lesions in the affected region. Ultrasonographic examination of the neck and right axillary region showed no signs of regional metastases. Under general anaesthesia, wide excision was performed. Entire tumour was removed, with the associated subcutaneous tissue and a part of fascia underneath. (Figure 2). Intraoperatively, we found that the muscle tissue was unaffected by the tumour. Defect was closed primarily by split thickness skin graft. The resected tissue was sent to the Department of Pathology for histopathological analysis. Results of the histopathological analysis confirmed the diagnosis of HP (Figure 3). The specimen showed no signs or elements of the neoplastic tissue on the edges of the resection lines. The patient was on the hospital treatment for 12 days after the surgery. During the routine

Fig. 1 – Large, well-circumscribed reddish growth on the right arm skin.

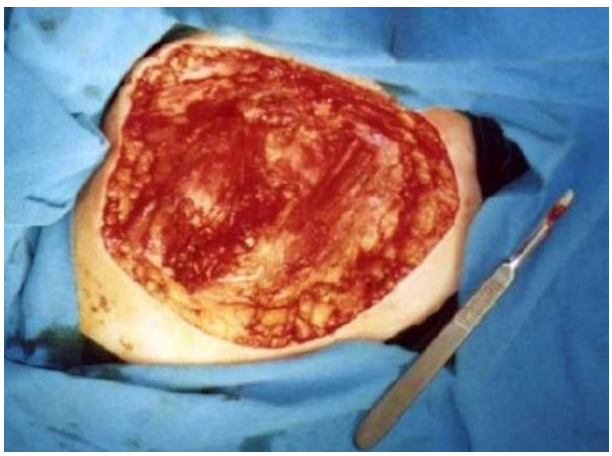

Fig. 2 – Wide excision of the lesion and post excisional skin and soft tissue defect.

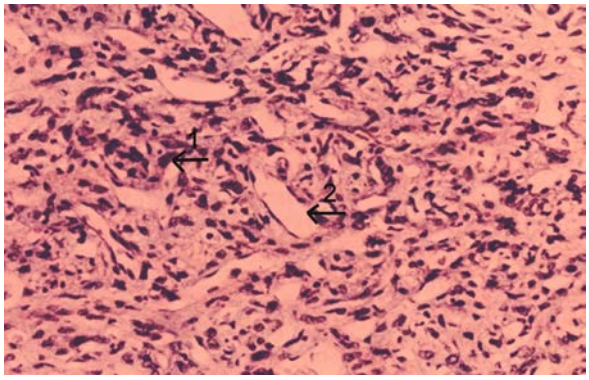

Fig. 3 –Arrow 1: tumour cells around vascular spaces (peritelial proliferation) in short fascicular arrangements; Arrow 2: numerous branched vascular spaces bounded by a single-row endothelium (haematoxylin and eosin staining, x10).

postoperative check-ups, which were performed regularly 3 years after the operation, we found no signs of tumour relapses, regional or systemic metastases. Functional and aesthetic result of the surgery were satisfactory (Figure 4).

Fig. 4 – Aesthetic and functional result one year after excision and skin grafting.

Discussion

HP manifest as slow growing, firm, and painless masses. Despite the fact that the uniform clinical presentation of HP does not exists, the pain is reported as a late symptom [14]. The reason for that phenomenon is most likely compression of the neurovascular structures. Absence of direct innervation of the tumour is most likely the reason why it is not uncommon that patients do not present with symptoms until the mass reaches considerable size. Beside the pain, enlarging of the tumour in our patient was the main reason for limited range of motion and a sense of tingling along the affected arm, because the tumour invaded the cutaneous nerves in the shoulder region of our patient. Regarding the upper extremity, a case of HP of the dorsal region of the hand was described in literature [13]. Since both are localized in the upper extremity, that case could be compared with our patient. However, the difference between them is manifested by various symptoms, as the tumours affected different regions of the upper extremity. Besides the fact that it is very important to distinguish the benign or malignant morphology of the tumour, which significantly influences the course of treatment, two clinical syndromes of HP (infantile and adult) have been described in the literature, depending on the age at which it occurs. Certain authors consider the infantile type of HP to occur before the age of 1, while others set the limit at 5 years. The cases that appear after those age marks were defined as adult types of HP [5]. Infantile and adult types of HP differ clinically and pathologically from one another. Infantile types occur more often as cutaneous and subcutaneous head and neck lesions, displaying benign behaviour despite the appearance of certain histological patterns such as hypercellularity, necrosis, bleeding and increased mitotic proliferation. There have been reports in the literature of multiple infantile HPs in the head and neck region [15]. The interesting fact in our case is that we found an adult form of HP with cutaneous and subcutaneous localization, which is, according to the literature, characteristic of the infantile type of this tumour. Enzinger and Smith [16] reported that 4 out of 9 infantile types of HP were morphologically benign in their study of 106 cases. Depending on the localization of the tumour, a large variety of symptoms were described in the same study. For example, tumours localized in the pelvic fossa and the retroperitoneum caused urinary retention, hydronephrosis, dysuria, nocturia, constipation and haematuria. Tumours situated at other sites, such as upper respiratory pathways caused epistaxis, cough, dyspnoea, while symptoms such as vomiting and distention occurred with digestive tract affection. The mentioned symptoms were a consequence of local tumour invasion. Furthermore, various paraneoplastic symptoms were described following the appearance of HP. Hypoglycaemia has been reported in about 5% of patients with HP, with the most frequent localization of the tumour in the retroperitoneum and the pelvic area. Benn et al. [17] have showed in 1990 that hypoglycaemia is most probably caused by the production of the insulin-like growth factor in the tumour. The most likely proposed mechanisms were increased tumour glucose uptake, decreased hepatic output and increased glucose tissue utilization. Another described paraneoplastic manifestation associated with HP is hypophosphatemic osteomalacia [18]. Also, there is a reported case of paraneoplastic rhinophyma-like nasal swelling with Leser-Trélat sign which was resolved postoperatively [19]. The sign of Leser-Trélat is described in the literature as an association of eruptive, pruritic, seborrheic keratoses with occult internal malignancy and any appearance of this sign should raise suspicion of an underlying malignancy. During the preoperative

diagnostics and routine postoperative check-ups during the follow-up period (three years), our patient showed no signs of metabolic disbalance or manifestations in other organ systems, even though certain paraneoplastic symptoms are reported in the literature. Histological analysis of the tumour showed pericytoma-like vascularization, which may be seen as a secondary vascular pattern in other mesenchymal lesions, hence the differentiation of benign or malignant forms of HP is made by exclusion [20]. That is essential to stand out, because HP, along with certain other neoplasms such as synovial sarcoma and solitary fibrous tumour, is not distinctly classified, and presents a problem in confirming the correct diagnosis due to pathologic similarities between these tumours. Histological confusion with synovial sarcoma and solitary fibrous tumour exists because of the same pericytoma-like vascularization pattern in all three neoplasms [1]. Histopathologically observed, benign forms of HP feature bland-oval or spindle cells, immersed in a reticulin network, and arranged around an elaborate gaping vasculature, without endothelial proliferation [20]. Perivascular hyalinization is commonly present, which was discovered and confirmed in our case, along with the other benign characteristics. On the other hand, HPs with aggressive, anaplastic features, such as atypia, high mitotic activity, haemorrhage and necrosis, are defined as malignant. Possible routes of HP metastasis could be explained by three pathways: direct extension, via the lymphatics, and hematogenous pathway, which is the most frequent route [21]. Considering the difficulty to predict biological behaviour of this tumour, it is useful to know that the prognostic value includes increased cellularity, anaplasia, necrosis, haemorrhage and prominent mitotic activity detected by microscope, which could be amplified using a proliferation index detected by immunohistochemical techniques. The proliferation index of 10% or greater may indicate a more aggressive type of this rare tumour [15]. The criteria for malignancy proposed by Enzinger and Smith [16] in classical HP identify overtly malignant or high-grade lesions but fail to address low-grade lesions. In their study, large-sized tumours (> 5 cm), increased the mitotic rate, high cellularity, presence of immature and pleomorphic tumour cells, and foci of haemorrhage and necrosis predicted a highly malignant course. Enzinger and Weiss [16] employed the term ”low-malignant potential“ for lesions with lower levels of mitotic activity, particularly if they have any degree of atypia and cellularity [22]. Metastases were noted in approximately 30% of patients, with a 5-year survival of 71%. The most common metastatic sites were the lungs, bones and liver [23]. Occurrence of metastases several years after the excision of the primary lesion is a frequent phenomenon regarding HP and therefore long-term follow-up is essential [24]. Current consensus regarding the treatment of HP is wide, radical surgical excision with the radiotherapy follow-up if the tumour shows malignant characteristics [25]. We performed wide surgical excision, with extirpation of the tumour in full and radical resection to clean edges, without adjuvant radiotherapy, since histopathological analysis showed benign characteristics of the tissue.

One of the main factors of increased surgical morbidity and mortality is the increased hypervascularization in the tumour region, which makes the treatment difficult. Since the tumour originates from cells which are a part of the vascular system and blood vessels, it is recommended to detect the type and extension of the circulation in the neoplasm, and, if necessary, perform the preoperative ligation of afferent vessels or vascular embolization which could reduce perioperative haemorrhage and facilitate further management [5]. During the diagnostics and treatment, it should be kept in mind to consider the possibility of complex vascular syndromes because HP originates from pericytes. For example, there may occur multiple haemangiomas and multiple organ cysts such as Von Hippel-Lindau or other anomalies. In this case, in addition to the radical surgical treatment, a multidisciplinary approach would be important.

Conclusion

Early histological diagnosis of HP is essential and wide surgical excision is recommended. Considering that there are no official clinical guides and protocols for HP management, more extensive research in the field and more described cases are needed to gain a better understanding of the issue. The fact that the occurrence of cutaneous and subcutaneous HP is exceptionally rare, give this particular case report great clinical significance and further our insight in the pathology of this neoplasm.

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Received on May 30, 2020
Accepted on July 6, 2020
Online First July, 2020

C O R R I G E N D U M

DOI: https://doi.org/10.2298/VSP2201099E

The article "Prevalence of vitamin D3 deficiency in patients with type 2 diabetes and protinuria" published in the December 2021 print issue of the Vojnosanitetski pregled (Vojnosanit Pregl 2021; 78(12): 1292–1302; https://doi.org/10.2298/VSP200220064S) by Tatjana Stojšić Vuksanović and Violeta Knežević, contains error with respect to the affiliation of Violeta Knežević. Instead of the existing affiliation "Clinical Center of Vojvodina, Clinic for Nephrology and Clinical Immunology, Novi Sad Serbia", there should be "University Clinical Center of Vojvodina, Clinic for Nephrology and Clinical Immunology, Novi Sad, Serbia".

The article has been corrected Online [1].

1. *Tatjana Stojšić Vuksanović, Violeta Knežević*. Prevalence of vitamin D3 deficiency in patients with type 2 diabetes and protinuria. Vojnosanit Pregl 2021; 78(12): 1292–1302.
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Rukopis se piše sa proredom 1,5 sa levom marginom od **4 cm**. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize i članci iz istorije medicine ne smeju prelaziti 16 stranica (bez priloga); aktuelne teme – deset, seminar praktičnog lekara – osam, kazuistika – šest, prethodna saopštenja – pet, a komentari i pisma uredniku – tri, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

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Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** rada, zahvalnost (po želji), literatura, prilozi.

1. ***Naslovna strana***

a) Poželjno je da naslov bude kratak, jasan i informativan i da odgovara sadržaju, podnaslove izbegavati.

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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.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost nadležnog etičkog komiteta.

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.

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Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u levom 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