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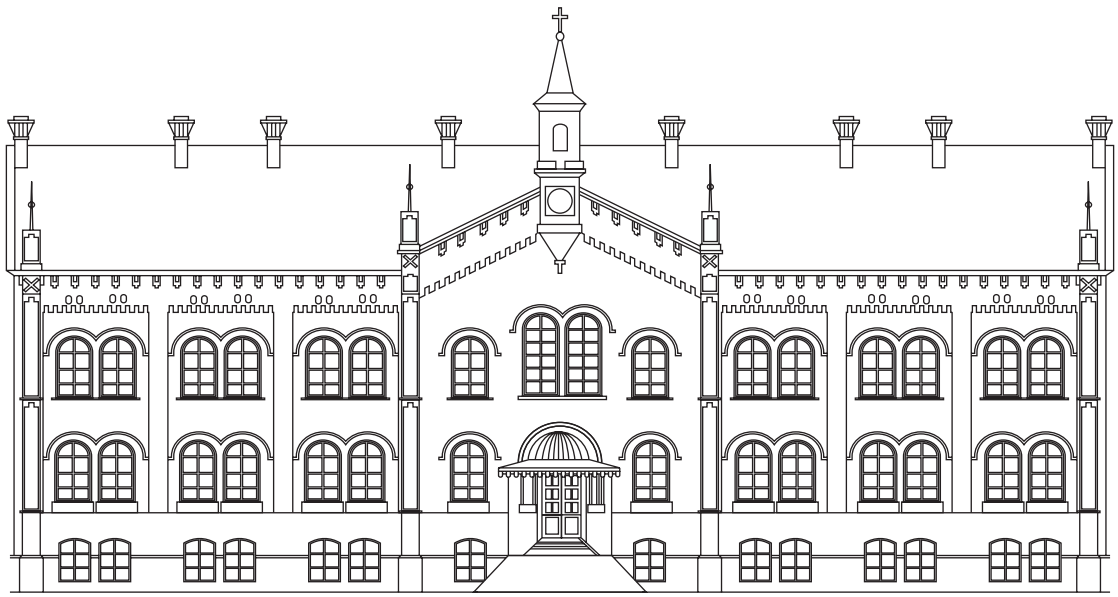
СРПСКИ АРХИВ
ЗА ЦЕЛОКУПНО ЛЕКАРСТВО

ЧАСОПИС СРПСКОГ ЛЕКАРСКОГ ДРУШТВА



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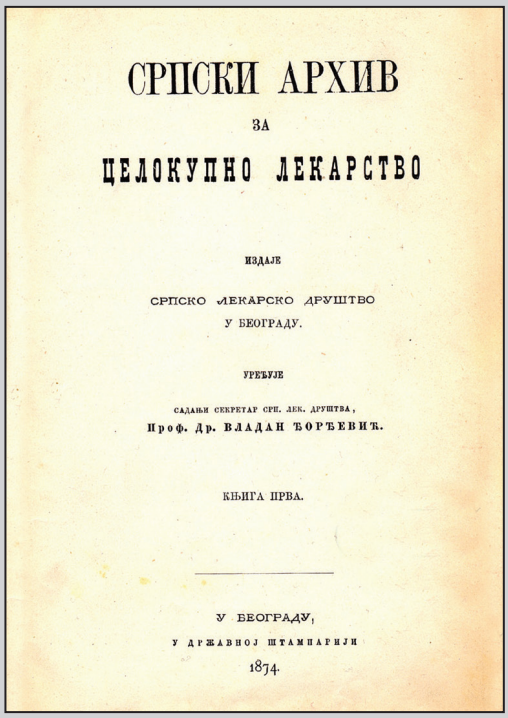

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

ИЗДАЈЕ

СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

УРЕЂУЈЕ

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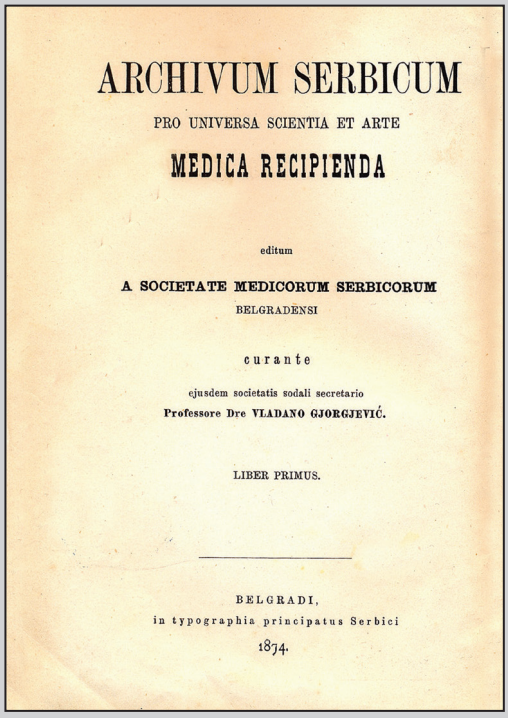
У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

Прва страна првог броја часописа на српском језику



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in typographia principatus Serbici

1874.

The title page of the first journal volume in Latin

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Српско лекарско друштво
Џорџа Вашингтона 19, 11000 Београд, Србија
Председник
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Интернет страна: http://www.sld.org.rs

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Српски архив за целокупно лекарство
Краљице Наталије 1, 11000 Београд, Србија
Телефон: +381 (0)11 409 27 76
+381 (0)11 409 44 79
Е-пошта: office@srpskiarhiv.rs
Интернет страна: www.srpskiarhiv.rs

ПРЕТПЛАТА И ЕКСПЕДИЦИЈА
Српско лекарско друштво
Џорџа Вашингтона 19, 11000 Београд, Србија
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EDITORIAL OFFICE
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САДРЖАЈ • CONTENTS

ORIGINAL ARTICLES • ОРИГИНАЛНИ РАДОВИ

Milica Jovanović-Medojević, Dragana Jovanović, Mina Medojević, Strahinja Nedić, Đorđe Stratimirović, Sanja Vujašković, Mirjana Vujašković
The prevalence and severity of molar incisor hypomineralization in group of students – a cross-sectional study 128–139
Милица Јовановић-Медојевић, Драгана Јовановић, Мина Медојевић, Страхиња Недић, Ђорђе Стратимировић, Сања Вујашковић, Мирјана Вујашковић
Заступљеност и тежина хипоминерализације молара и секутића у групи студената – студија пресека

Igor Đukić, Zoran Jezdić, Mirsad Kadić, Anesa Pirović Kadić, Ognjenka Janković, Aleksandra Radulović, Ivan Arsić, Aleksandar Jakovljević, Nenad Nedeljković
Exploiting maxillary sinus dimensions for sexual determination in the Bosnian and Herzegovinian population 140–145
Ігор Ђукић, Зоран Јездић, Мирсад Кадић, Анеса Пировић Кадић, Огњенка Јанковић, Александра Радуловић, Иван Арсић, Александар Јаковљевић, Ненад Недељковић
Коришћење димензија максиларног синуса за одређивање пола у босанскохерцеговачкој популацији

Uroš Anđelić, Marko Uzelac, Filip Filipović, Mihailo Ille, Marija Đurić, Jelena Jadžić
Trabecular microcalli in lumbar vertebrae of adult men with alcohol-associated liver disease: postmortem micro-computed tomography assessment 146–152
Урош Анђелић, Марко Узелац, Филип Филиповић, Михаило Илле, Марија Ђурић, Јелена Јаџић
Трабекуларни микрокалуси слабинских кичмених пршљенова код одраслих мушкараца са алкохолном болести јетре: постмортална анализа методом микро-компјутеризоване томографије

Liuxiu Yu, Mingfeng Xiong, Xiaoyu Li, Chunhong Wu, Liqin Wang, Yao Gao, Minxia Long, Qin Gan
Efficacy of Xiaozhoutian fire dragon moxibustion combined with traditional Chinese medicine on polycystic ovary syndrome with kidney yang deficiency – a randomized controlled trial 153–162
Љусју Ју, Минфенг Сјунг, Сјаоју Ли, Чунхонг Ву, Лићин Ванг, Јао Гао, Минсја Лонг, Ћин Ган
Ефикасност моксибустије Сјаоцоутијан ватреног змаја у комбинацији са традиционалном кинеском медицином на синдром полицистичних јајника са недостатком јанга бубрега – рандомизовано контролисано испитивање

Saša S. Živić, Ivan Nikolić, Marko Jović, Vladimir Petrović, Dragan C. Bogdanović, Vladimir Stojiljković, Aleksandar Kamenov
Morphometric characteristics of the great saphenous vein as graft for surgical myocardial revascularization in relation to sex 163–169
Саша С. Живић, Иван Николић, Марко Јовић, Владимир Петровић, Драган Ц. Богдановић, Владимир Стојиљковић, Александар Каменов
Морфометријске карактеристике велике сафенске вене као графта за хируршку реваскуларизацију миокарда у односу на пол

Željka Rogač, Aleksandar Dimitrijević, Ivana Andrić, Vesna Miranović, Dejan Stevanović
The impact of certain anti-seizure medications on cognitive status, behavior, anxiety, and depression in school-aged children with newly diagnosed epilepsy – a six-month follow-up study 170–175
Жељка Роґач, Александар Димитријевић, Ивана Андрић, Весна Мирановић, Дејан Стевановић
Утицај појединих антиепилептичких лекова на когнитивни статус, понашање, анксиозност и депресију код деце школског узраста са новодијагностикованом епилепсијом – студија шестомесечног праћења

Jera Grabnar, Rina R. Rus
Renal injury in children with a congenital solitary kidney – a single center experience 176–180
Јера Грабнар, Рина Р. Рус
Оштећење бубрега код деце са урођеним солитарним бубрегом – искуство једног центра

Marina Nikitović, Aleksandar Stepanović, Tatjana Arsenijević, Nina Petrović
Relation of oncogenic microRNA-10b and microRNA-21 to glioblastoma size and localization. 181–186
Марина Никитовић, Александар Степановић, Татјана Арсенијевић, Нина Петровић
Повезаност онкогених микрорнк-10б и микрорнк-21 са величином и локализацијом глиобластома

Xiaomei Huang, Hanxue Cai, Wen Zeng, Xueqin Zeng, Xinhong Xiao, Yi Liu, Yanhong Xu, Xiaomei Liu, Qinghua Zhong
Effect of auricular point acupressure combined with three-step analgesic therapy on cancer pain 187–192
Сјаомеј Хуанī, Ханшуе Цај, Вен Ценī, Сјуецин Ценī, Сјинхонī Сјао, Ји Љу, Јанхонī Сју, Сјаомеј Љу, Ћинīхуа Јонī
Ефекат комбинације акупресуре аурикуларних тачака и тростепене аналгетске терапије на бол код оболелих од карцинома

CASE REPORTS • ПРИКАЗИ БОЛЕСНИКА

Sofija Jovanović, Tijana Tomić, Nemanja Bidžić, Miloš Raspopović, Dragan Vasin
Familial adenomatous polyposis and colorectal cancer – how sensitive is computed tomography in detecting the underlying disease? 193–196
Софија Јовановић, Тијана Томић, Немања Бицић, Милош Расīоповић, Драīан Васин
Породична аденоматозна полипоза и колоректални карцином – колико је компјутеризована томографија осетљива метода за откривање основне болести?

Bojan Milenković, Miljan Bilanović, Slađan Timotijević, Miroslav Tatić, Đorđe Kolak
Isolated ipsilateral shoulder and elbow dislocation 197–200
Бојан Миленковић, Миљан Биланović, Слађан Тимоīијевић, Мирослав Таīић, Ђорђе Колак
Изолована ипсилатерална луксација зглоба рамена и зглоба лакта

Aleksandar Sretenović, Đorđe Topličić, Rastko Sava Jeremić, Dragana Vujović
Biliary atresia associated with intestinal malrotation – unusual intraoperative presentation 201–204
Александар Срећеновић, Ђорђе Тоīличић, Расīко Сава Јеремић, Драīана Вујовић
Билијарна атрезија повезана са малротацијом црева – необична интраоперативна презентација

Nikola Pantić, Milica Stojiljković, Lenka Grujičić, Dragana Šobić Šaranović, Vera Artiko
^{18}F-fluorodeoxyglucose positron emission tomography / computed tomography in the diagnosis of secondary malignancies in a patient with Hodgkin lymphoma 205–207
Никола Панīић, Милица Сīојиљковић, Ленка Грујичић, Драīана Шобић Шарановић, Вера Арīико
Позитронска емисиона и компјутеризована томографија са ^{18}F-флуородеоксиглукозом у дијагностици секундарних малигнитета код болесника са Хоџкиновим лимфомом

HISTORY OF MEDICINE • ИСТОРИЈА МЕДИЦИНЕ

Zoran Vacić
Guidelines for the preparation and dosage of medicines, instructions for their use, and methods of application according to the *Hilandar Medical Codex No. 517* 208–212
Зоран Вацић
Смернице за израду и дозирање лекова, упутства за употребу и средства за апликацију лека у *Хиландарском медицинском кодексу* бр. 517

IN MEMORIAM

Milan T. Stojičić, Maja Nikolić Živanović
Radmilo Rončević (January 20, 1938 – January 18, 2024) 213–214

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The prevalence and severity of molar incisor hypomineralization in group of students – a cross-sectional study

Milica Jovanović-Medojević[1], Dragana Jovanović[2], Mina Medojević[3,4], Strahinja Nedić[1], Đorđe Stratimirović[5], Sanja Vujašković[1], Mirjana Vujašković[1]

[1]University of Belgrade, School of Dental Medicine, Department for Restorative Dentistry and Endodontics, Belgrade, Serbia;
[2]University of Belgrade, School of Dental Medicine, Belgrade, Serbia;
[3]Université de Bordeaux, Institut National de la Santé et de la Recherche Médicale, BioTis, U1026, Bordeaux, France;
[4]University of Belgrade, School of Dental Medicine, Implant-Research Center, Belgrade, Serbia;
[5]University of Belgrade, School of Dental Medicine, Department of Basic Sciences, Belgrade, Serbia

SUMMARY

Introduction/Objective The aim of this study was to investigate the prevalence and conditions of teeth with molar incisor hypomineralization (MIH) in the early adult population.

Methods A cross-sectional study was conducted involving 298 students from the School of Dental Medicine, University of Belgrade, from the third to the fifth year of study. The presence of MIH was registered on the basis of European Academy of Paediatric Dentistry diagnostic criteria, and oral health and caries risk were assessed by standard dental examination and determination of DMF index values. The MIH sample was divided into two groups: untreated teeth (the estimation of the degree of hypomineralization was based on the color, location, and size of the hypomineralized part of the enamel) and treated teeth (where types of preventive and various restorative treatments were registered). Statistical analysis was performed using the χ^2 test ($p < 0.05$).

Results MIH was observed in 90 subjects (30.2%). The results of the oral health examination showed that the average DMF index was medium risk (7.66) for the MIH sample. MIH-untreated teeth showed a mild or moderate degree of hypomineralization. The presence of extensive carious lesions, secondary caries, as well as the loss of the first permanent molars was statistically significantly higher ($p < 0.05$) compared to the sample without MIH changes.

Conclusion The study results confirmed the problem in recognizing and adequately treating MIH-altered teeth.

Keywords: molar incisor hypomineralization; MIH; student population; cross-sectional study

INTRODUCTION

Hypomineralization of molars and incisors (MIH) is a developmental enamel disorder that affects first permanent molars and often permanent incisors [1]. The clinical picture of MIH depends on its severity and can range from opaque white, cream-colored markings or yellow-brown color, superficial enamel defects to atypical caries located on at least one permanent molar [2].

MIH is a qualitative enamel defect caused by reduced mineralization, which, according to research by Crombie et al. [3], can be up to 58.8%. The mineralization disorder occurs at the level of the enamel-dentine junction and spreads towards the surface of the tooth. Pathogenetically, MIH results from dysfunctional ameloblast resorption and inhibition of proteolytic enzymes, which results in retention of enamel proteins, disruption of hydroxyapatite crystal growth and enamel maturation [4]. The enamel of hypomineralized teeth is structurally compromised due to higher protein and water content, altered color, increased porosity and irregularly organized hydroxyapatite crystals [5]. Mechanical property tests (hardness and modulus of elasticity) of enamel affected by MIH reveal significantly lower values compared to healthy enamel [3]. The consequences of this enamel structure are increased tooth sensitivity (especially in the early eruptive phase), increased accumulation of dental plaque and, consequently, a tendency to rapid caries development [6]. Therefore, MIH represents a public health concern that negatively affects the oral health of patients, as well as a significant therapeutic challenge for clinicians [7, 8].

Although MIH can be diagnosed in childhood at the time of eruption of permanent first molars and central incisors (6–8 years), clinicians are often faced with the consequences of unrecognized MIH lesions that have not been adequately treated [4]. MIH is often misdiagnosed or diagnosed too late, so the therapy of hypomineralized teeth is delayed and

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Correspondence to:
Milica JOVANOVIĆ-MEDOJEVIĆ
University of Belgrade
School of Dental Medicine
Department for Restorative Dentistry and Endodontics
Rankeova 4
11000 Belgrade, Serbia.
medojevic.milica@gmail.com

inappropriate [9]. Due to extreme sensitivity and difficulty in maintaining oral hygiene, patients with hypomineralization avoid regular dental check-ups, which, combined with difficulties in achieving proper anesthesia, consequently deepen dental anxiety [9, 10]. Hypomineralized teeth are sensitive and painful, and their therapy is therefore complex and specific [10]. The porous structure of enamel with weakly bonded crystals and irregular arrangement of enamel prisms makes adhesion difficult, compromising the success of restorative treatments, which often results in the occurrence of subacute pulpitis [11]. Therefore, MIH patients require significantly more dental interventions compared to patients without MIH [12].

Therapeutic approaches for hypomineralized teeth can be implemented in three directions: prevention, restorative procedures (with or without endodontic therapy), and extractions. Preventive measures are implemented through adequate risk assessment, early diagnosis, remineralization, desensitization, fissure filling, education on maintaining proper oral hygiene, and the benefits of anticariogenic nutrition. Materials used in restorative procedures are most often glass ionomer cements (GIC), resin-modified GIC, compomers, composite materials, metal crowns, and onlays [13–16].

After adequate preventive and restorative procedures have been carried out, it is necessary to constantly control the marginal integrity of restorations (due to frequent enamel fractures) and to ensure that the patient is adhering to an adequate hygienic–dietary regime [15, 16, 17].

Studies on the prevalence and degree of hypomineralization, as well as applied therapeutic procedures of MIH, were conducted in children and their results indicate a different prevalence of MIH (2.8–40.2%) [18]. Limited data exist on monitoring the success of applied preventive and therapeutic measures, as well as the follow-up of MIH-affected teeth in early adulthood. It is necessary to analyze the success of implemented preventive measures, methods of therapy, as well as the stability and adequacy of restorations using certain dental materials in the treatment of MIH.

The aim of this study is to examine the prevalence and condition of hypomineralized first permanent molars and incisors in the population of young adult patients (students of the School of Dental Medicine, University of Belgrade).

METHODS

Data source

The present study was designed as an observational cross-sectional study and carried out during the summer semester (March 14th – May 13th) of the academic year 2021/2022. The study protocol was approved by the Ethics Committee of the School of Dental Medicine, University of Belgrade, Belgrade, Serbia (No 36/31) in accordance with the Declaration of Helsinki.

Participants

Participants in this study were 298 students (223 female and 75 male) in the sixth, eighth, and tenth semester attending the School of Dental Medicine, University of Belgrade, Belgrade, Serbia, who voluntarily gave and signed their written consent (available in the Appendix) to participate in the research. Students born between 1993 and 2001 participated in the study, with the majority of participants born between 1998 and 2000, resulting in 157 students (more than 50%).

All students were divided into two groups: a sample without MIH and a sample in which MIH was diagnosed. Eligibility criteria were as follows: all students who showed at least one tooth affected by molar-incisor hypomineralization were referred for inclusion in the study and categorized in the MIH group. Exclusion criteria were the following: patients undergoing orthodontic treatment with a fixed appliance, which did not allow an adequate clinical examination. Students with other developmental defects of teeth (fluorosis, amelogenesis imperfecta, and dentinogenesis imperfecta), or syndromes associated with enamel formation defects were classified in the group without MIH.

Recording procedure

The clinical examination was performed in a dental clinic setting, using a standard dental mirror and probe by one examiner, assisted by two students. Data on the status of all teeth were recorded, classified, and entered into a modified WHO epidemiological survey record from 2013 [19]. After a clinical dental examination of the dental status of all participants, an MIH sample was selected, consisting of subjects in whom the diagnosis of hypomineralized molars and incisors was confirmed. The presence of MIH was registered on the basis of European Academy of Paediatric Dentistry diagnostic criteria:

- Demarcated opacities,
- Enamel disintegration – posteruptive enamel breakdown,
- Atypical restorations,
- Extraction due to MIH,
- Retention or non-eruption of molars and incisors,
- Larger and atypical carious lesions with blurring along the edges of the cavity [20].

Lesions smaller than 1 mm were not verified as MIH, nor were changes in the form of opacity observed only on the incisors, without molar involvement [20].

The condition of all teeth, including first permanent molars and upper central incisors, was recorded in the questionnaires for both groups (with or without MIH) (questionnaires available in the Appendix). The occurrence and size of carious lesions (primary and secondary) were noted, as well as the presence of preventive treatments (sealants), restoration of examined teeth (occurrence and size of the fillings, prosthodontic treatment), endodontic treatments, and the number of extracted teeth. Oral health and caries risk were assessed by standard dental examination

and determination of DMF-T (Decayed, Missing, Filled Teeth) index values [21].

The sample with MIH was divided into two groups: untreated teeth and teeth that had undergone preventive and restorative treatment. In untreated hypomineralized teeth, information about color (white, yellow, or brown), localization (vestibular or oral surfaces and/or occlusal or incisal) and size of the lesion (< 30% of the tooth surface (mild defects), 31–49% of the tooth surface (moderate defects), > 50% of the tooth surface (severe defects)) were recorded and, based on them, the degree of hypomineralization was assessed [17].

Table 1. Distribution of color, localization and size of lesions of molar incisor hypomineralization (MIH)-affected teeth that were not treated

Teeth with MIH		16	11	21	26	36	46	Total N (%)
Color	White	24	35	36	18	14	22	149 (87)
	Yellow	2	1	1	2	3	7	16 (9)
	Brown	1			1	2	3	7 (4)
Localization	Vestibular/oral	14	12	14	11	11	18	80 (46)
	Occlusal/incisal	13	24	23	10	8	14	92 (54)
Size	< 30%	24	35	37	18	17	28	172 (92)
	31–49%	3	1		3	2	4	13 (8)
	> 50%							

Statistical analyses

Statistical analysis of the obtained data was performed using the χ^2 test for independent samples at a confidence level of 5% ($\alpha = 0.05$).

RESULTS

In the population of 298 students in the third, fourth, and fifth year of basic integrated studies at the School of Dental Medicine, University of Belgrade, Belgrade, Serbia, MIH was observed in 90 subjects, representing 30.2% (75 female, 15 male). In the group with MIH (90 students), nine subjects had no caries, fillings, or non-orthodontic extractions.

The color distribution, localization, and size of enamel defects are shown in Table 1. Among MIH-diagnosed teeth without any preventive and therapeutic procedures, a mild (92%) or moderate degree of hypomineralization (8%) was observed, with mostly white spots (87%) that affected the vestibular/oral (46%), that is, occlusal/incisal (54%) surfaces of the teeth relatively equally.

The results of the oral health examination showed that the DMF for the sample with MIH (D – 37, M – 55, F – 589) / 90 = 7.66. The results of the analysis of teeth affected by MIH in which different therapeutic procedures were applied, revealed the presence of extensive caries lesions, secondary caries, as well as the loss of teeth affected by MIH that was statistically significantly higher ($p < 0.05$) compared to the sample without MIH changes (Tables 2 and 3).

A statistically significant difference was observed in the number of surfaces affected by fillings of the first permanent molars in the sample with MIH compared to molars not affected by hypomineralization (Table 4 and Figure 1).

DISCUSSION

The prevalence of MIH in our study was 30.2%. The degree of representation is somewhat higher compared to the results of epidemiological studies, which reported a rate of 3.6–25% in European countries [22, 23]. However, the results of the Jälevik [24] systematic review show a wider variation in the prevalence of MIH (2.4–40.2%). There are no official data on the prevalence of MIH for Serbia, although it is estimated to be around 19.5% [25]. The reasons for the higher degree of MIH in this population can be found in the turbulent time in which the subjects were born (time of NATO bombing and exposure to various stressful and toxic factors) [1–4]. This increase in MIH is in agreement with the results of an epidemiological pilot study by Elzein et al. [26], who observed a positive correlation in the prevalence of MIH in the bombed areas of Lebanon.

The sex of the students was not significant in the finding of MIH, although a greater number of women showed MIH changes (75 female and 15 male), which reflected the structure of the sample (223 female and 75 male).

It is fully expected that teeth in which MIH has been observed, where preventive and therapeutic procedures have not been carried out, show mostly isolated mild

Table 2. Distribution of preventive and therapeutic procedures in a sample with and without molar incisor hypomineralization (MIH) on permanent first molars

Procedure	Tested teeth							
	16		26		36		46	
Preventive and therapeutic method	without MIH (208)	with MIH (90)	without MIH (208)	with MIH (90)	without MIH (208)	with MIH (90)	without MIH (208)	with MIH (90)
Fissure sealing	52 (25%)	13 (14.4%)		10 (9%)	52 (25%)	10 (9%)	60 (28.8%)	13 (14.4%)
Temporary fill				1 (1.1%)				
DMG Icon								
Endodontic therapy	2 (1%)		2 (1%)		1 (0.5%)	2 (1%)	1 (0.5%)	
Crowns inlay onlay veneers						1 (0.5%)		
Non-orthodontic extractions	4 (1.9%)	5 (5.6%)	2 (1%)	3 (3.3%)	3 (1.4%)	6 (2.9%)	3 (1.4%)	13 (14.4%)
Caries		3 (3.3%)	2 (1%)	4 (4.4%)				
Secondary caries	2 (1%)	4 (4.4%)	2 (1%)	4 (4.4%)	1 (0.5%)	2 (1%)	1 (0.5%)	4 (4.4%)

Table 3. Distribution of preventive and therapeutic procedures in a sample with and without molar incisor hypomineralization (MIH)on permanent upper central incisors

Procedure	Tested teeth			
	11		21	
Preventive and therapeutic method	without MIH (208)	with MIH (90)	without MIH (208)	with MIH (90)
Fissure sealing				
Temporary fill				
DMG Icon		1 (1.1%)		2 (1%)
Endodontic therapy	1 (0.5%)	4 (4.4%)		4 (4.4%)
Crowns inlay onlay veneers	1 (0.5%)	2 (1%)	1 (0.5%)	2 (1%)
Non-orthodontic extractions				
Caries	2 (1%)		1 (0.5%)	
Secondary caries				

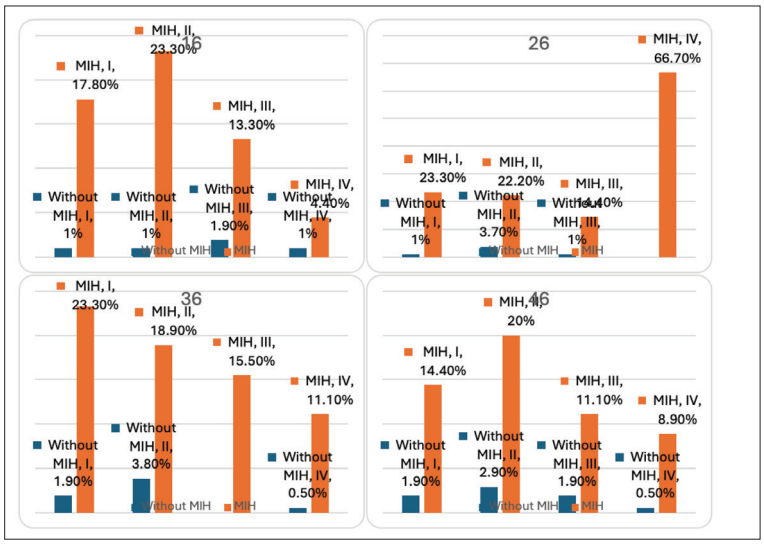

Figure 1. Distribution number of surfaces with fillings on the first permanent molars

or moderate degree of hypomineralization, with mostly white spots. This population includes 10% of BO students (without caries, fillings, and extractions due to caries). Therefore, it is extremely important to recognize the degree of these hypomineralizing changes, in order to apply adequate preventive and therapeutic measures. A mild or moderate degree of hypomineralization with adequate maintenance of oral hygiene enables a healthy set of teeth.

The increased degree of caries risk in the MIH sample (7.66, i.e., medium caries risk) indicates a high vulnerability of the MIH population to the occurrence of carious lesions. The presentation of different preventive and therapeutic procedures in the sample with and without MIH on the first permanent molars and central upper incisors indicates an increased number of primary and secondary carious lesions in the MIH sample compared to students without this hypomineralization. Hypomineralized molars have a five- to 10-times greater need for dental treatment than molars without MIH [13]. The restoration of hypomineralized teeth very often presents a problem, especially in defining the edges of the cavity (hypomineralized parts of the enamel cause the appearance of marginal permeability and endanger the success of the restoration). It is recommended to include the complete porous enamel in the cavity, but with the maximum preservation of healthy enamel that has changed color [26]. For this reason, it is extremely important to recognize MIH changes and start increased prevention as soon as MIH teeth emerge, due to their propensity for posteruptive enamel deterioration and higher prevalence of carious lesions. The frequent occurrence of secondary caries and tooth crown fractures requires more frequent dental controls with monitoring of the success of the implemented preventive and therapeutic procedures.

Defects on the incisors are usually milder than the changes on the molars, and the mild, mostly yellow-white changes are discolored over time by food and drink dyes and are less noticeable with age. Resin infiltration technique (DMG Icon; DMG America LLC, Ridgefield Park, NJ, USA) was performed on three teeth of the MIH population in order to improve the aesthetic properties of these teeth. This preparation improves optical properties by acting on translucency (the refractive index of the resin infiltrate (1.52) is close to the index of healthy enamel (1.62)) [19]. Preparation for aesthetic fillings of hypomineralized incisors should be as conservative as possible, and the degree of treatment depends on the patient's age, aesthetic concern, and interest of the patients themselves, as well as the severity of the lesion.

Table 4. Distribution number of surfaces with fillings on teeth 16, 26, 36, 46, 11, 21 in the sample with and without molar incisor hypomineralization (MIH)

Tested teeth	16		26		36		46		11		21	
Number of surfaces with fillings	without MIH (208)	with MIH (90)	without MIH (208)	with MIH (90)	without MIH (208)	with MIH (90)	without MIH (208)	with MIH (90)	without MIH (208)	with MIH (90)	without MIH (208)	With MIH (90)
I	2 (1%)	16 (17.8%)	2 (1%)	21 (23.3%)	4 (1.9%)	21 (23.3%)	4 (1.9%)	13 (14.4%)	2 (1%)	2 (2.2%)	4 (1.9%)	4 (4.4%)
II	2 (1%)	2 1 (23.3%)	7 (3.7%)	20 (22.2%)	8 (3.8%)	17 (18.9%)	6 (2.9%)	18 (20%)		1 (1.1%)		1 (1.1%)
III	4 (1.9%)	12 (13.3%)	2 (1%)	13 (14.4%)		14 (15.5%)	4 (1.9%)	10 (11.1%)		2 (2.2%)		2 (2.2%)
IV	2 (1%)	4 (4.4%)		6 (66.7%)	1 (0.5%)	10 (11.1%)	1 (0.5%)	8 (8.9%)				

However, the incisal edge of MIH central incisors tends more often to have post-eruptive fractures, which represents a challenge in restoration; thus, significantly more endodontic treatments were performed on the central incisors of the MIH population compared to students without MIH.

Also, the size of the restorations, that is, the number of tooth surfaces included in the restoration is significantly higher in the population of students with MIH. The large area of hypomineralized first permanent molars is probably a consequence of higher enamel porosity and lower mechanical resistance, especially in severe MIH lesions. First permanent molars as occlusion carrier teeth must be adequately restored prosthetically in order to prevent pathological fractures of these teeth.

Endodontic treatment of first permanent molars with MIH does not show a higher degree of frequency compared to students without MIH. The reason is probably in the age of the patients. Based on the anamnestic data obtained from the subjects, it was found that the MIH-affected molars were the cause of their dental problems from the moment they erupted. They were restored with fillings that did not persist long in the cavity, with frequent occurrence of secondary caries and crown fractures. Complex and long-term endodontic therapies were carried out on such teeth, but they usually had to be extracted. A systematic review by Taylor et al. [27] showed that partial and coronal pulpotomies have both short- and long-term high success rates, while conventional pulpectomy or regenerative techniques have limited success in MIH molars. Thus, partial or coronal pulpotomies can be considered as a potential endodontic treatment option for molars affected by MIH.

The presentation of different therapeutic procedures in the sample with and without MIH on the first permanent molars indicates an increased number of non-orthodontic extractions in the MIH sample compared to students without this hypomineralization. Extracted first permanent molars have a significant impact on adequate occlusion, and expensive implant-prosthetic solutions are necessary. Extraction of first permanent molars with severe hypomineralization changes and a poor prognosis must be considered at an early age based on a comprehensive dental evaluation. A multidisciplinary approach that includes the opinion of both the pediatric dentist and the orthodontist is necessary to enable balance and compensation of the bite [28].

To the best of our knowledge, this is the first study that investigated the prevalence and conditions of teeth with molar and incisor hypomineralization in the student (young adult) population in Serbia. This study enabled the evaluation of changes in the oral health of patients with MIH over time and the possible formation of priorities and ways of directing existing resources for prevention and therapy.

CONCLUSION

The results of this study confirmed the problem in recognizing teeth affected by molar incisor hypomineralization and their adequate therapy. During the treatment of MIH teeth, the degree of hypomineralized changes and their long-term prognosis must be taken into account, as well as specific characteristics in the form of increased pain sensitivity of such teeth. Early diagnosis, adequate therapy in the post-eruptive phase, active application of preventive measures and regular check-ups of young adult patients are extremely important for the preservation of teeth affected by hypomineralization.

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Учесталост и тежина хипоминерализације молара и секутића у студентској популацији – студија пресека

Милица Јовановић-Медојевић[1], Драгана Јовановић[2], Мина Медојевић[3,4], Страхиња Недић[1], Ђорђе Стратимировић[5], Сања Вујашковић[1], Мирјана Вујашковић[1]

[1]Универзитет у Београду, Стоматолошки факултет, Клиника за болести зуба и ендодонцију, Београд, Србија;
[2]Универзитет у Београду, Стоматолошки факултет, Београд, Србија;
[3]Универзитет у Бордоу, Медицински истраживачки центар „БиоТис", У1026, Бордо, Француска;
[4]Универзитет у Београду, Стоматолошки факултет, Имплантолошко-истраживачки центар, Београд, Србија;
[5]Универзитет у Београду, Стоматолошки факултет, Катедра за нститутске предмете, Београд, Србија

САЖЕТАК

Увод/Циљ Циљ овог истраживања био је да се испитају учесталост и стање хипоминерализованих првих сталних молара (*molar incisor hypomineralization – MIH*) у студентској популацији.

Методе Епидемиолошка студија пресека спроведена је након добијања писане сагласности 298 испитаника – студената Стоматолошког факултета Универзитета у Београду од III до V године студија. Присуство *MIH*-а регистровано је на основу критеријума Европске академије за дечју стоматологију, а стандардним стоматолошким прегледом и одређивањем вредности индекса КЕП процењен је ризик за орално здравље и каријес. Узорак са *MIH*-ом подељен је у две групе: нелечени зуби (на којима је на основу боје, локализације и величине хипоминерализованог дела глеђи процењен степен хипоминерализације) и лечени зуби (на којима су регистровани превентивни и различити реставративни третмани). Подаци су статистички анализирани тестом χ^2 ($\alpha = 0,05$).

Резултати *MIH* је уочен код 90 испитаника (30,2%). Резултати оралног здравственог прегледа показали су да је КЕП индекс за узорак са *MIH*-ом 7,66. Нетретирани *MIH* зуби показали су благи или умерени степен хипоминерализације. Присуство екстензивних каријесних лезија, секундарног каријеса и екстракција првих сталних молара било је статистички значајно веће ($p < 0,05$) у поређењу са зубима без промена *MIH*.

Закључак Резултати студије су потврдили проблем у препознавању и адекватном лечењу зуба измењених *MIH*-ом.

Кључне речи: хипоминерализација првих молара и секутића; *MIH*; студентска популација; студија пресека

Informed Consent of the Patient

Researchers:
Prof. Dr. Mirjana Vujašković
Assoc. Prof. Dr. Milica Jovanović-Medojević
Student Dragana Jovanović
Student Katarina Teofilović

Study: Dental status of teeth affected by Molar-incisor hypomineralisation in students' population

With this document, we wish to inform you about the aim and significance of this research. Please read this document carefully, and if you have any additional questions, feel free to ask the researchers.
This study has been approved by the Ethics Committee of the Faculty of Dentistry, University of Belgrade, under the number ……

What is MIH?
Molar-incisor hypomineralization (MIH) is a developmental disorder that most commonly affects the first permanent molars and incisors. Healthy enamel is a highly mineralized hard dental tissue, composed of 96% inorganic materials, 1% organic materials, and 3% water. MIH is a qualitative enamel defect caused by reduced mineralization (lower percentages of apatite, calcium, and phosphate), making the enamel of these teeth dysfunctional due to higher protein and water content. Hypomineralized enamel has an altered color, is porous, prone to fractures, and more susceptible to rapid caries development.

What is the goal-aim of the study?
The goal of this study is to identify various etiological factors involved in the development of MIH, as well as to assess the application of different therapeutic methods and the survival rate of MIH-affected teeth in younger adult patients, specifically in the student population of the Faculty of Dentistry.

What data will be collected in this study?
Data will be collected on potential etiological factors that caused the development of dental hypomineralization, as well as the size, location, and color of such lesions. The type of therapy applied to the hypomineralized teeth will also be noted.

How will the data be handled?
At the end of the study, the results will be published in a scientific journal. In this article, no participant will be identifiable. Data will be preserved for an indefinite time.

CONSENT FORM
I am well informed about this study. I have thoroughly read this information. I had the opportunity to ask any questions regarding this research, and they were answered to my satisfaction. I agree to participate in this study. I understand that I can withdraw my consent at any time without any justification.

Name and last name: .....
Date of Birth: .....
Signature: .....
Date: .....

By signing this, I confirm that I have been properly informed, both orally and in writing, about the research.

Clinical research record (patients without MIH)

Subject number _____ Date of clinical examination _____

Dental record number _____

Patient's initials _____ Gender 1- M 2 - F

Date of birth _____

Smoker: 1- No 2 - Yes _____cigarettes per day

WHO Epidemiological dental record 2013.

Dental Status by Tooth Surfaces:

	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
Occ.																
Mes.																
Buc.																
Dis.																
Oral.																

	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
Occ.																
Mes.																
Buc.																
Dis.																
Oral.																

Parameters recorded on different tooth surfaces and entered into the Status:

- **0 = healthy**
- **1 = cavity**
- **2 = secondary cavity**
- **3 = filling without caries**
- **4 = missing due to caries**
- **5 = missing for other reasons**
- **6 = sealed fissures**
- **7 = fixed prosthetic restorations/crowns, dental implants, veneers**
- **8 = unerupted**
- **9 = extracted for orthodontic reasons**

Additional notes

_____
_____
_____
_____
_____
_____

Clinical research record (patients with MIH)

Subject number _____ Date of clinical examination _____

Dental record number _____

Patient's initials _____ Gender 1- M 2 - F

Date of birth _____

Smoker: 1- No 2 - Yes _____cigarettes per day

WHO Epidemiological dental record 2013.

Dental Status by Tooth Surfaces:

	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
Occ.																
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- **8 = unerupted**
- **9 = extracted for orthodontic reasons**

Additional notes

_____
_____
_____
_____
_____

Table 1. Color, localization, and size of lesions on MIH-affected teeth that have not been treated

Tooth affected by MIH		17	16	11	21	26	27	36	37	46	47
Color	White										
	Yellowish										
	Brownish										
Localisation	Vestibular/oral										
	Occlusal/incisal										
Size	< 30%										
	31-49%										
	> 50%										

Legend: **Color** (white, yellowish, or brownish discoloration), **Localization** (vestibular and oral surfaces and/or occlusal and incisal surfaces), **Size of the lesion**:

- < 30% of the tooth surface (mild defects)
- 31-49% of the tooth surface (moderate defects)
- 50% of the tooth surface (advanced defects)

Table 2. Treatment Methods for Hypomineralized Teeth

MIH affected tooth	16	11	21	26	36	46
Fissure sealing						
Temporary filling (perseverance of vitality or endodontic treatment)						
Composite or GIC filling (Black classification)						
Endodontic treatment (quality assessment)						
Crown, inlay, onlay						
Extraction						

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UDC: 616.314.3/.5-008.6-057.875(497.6)

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Exploiting maxillary sinus dimensions for sexual determination in the Bosnian and Herzegovinian population

Igor Đukić[1], Zoran Jezdić[2], Mirsad Kadić[3], Anesa Pirović Kadić[4], Ognjenka Janković[5], Aleksandra Radulović[6], Ivan Arsić[7], Aleksandar Jakovljević[8,9] Nenad Nedeljković[7]

[1]University of Banja Luka, Faculty of Medicine, Department of Oral Surgery, Banja Luka, Republic of Srpska, Bosnia and Herzegovina;
[2]University of Belgrade, School of Dental Medicine, Department of Maxillofacial Surgery, Belgrade, Serbia;
[3]Sarajevo School of Science and Technology, Department of Dentistry, Sarajevo, Federation of Bosnia and Herzegovina, Bosnia and Herzegovina;
[4]University of Banja Luka, Faculty of Medicine, Banja Luka, Republic of Srpska, Bosnia and Herzegovina;
[5]University of Banja Luka, Faculty of Medicine, Department of Restorative Odontology and Endodontics, Banja Luka, Republic of Srpska, Bosnia and Herzegovina;
[6]University of Banja Luka, Faculty of Medicine, Department of Prosthodontics, Banja Luka, Republic of Srpska, Bosnia and Herzegovina;
[7]University of Belgrade, School of Dental Medicine, Department of Orthodontics, Belgrade, Serbia;
[8]University of Belgrade, School of Dental Medicine, Department of Pathophysiology, Belgrade, Serbia;
[9]University of Belgrade, School of Dental Medicine, Implant-Research Center, Belgrade, Serbia

SUMMARY

Introduction/Objective This study aimed to evaluate sex assessment using cone-beam computed tomography (CBCT) images by analyzing the dimensions and volume of the maxillary sinus. Additionally, the study aimed to develop prediction formulas for sex assessment and assess their accuracy for the population of Bosnia and Herzegovina.

Methods A total of 150 CBCT images were analyzed, comprising 73 males and 77 females, aged 20–69 years. The dimensions of the maxillary sinuses – specifically, the mediolateral, superoinferior, and anteroposterior measurements – along with their volumes, were assessed using Romexis software. Measurements were taken from both axial and coronal projections. To develop gender prediction formulas, multiple regression analysis was employed. The accuracy of sex prediction was evaluated using discriminant functional analysis.

Results Significant differences ($p < 0.05$) in sinus dimensions and volume were observed between sexes. Males had larger maxillary sinuses: left sinus volume was 27.60 ± 6.87 cm^3 in males versus 22.10 ± 5.77 cm^3 in females and right sinus volume was 27.48 ± 7.02 cm^3 in males versus 21.65 ± 5.42 cm^3 in females. The right maxillary sinus provided the highest accuracy for sex prediction (75%), followed by the left sinus (71%).

Conclusion The dimensions and volume of the maxillary sinus vary significantly between sexes, with males typically having larger sinuses. A gender prediction formula based on the right maxillary sinus offers the highest accuracy for sex prediction, achieving 75%. This formula could be a valuable tool in forensic applications specific to the Bosnian population when other methods are not available.

Keywords: maxillary sinus; sex determination; forensic identification; CBCT; sexual dimorphism

INTRODUCTION

The maxillary sinus is a pneumatized hollow space located in the central portion of the upper jaw. Its size varies among individuals and can be influenced by factors such as age, sex, the level of pneumatization, and early tooth loss. The exact function of the maxillary sinus is still not fully understood, but there are several theories regarding its role. It is thought to help minimize the weight of the head, contribute to voice resonance, and prepare air intake by heating and humidifying the air [1].

This research examines the maxillary sinus as a valuable anatomical feature for assessing sex based on its dimensions. Sex assessment is a crucial area in dental forensics, playing a significant role in identifying unknown individuals who may be victims of bomb explosions, natural disasters, or other types of accidents. It also contributes to establishing a person's identity when identity information is lacking. In addition to forensic applications, sex assessment is also relevant in archaeological, biological, and anthropological studies. [2, 3]. Forensic experts face significant challenges in sex assessment when only skeletal fragments of individuals are available. The most critical aspect of anthropological examination is accurately determining sex. Due to the knowledge of gender, half of the possibilities are eliminated, as sex is a two-option framework, meaning it can be either male or female. Accurate sex assessment is essential because it significantly influences factors such as age and physical constitution. This information is valuable for researchers across various

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Correspondence to:
Zoran JEZDIĆ
University of Belgrade
School of Dental Medicine
Department of Maxillofacial Surgery
Starine Novaka 5
11000 Belgrade
Serbia
zoran.jezdic@stomf.bg.ac.rs

fields, including medical sciences, anthropology (particularly in the examination of skeletal remains), criminology (for identifying undocumented individuals, such as in major accidents where identification is challenging), forensic medicine, archaeology, and others [4, 5, 6].

There are several methods for assessing the sex of unidentified individuals based on skeletal remains and their anatomical features. These methods include measuring the length and height of the head, the distance between the basion and the prosthion, and the circumference of the head. Other measurements involve the length of the supraorbital edge, the size of the mastoid process and the mandibular ramus, the shape and length of the palate, and the circumference of the occipital condyle, among others. [3]. Unlike other anatomical features used for sex assessment, the maxillary sinus of individuals exposed to fire or other disasters invariably remains intact. This characteristic establishes the maxillary sinus as a crucial element in forensic dentistry [4, 5].

The aim of this study was to evaluate sex differences using cone-beam computed tomography (CBCT) images to determine the dimensions and volume of the maxillary sinus. Additionally, the study aimed to develop formulas for sex prediction and assess their accuracy for the population of Bosnia and Herzegovina.

METHODS

The study was designed to measure the mediolateral (ML), superoinferior (SI) and anteroposterior (AP) linear dimensions and the volume of both right and left maxillary sinuses using CBCT images. A total of 150 CBCT images including 73 males and 77 females aged 20 to 69 from the existing database of the Dental Clinic Specialist Center, Faculty of Medicine, University of Banja Luka were observed in this study. CBCT images were taken using Planmeca Viso® G7 plus (Planmeca Group, Helsinki, Finland) following exposure parameter settings 90kW, 4–10mA,14s and images acquisition at 0.2 mm voxel size. CBCT images were selected based on strict inclusion and exclusion criteria. The inclusion criteria ensured that only images of individuals with intact maxillary sinuses were included. Exclusion criteria included individuals under 20 years of age, as maxillary sinus development is typically completed by the age of 18–20 years [7]. Additionally, images were excluded if any pathological conditions were present, such as maxillary sinus pathology, facial deformities involving the maxilla, deep caries, periapical abscesses, odontogenic cysts, tumors, or significant bone loss due to periodontal disease. These criteria were applied to ensure the selection of healthy, anatomically intact cases, thus minimizing the impact of potential factors that could compromise the research results. Considering the retrospective design of the study, the CBCT images were originally acquired for various clinical purposes. The subjects' written consent was obtained, according to the Declaration of Helsinki, and the study has been approved by the local ethics committee (Ethics Committee for Research on Humans and Biological Materials, Faculty of Medicine, University of Banja Luka, under approval number No.18/4. 227/24).

Performed measurements were operated in the software Romexis 6.0 (Planmeca Group). The ML and AP dimensions of right and left maxillary sinuses were measured on axial projection while the SI dimensions of right and left maxillary sinuses were measured on coronal projection. On the axial and coronal projections, the slices of CBCT image whose ML, AP, and SI dimensions had the greatest dimensions of the left and right maxillary sinus, were chosen as the reference for measurements.

The ML dimension was defined as the longest distance perpendicular from the medial wall of the sinus to the most lateral wall of the lateral process of the maxillary sinus in the axial projection (Figure 1). The AP was defined as the longest distance from the most anterior point to the most posterior point of the medial wall in the axial projection (Figure 2). The SI was defined as the longest distance from the lowest point of the sinus floor to the highest point of the sinus roof in the coronal projection (Figure 3). The measurements were performed by two trained examiners, who calibrated and cross-verified their results to ensure accuracy and consistency.

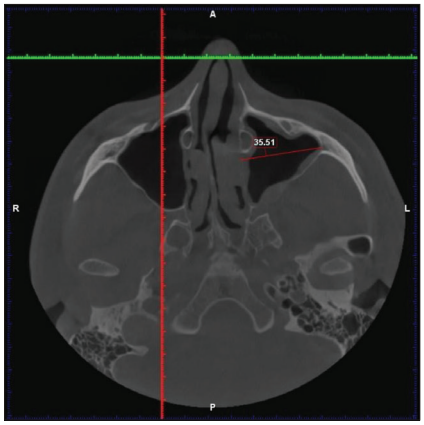

Figure 1. Mediolateral dimension of the maxillary sinus on axial projection of cone-beam computed tomography

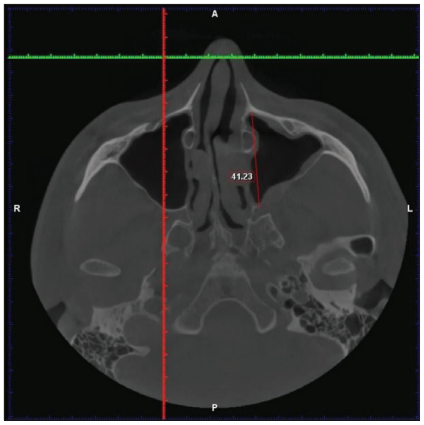

Figure 2. Anteroposterior dimension of the maxillary sinus on axial projection of cone-beam computed tomography

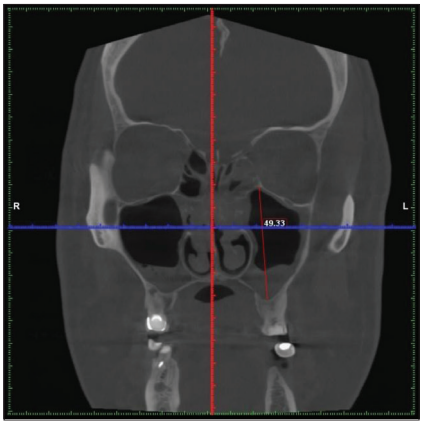

Figure 3. Superoinferior dimension of the maxillary sinus on coronal projection of cone-beam computed tomography

The volume of each maxillary sinus was calculated using the following equation [4]:

$$\text{Volume} = (\text{ML} \times \text{AP} \times \text{SI} \times 0.5)$$

The data collected were entered into Microsoft Excel 2017 (Microsoft Corporation, Redmond, WA, USA) and statistically analyzed using IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp., Armonk, NY, USA). The mean values of all obtained dimensions, standard deviations, and statistical significance ($p < 0.05$) were calculated. Multiple regression formulas were created to predict sex based on the measured dimensions. If the result of the formula was positive, the predicted sex was male; if negative, female. The accuracy of sex prediction was assessed using discriminant functional analysis, with results expressed as percentages and displayed in tabular form. Statistical significance was set at $p < 0.05$ for standard significance, $p < 0.001$ for highly significant results, and $p > 0.05$ was considered non-significant.

RESULTS

The mean values and standard deviations of the dimensions of the left and right maxillary sinuses revealed statistically significant differences between males and females. A statistically significant difference ($p < 0.001$) was observed in all dimensions of both maxillary sinuses between males and females, except for the AP dimension of the left maxillary sinus (APL) (Table 1).

Table 1. Mean dimensions of maxillary sinus and their standard deviation (SD)

Dimensions	Male	Female	p-value
	Mean (mm) ± SD	Mean (mm) ± SD	
MLL	3.26 ± 0.42	3.01 ± 0.44	< 0.001
SIL	4.23 ± 0.45	3.84 ± 0.43	< 0.001
APL	3.93 ± 0.36	3.75 ± 0.32	< 0.002
MLR	3.24 ± 0.41	2.97 ± 0.43	< 0.001
SIR	4.23 ± 0.53	3.84 ± 0.42	< 0.001
APR	3.94 ± 0.35	3.74 ± 0.34	< 0.001

MLL – mediolateral dimension of the left maxillary sinus; SIL – superoinferior dimension of the left maxillary sinus; APL – anteroposterior dimension of the left maxillary sinus; MLR – mediolateral dimension of the right maxillary sinus; SIR – superoinferior dimension of the right maxillary sinus; APR – anteroposterior dimension of the right maxillary sinus

It was noted in comparison between the right and left maxillary sinus that the all dimensions of left maxillary sinus were marginally larger than the right maxillary sinus in females. On the contrary, in males, the right maxillary sinus was marginally larger in dimensions than the left maxillary sinus, apart from the ML dimensions. The ML dimension of the right maxillary sinus was less than ML dimension of the left maxillary sinus in males. However, these all-dimensions differences were statistically insignificant (Table 2).

It was observed that the volume of the left maxillary sinus (VL) was marginally larger than the volume of the right maxillary sinus (VR) in males, while in females, the VL was smaller than the VR. Conversely, the VR was marginally larger than the VL in females, and smaller than the VL in males. However, these differences in volume were statistically insignificant. When comparing between genders, the volume of both maxillary sinuses was larger in males than in females, and this difference was statistically significant (Table 3).

Table 2. Comparison of dimensions of the left and right maxillary sinuses between males and females

Sex	Dimensions	Mean (mm) ± SD**		p-value
		Right maxillary sinus dimensions	Left maxillary sinus dimensions	
Males	ML	3.24 ± 0.41	3.26 ± 0.42	> 0.05
	SI	4.23 ± 0.53	4.23 ± 0.45	> 0.05
	AP	3.94 ± 0.35	3.93 ± 0.36	> 0.05
Females	ML	2.97 ± 0.43	3.01 ± 0.44	> 0.05
	SI	3.84 ± 0.42	3.84 ± 0.43	> 0.05
	AP	3.74 ± 0.34	3.75 ± 0.32	> 0.05

ML – mediolateral dimension of the maxillary sinus on axial projection of cone-beam computed tomography (CBCT); SI – superoinferior dimension of the maxillary sinus on coronal projection of CBCT; AP – anteroposterior dimension of the maxillary sinus on axial projection of CBCT; SD – standard deviation

Table 3. Comparison of mean dimensions of volume of right and left maxillary sinuses with standard deviation (SD) between males and females

Sex	Mean (mm3) + SD		p-value
	Right maxillary sinus volume	Left maxillary sinus volume	
Males	27.48 ± 7.02	27.60 ± 6.87	> 0.05
Females	21.65 ± 5.42	22.10 ± 5.77	> 0.05
p-value	< 0.001	< 0.001	

Gender prediction formulas (*Formula 1*, *Formula 2*, *Formula 3*) were developed using multiple regression analysis, based on the dimensions and volume of both the right and left maxillary sinuses.

Formula 1. Sex prediction formula based on dimensions and volume of the left maxillary sinus.

Predicted Sex = 6.44 – 0.55 × SIL – 1.60 × MLL – 1.33 × APL + 0.24 × VL

Where:

- SIL = SI dimension of the left maxillary sinus
- MLL = ML dimension of the left maxillary sinus
- APL = AP dimension of the left maxillary sinus
- VL = Volume of the left maxillary sinus

Formula 2. Sex prediction formula based on dimensions and volume of the right maxillary sinus.

Predicted Sex = 5.66 – 0.67 × SIR – 1.28 × MLR – 1.16 × APR + 0.22 × VR

Where:

- SIR = SI dimension of the right maxillary sinus
- MLR = ML dimension of the right maxillary sinus
- APR = AP dimension of the right maxillary sinus
- VR = Volume of the right maxillary sinus

Formula 3. Sex prediction formula based on dimensions and volume of both maxillary sinuses.

Predicted Sex = 7.16 – 0.03 × SIL – 1.25 ×MLL – 0.81 × APL + 0.13 × VL – 0.69 × SIR – 0.14 × MLR – 0.62 × APR + 0.13 × VR

Where:

- SIL = SI dimension of the left maxillary sinus
- MLL = ML dimension of the left maxillary sinus
- APL = AP dimension of the left maxillary sinus
- VL = Volume of the left maxillary sinus
- SIR = SI dimension of the right maxillary sinus
- MLR = ML dimension of the right maxillary sinus
- APR = AP dimension of the right maxillary sinus
- VR = Volume of the right maxillary sinus

The sex prediction based on the dimensions and volume of the maxillary sinuses, as shown in Table 4, ranged from a minimum accuracy of 64% for males in the left maxillary sinus to a maximum of 83% for females using both sinuses combined.

Table 4. Classification results of discriminant functional analysis of accuracy of sex predicted

Parameters	Sex predicted (%)		
	Male	Female	Both sexes
Dimensions and volume of right maxillary sinus	70	81	75
Dimensions and volume of left maxillary sinus	64	78	71
Dimensions and volume of both maxillary sinus	64	83	74

DISCUSSION

In dental forensics, sex assessment is a critical step in the identification process, particularly when there is limited information available about the deceased. It is often the first priority for forensic experts in cases such as accidents, chemical and nuclear explosions, natural disasters, and similar situations [8]. The maxillary sinus serves as a valuable alternative for sex assessment when other methods are impractical or impossible to use. Skeletal analysis, particularly of pelvic morphology, is widely considered the gold standard for determining sex due to its high accuracy. However, in cases where remains are fragmented or incomplete, the pelvis or other skeletal markers may not be available or suitable for analysis. Since the maxillary sinus is well-protected within the craniofacial skeleton, it is often preserved even in instances of significant trauma or decomposition, making it an important option for assessment [9, 10, 11].

Dental structures, particularly teeth, are often used for sex assessment due to their durability and availability. However, prolonged exposure to environmental factors, diseases, or wear can lead to significant degradation of these dental elements. In contrast, the maxillary sinus remains largely unaffected by such conditions, making it a more reliable structure for analysis. Furthermore, advanced imaging techniques, such as CBCT, have facilitated precise measurements of maxillary sinus dimensions, enhancing its utility in forensic identification [12, 13, 14].

The results of this study indicate that the dimensions of the left and right maxillary sinuses (specifically MLL, SIL, APL, VL, MLR, SIR, APR, and VR) are larger in males than in females, with these differences being statistically significant. Previous studies by Gamba et al. [9], Ayyildiz and Akgunlu [10], Mathew and Jacob [11], Elbaz et al. [12], Rani et al. [13], and Jaideepa et al. [14] have also reported that male maxillary sinuses are significantly larger than those of females. These findings align with the results of this study and provide additional validation. Mathew and Jacob [11] utilized CBCT technology, which offers greater precision compared to the two-dimensional methods employed by Elbaz et al. [12]. Rani et al. [13] noted the influence of age, while Elbaz et al. [12] focused on an Egyptian population, suggesting potential ethnic variations. Conversely, other studies by Barros et al. [15], Belgin et al. [16], Gulec et al. [17], and Saccucci et al. [18] did not find statistically significant differences in maxillary sinus dimensions between sexes. Barros et al. [15] included subjects of varying ages and nutritional statuses, which could contribute to these inconsistent findings. While Gulec et al. [17] used CBCT, their sample size was smaller. Belgin et al. [16], on the other hand, relied on traditional radiographic methods, which may have affected accuracy.

This study clearly demonstrates that the height of the maxillary sinus is the most reliable distinguishing factor between sexes, aligning with the findings of Teixeira et al. [19], Mathew and Jacob [11], Kannampurath et al. [20], and Paknahad et al. [21]. Teixeira et al. [19] specifically noted that maxillary sinus height is the most effective parameter for sex assessment. Similarly, the CBCT-based study by Mathew and Jacob [11] closely aligns with our findings, underscoring the importance of maxillary sinus height. Kannampurath et al. [20] further validate maxillary sinus height as a reliable marker in Asian populations, highlighting the consistency of this observation across different groups. Additionally, Paknahad et al. [21] confirmed the significance of maxillary sinus height while noting variability based on the population sample studied.

Studies by Aşantoğrol and Coşgunarslan [22] along with Dhandapany et al. [23] confirm statistically significant correlations between maxillary sinus volume and sex, which supports our findings. Aşantoğrol and Coşgunarslan [22] highlighted the impact of anatomical variations on maxillary sinus volume, making this information relevant for the broader applicability of our results. Dhandapany et al. [23] also emphasized the potential of artificial intelligence in analyzing maxillary sinus volumes, suggesting that it could improve both accuracy and applicability.

Our results indicate that the highest accuracy for sex prediction was achieved using the dimensions and volume of the right maxillary sinus, with an accuracy rate of 75%. This is followed by the dimensions and volume of both maxillary sinuses together at 74%, and lastly, the dimensions and volume of the left maxillary sinus, which showed an accuracy of 71%. These findings align with those of Teixeira et al. [19], Prabhat et al. [24], and Wanzeler et al. [25], but contrast with the results reported by Teke et al. [3]. Teixeira et al. [19] found an overall accuracy rate

of 73.6% for sex prediction based on similar parameters, including distance between sinuses. Prabhat et al. [24] reported a higher accuracy for the right maxillary sinus at 80%, compared to 73.3% for the left maxillary sinus, which is consistent with our findings. Wanzeler et al. [25] demonstrated even greater accuracy for the right maxillary sinus, at 80.37%, and noted high accuracy when analyzing both maxillary sinuses together, at 84.66%.

Teke et al. [3] found lower overall accuracy for both maxillary sinuses combined (69.3%) likely due to their smaller sample size and older methodology.

Studies by Teixeira et al. [19], Prabhat et al. [24] and Wanzeler et al. [25] support similar accuracy rates across various populations, indicating the potential universality of these methods. However, variability in findings by Gamba et al [9] and Barros et al. [15] highlights the importance of further validation in different ethnic groups.

The limitation of this study is the exclusion of patients aged 19 years and younger, which may affect the accuracy of sex prediction using the formula created in this study when applied to a younger population.

CONCLUSION

The results of this study revealed significant differences in the dimensions and volume of the left and right maxillary sinuses between the sexes. Male sinuses were found to be larger than those of females, leading to the conclusion that maxillary sinus dimensions can be used for sex assessment. The highest accuracy in sex prediction was observed for the dimensions and volume of the right maxillary sinus, achieving an accuracy rate of 75%. The formulas developed for sex prediction suggest that this method may be beneficial for forensic sex assessment in the population of Bosnia and Herzegovina, especially in cases where other, more reliable methods are not applicable. Future research should aim to validate these formulas in various populations to evaluate their generalizability and accuracy across different demographic groups.

Conflict of interest: None declared.

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Коришћење димензија максиларног синуса за одређивање пола у босанскохерцеговачкој популацији

Игор Ђукић[1], Зоран Јездић[2], Мирсад Кадић[3], Анеса Пировић Кадић[4], Огњенка Јанковић[5], Александра Радуловић[6], Иван Арсић[7], Александар Јаковљевић[8,9], Ненад Недељковић[7]

[1]Универзитет у Бањој Луци, Медицински факултет, Клиника за оралну хирургију, Бања Лука, Република Српска, Босна и Херцеговина;
[2]Универзитет у Београду, Стоматолошки факултет, Клиника за максилофацијалну хирургију, Београд, Србија;
[3]Универзитет „Сарајевска школа за науку и технологију", Одсек за стоматологију, Сарајево, Федерација Босне и Херцеговине, Босна и Херцеговина;
[4]Универзитет у Бањој Луци, Медицински факултет, Бања Лука, Босна и Херцеговина;
[5]Универзитет у Бањој Луци, Клиника за болести зуба и ендодонцију, Медицински факултет, Бања Лука, Република Српска, Босна и Херцеговина;
[6]Универзитет у Бањој Луци, Медицински факултет, Клиника за протетику, Бања Лука, Република Српска, Босна и Херцеговина;
[7]Универзитет у Београду, Стоматолошки факултет, Клиника за ортопедију вилица, Београд, Србија;
[8]Универзитет у Београду, Стоматолошки факултет, Патолошка физиологија, Београд, Србија;
[9]Универзитет у Београду, Стоматолошки факултет, Имплантолошко-истраживачки центар, Београд, Србија

САЖЕТАК

Увод/Циљ Циљ овог истраживања био је да се процени пол на основу снимака компјутерске томографије са конусним снопом анализом димензија и волумена максиларног синуса, као и да се развију формуле за предвиђање пола, уз одређивање њихове тачности за популацију Босне и Херцеговине.

Методе Анализирано је укупно 150 снимака компјутерске томографије са конусним снопом (73 мушкарца, 77 жена) у доби од 20 до 69 година. Димензије (медиолатералне, супероиферионе и антеропостериорне) и волумен максиларних синуса мерени су коришћењем софтвера *Romexis*. Мерења су извршена на аксијалним и коронарним пројекцијама. Формуле за предвиђање пола генерисане су коришћењем вишеструке регресионе анализе. За процену тачности предвиђања пола коришћена је дискриминантна функционална анализа.

Резултати Уочене су значајне разлике ($p < 0,05$) у димензијама синуса и волумену између полова. Мушкарци су имали веће максиларне синусе: волумен левог синуса био је 27,60 ± 6,87 cm^3 код мушкараца наспрам 22,1 ± 5,77 cm^3 код жена, док је волумен десног синуса био 27,48 ± 7,02 cm^3 код мушкараца наспрам 21,65 ± 5,42 cm^3 код жена. Десни максиларни синус пружио је највишу тачност у предвиђању пола (75%), док је леви синус имао тачност од 71%.

Закључак Димензије и волумен максиларног синуса значајно се разликују између полова, при чему мушкарци имају веће синусе. Формула за предвиђање пола заснована на десном максиларном синусу пружа најтачнију процену пола (75%) и може бити користан алат у форензичким применама за популацију Босне и Херцеговине када други методи нису доступни.

Кључне речи: максиларни синус; одређивање пола; форензичка идентификација; *CBCT*; сексуални диморфизам

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Trabecular microcalli in lumbar vertebrae of adult men with alcohol-associated liver disease: postmortem micro-computed tomography assessment

Uroš Anđelić[1], Marko Uzelac[1], Filip Filipović[1], Mihailo Ille[1,2], Marija Đurić[3], Jelena Jadžić[3]

[1]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[2]University Clinical Center of Serbia, Orthopedic and Traumatology Clinic, Belgrade, Serbia;
[3]University of Belgrade, Faculty of Medicine, Center of Bone Biology, Belgrade, Serbia

SUMMARY

Introduction/Objective Increased fracture risk was previously associated with alcohol-associated liver disease (AALD), but contemporary literature lacks the assessment of the micro-fracture healing events (microcalli) in these individuals. We aimed to quantify microcalli in a trabecular compartment of lumbar vertebrae obtained from individuals with pathohistological confirmation of AALD.

Methods We used high-resolution micro-computed tomography scanning to evaluate the density of trabecular microcalli in the anterior mid-transverse portion of lumbar vertebral bodies collected from 32 male adult cadaveric donors (age range: 33–75 years), divided into the AALD group (n = 13) and the control group (n = 19). Pathohistological analysis indicated that seven individuals had the initial AALD stage (fatty liver disease), while six individuals had end-stage AALD (alcoholic liver cirrhosis).

Results A declining trend in the density of trabecular microcalli was noted in the AALD group ($1.8 \pm 1.7/mm^3$) compared to the control ($3.3 \pm 2.6/mm^3$), but without reaching statistical significance ($p = 0.080$, Student's t-test). The density of trabecular microcalli was not significantly different between initial and end-stage AALD ($p > 0.05$; ANOVA with Bonferroni correction). Pearson correlation indicated that a decreasing trend in the density of trabecular microcalli was associated with the deteriorated trabecular microarchitecture of the AALD group.

Conclusions The density of trabecular microcalli was not significantly altered in the lumbar vertebrae of men with different stages of AALD, suggesting that AALD does not have a substantial impact on the healing process of trabecular micro-fractures and the formation of trabecular microcalli in the lumbar vertebrae. However, future studies are required to confirm our findings.

Keywords: alcohol-associated liver disease; micro-fracture healing event; microcallus; micro-CT; lumbar vertebrae; men

INTRODUCTION

The adverse impacts of heavy alcohol consumption on population health led the World Health Organization to define standard alcoholic units (i.e., standard drinks) as 10 g of pure ethanol, with both men and women advised not to exceed two standard drinks per day [1]. This definition has not yet been universally adopted, resulting in varying governmental standards and guidelines across different countries [1]. Continuous consumption of more than three standard drinks per day in men and more than two drinks per day in women for more than five years increases the risk of developing alcohol-associated liver disease (AALD) [2]. Chronic alcohol consumption, regardless of the type of alcoholic beverage, disrupts liver metabolic functions, which results in hepatocytic lipid accumulation, causing the appearance of the initial pathohistological AALD manifestation – fatty liver disease (FLD) i.e., steatosis [3]. With further AALD development, acute and/or chronic alcoholic steatohepatitis may occur, which sets a structural base for progressive liver fibrosis [2]. Finally, irreversible end-stage AALD, known as alcoholic liver cirrhosis (ALC), could be developed by fibrous tissue accumulation and nodular organization of liver parenchyma, making these individuals more prone to hepatocellular carcinoma [3].

While contemporary research in AALD focuses mainly on liver-related clinical manifestations, extrahepatic complications are often neglected [4]. Due to damage of the liver-bone bidirectional crosstalk, frequently neglected AALD-induced extrahepatic complications are skeletal alterations, initially classified as hepatic osteodystrophy [4, 5]. Hepatic osteodystrophy is a clinical term that refers to a spectrum of skeletal abnormalities, including osteopenia, osteoporosis, and osteomalacia, depending on the severity and underlying pathophysiological mechanism [4, 5]. However, more recent studies suggest that osteomalacia, a condition characterized by poor bone mineralization and accumulation of unmineralized osteoid, rarely exists in AALD [6, 7]. On the other hand, osteopenia and osteoporosis are reported to affect up to one-half of patients with AALD, which was demonstrated with an increased fracture risk, primarily affecting the lumbar vertebrae [8, 9].

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Correspondence to:
Jelena JADŽIĆ
University of Belgrade
Faculty of Medicine
Center of Bone Biology
Dr. Subotića 4/2
11000, Belgrade
Serbia
jelena.jadzic@med.bg.ac.rs

A major problem in the clinical management of increased bone fragility is its asymptomatic nature, which causes delays in therapeutic and preventive measures [8]. Since osteoporosis and bone fragility are more frequently observed in postmenopausal women, and AALD is more frequent in men, it is essential to note that AALD may cause a change in the sex-specificity of fracture risk [10]. Additionally, a particularly concerning observation is the AALD-induced shift toward earlier fracture occurrence in younger individuals [8, 11]. Further, the bone fracture mortality rate is substantially higher in patients with AALD [12], indicating the urgent need for adequate bone-assessing tools in the clinical management of these patients [8].

Since direct analysis of bone fracture risk is often challenging, many studies rely on measuring various surrogate markers of increased bone fragility [13]. The "gold standard" in the clinical assessment of bone fragility is the measurement of bone mineral density (BMD) using dual-energy X-ray absorptiometry [13]. Although this method provides reliable data on population-based fracture risk, it is known that BMD alterations can only partially explain increased bone fragility in each patient [14]. Therefore, modern research focuses on analyzing other characteristics at various hierarchical levels of bone tissue organization to understand further the increased bone fragility in the elderly and individuals with chronic diseases [15]. Previous studies reported that micro-scale alterations in the trabecular part of the lumbar vertebrae (thinner and less numbered trabeculae with reduced mechanical properties, deteriorated osteocytic network, and altered bone marrow adiposity) contribute to increased bone fragility in AALD [4, 9, 16]. Furthermore, it has been known that microdamage accumulation, including microcracks and micro-fractures, contributes to the aging-related bone strength decline [17]. Previous research indicates that bone microdamage is typically healed in the form of globular, woven bone formations known as microcalli [17, 18]. Still, bone micro-fracture healing events have not been previously investigated in AALD. Therefore, this study aimed to quantify the density of trabecular micro-fracture healing events (microcalli) in male individuals with pathohistological confirmation of AALD. Additionally, to analyze the effect of the liver disease stage on the density of trabecular microcalli, bone samples collected from individuals with initial and end-stage AALD were compared. Lastly, we aimed to estimate the potential association between micro-fracture healing events (microcalli) and disruption of trabecular microarchitecture in lumbar vertebrae of male individuals with pathohistological confirmation of AALD.

METHODS

Study design and study material

This cross-sectional study encompassed micro-computed tomography (micro-CT) images stored in the digital repository of the Center of Bone Biology, Faculty of Medicine, University of Belgrade (MFUB). Briefly, this study involved the analysis of micro-CT scans of 32 anterior mid-transverse portions of the first lumbar vertebrae that were collected during routine autopsies at the Institute of Forensic Medicine, MFUB, from deceased male adults (aged 33–75 years). The sample collection was approved by the MFUB Ethics Committee (approval no. 1322/V-1, approval date: 20.05.2021).

The collected bone samples were divided into the following groups:

1) AALD group (samples of male adults with different AALD stages, n = 13);

2) control group [samples of healthy male adults of the same age (± 5 years) without liver disease, n = 19].

The AALD group included bone samples from adult men with macroscopically visible signs of chronic alcoholism, with the visible presence of pathologically altered liver tissue at autopsy and positive hetero-anamnestic data on long-term alcohol consumption (more than three units of alcohol per day for more than five years) [9]. Pathohistological analysis of liver tissue confirmed the presence of different stages of AALD in these individuals (Figure 1), revealing that the initial AALD stage was

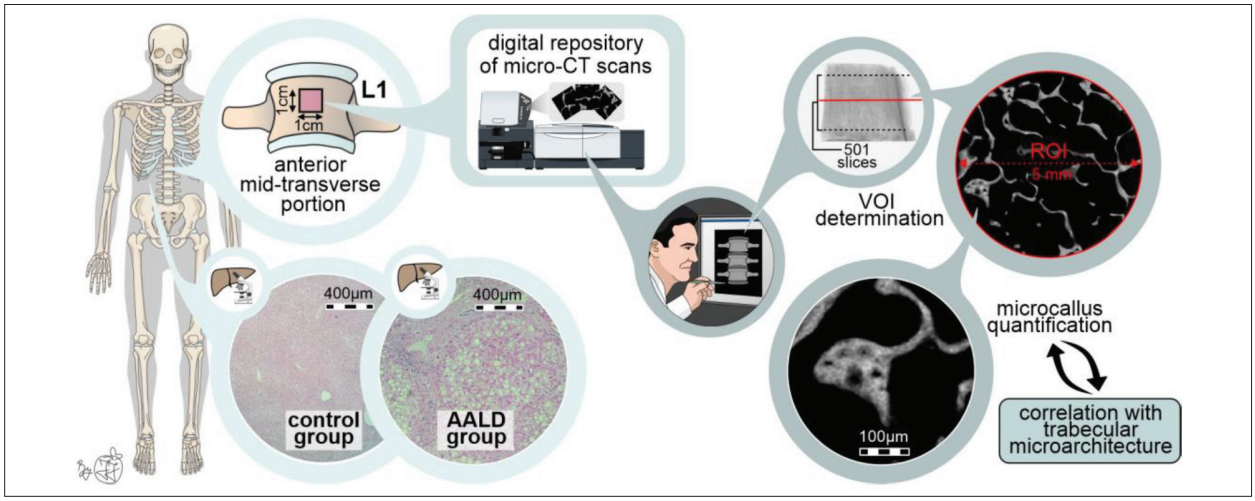

Figure 1. Schematic representation of methodology used in the present study; the figure is hand-generated using vector graphic editor software (CorelDRAW Graphics Suite v.X5, Ottawa, Canada) and represents the author's original work; AALD – alcohol-associated liver disease; L1 – first lumbar vertebra; micro-CT – micro-computed tomography; VOI – volume of interest; ROI – region of interest

present in seven individuals (FLD group), while the end-stage AALD was present in six individuals (ALC group). The control group included healthy age-matched men (± 5 years) without macroscopically visible liver disorders and with negative hetero-anamnestic data about long-term alcohol consumption. Pathohistological analysis of liver tissue confirmed the absence of pathological changes in liver tissue in these individuals (Figure 1).

Study exclusion criteria were a positive history of endocrine and metabolic diseases affecting the skeletal system (e.g., hyperparathyroidism, hypogonadism, thyroid function disorder, diabetes, chronic kidney disease), hereditary musculoskeletal changes, and the presence of solitary and/or metastatic cancerous, infectious and/or inflammatory lesions, as well as the use of drugs that significantly affect bone metabolism (e.g., antiepileptics, cytostatics, corticosteroids, hormonal therapy, vitamin D, and bisphosphonates). Also, clinical or hetero-anamnestic data on intravenous drug abuse and conditions characterized by immobility were criteria for exclusion from the study in potential donors of all groups.

Our micro-CT scanning was performed according to previously published recommendations to ensure adequate imaging quality standards [19]. Using 3D-histomorphometry software (Bruker CTAn Micro-CT Software 2020 1.20.30.0, Bruker, Billerica, MA, USA), all assessments were standardized so that the central 501 sections were analyzed (central section ± 250 sections, Figure 1). Two researchers, independently of each other, manually analyzed the presence of trabecular microcalli in the selected trabecular volume of interest (VOI). These VOIs were generated using centrally-positioned circular regions of interest (ROI), with a diameter of 5 mm (Figure 1). The microcallus quantification was done based on previous reports [18]. In short, trabecular microcalli were defined as globular formations made of woven bone (Figure 1), and its density was quantified per unit of vertebral bone volume (#/mm^3), while the mean results of the two investigators were used for statistical analysis. Further, to estimate the association between microcalli and trabecular microarchitecture, standard vertebral microarchitectural parameters [bone volume ratio (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), and trabecular pattern factor (Tb.Pf)] were generated using 3D histomorphometry software (Bruker CTAn Micro-CT Software 2020 1.20.30.0, Bruker), as previously described [20].

Statistical analysis

Since the Kolmogorov–Smirnov test confirmed a normal distribution, the Student's t-test for two independent samples was used to assess the significance of the intergroup differences, with a significance level of 5% and a 95% confidence interval ($p < 0.05$). A one-way analysis of variance (ANOVA) with a Bonferroni *post hoc* test was used to assess the significance of the difference between different AALD stages and the control group. Further, the Pearson correlation assessment estimated the correlation between the density of trabecular microcalli and trabecular microarchitectural parameters in the obtained vertebral samples. Non-commercially available statistical EZR software (Easy R on R Commander) was used for data analysis.

RESULTS

The demographic data of included individuals and results of pathohistological assessment of liver tissue samples were presented in Table 1.

Table 1. Basic autopsy data and pathohistological assessment of liver tissue samples

Demographic data	AALD group	Control group
Age (years)	58 ± 13	59 ± 6
BMI (kg/m^2)	25.6 ± 7.1	25.7 ±2.8
Hip BMD in osteoporotic range (n)	2/13	1/19
Hip BMD in osteopenic range (n)	6/13	4/19
Previous bone fracture (n)	0/13	0/19
Pathohistological assessment of liver tissue		
Piecemeal necrosis		
None (n)	2/13	5/19
Mild (n)	1/13	10/19
Moderate (n)	2/13	2/19
Marked (n)	2/13	2/19
Moderate + bridging necrosis (n)	3/13	0/19
Marked + bridging necrosis (n)	2/13	0/19
Multilobular necrosis (n)	1/13	0/19
Intralobular degeneration and focal necrosis		
None (n)	2/13	19/19
Mild (n)	7/13	0/19
Moderate (n)	3/13	0/19
Marked (n)	1/13	0/19
Portal inflammation		
None (n)	4/13	7/19
Mild (n)	6/13	8/19
Moderate (n)	2/13	4/19
Marked (n)	1/13	0/19
Steatosis		
None (i.e. < 5%, n)	1/13	11/19
Mild (i.e. 5–33%, n)	2/13	6/19
Moderate (i.e. 34–66%, n)	4/13	2/19
Marked (i.e. > 66%, n)	6/13	0/19
Fibrosis		
No fibrosis (n)	5/13	14/19
Fibrous portal expansion (n)	1/13	5/19
Bridging fibrosis (n)	0/13	0/19
Cirrhosis (n)	7/13	0/19

AALD – alcohol-associated liver disease; BMI – body mass index; BMD – bone mineral density

Micro-CT revealed the presence of a total of 86 trabecular microcalli in the analyzed samples. Representative micro-CT findings of trabecular microcalli in lumbar vertebrae of individuals with AALD are shown in Figure 2. Trabecular microcalli were absent in 38.4% of the AALD group and 26.3% of control samples.

As shown in Figure 3A, our data indicate a trend toward a decrease in the density of trabecular microcalli in the AALD group (1.8 ± 1.7/mm^3) compared to controls

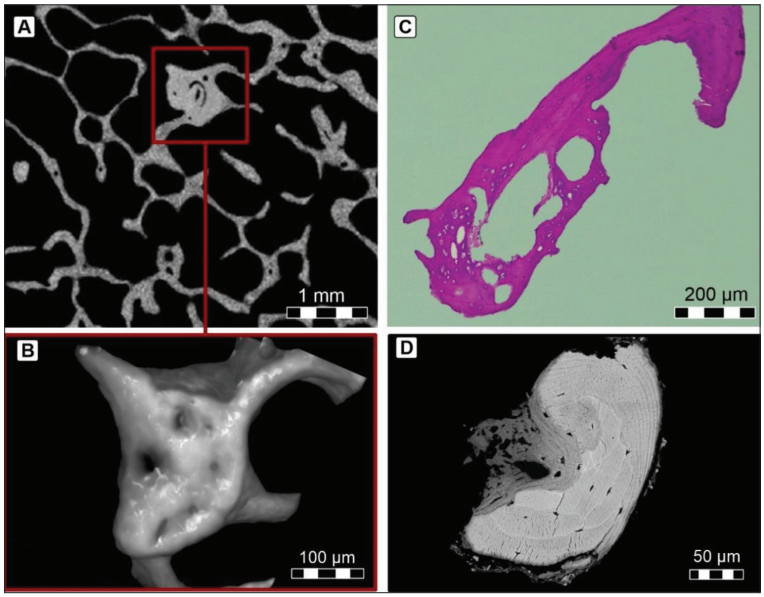

Figure 2. Representative findings of trabecular microcalli in individuals with alcohol-associated liver disease, obtained by micro-computed tomography (A – transverse cross-section, B – 3D reconstruction), C – optic microscopy, and D – quantitative back-scattered electron imaging; the figure represents the author's original work, arranged using vector graphic editor software (CorelDRAW Graphics Suite v.X5, Ottawa, Canada)

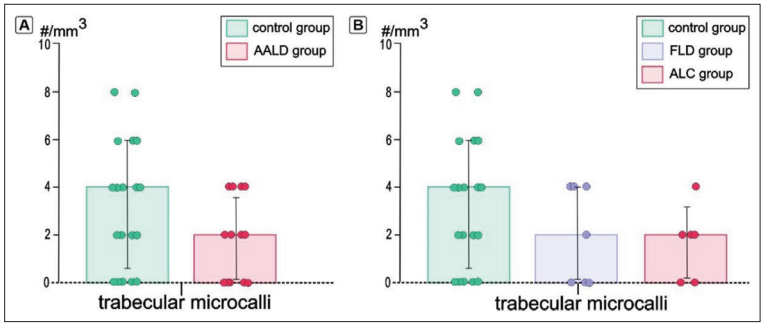

Figure 3. Comparative assessment of trabecular microcalli in individuals with alcohol-associated liver disease and the control group (A) and comparative assessment of trabecular microcalli in individuals with various stages of the disease (B); the statistical significance of the inter-group difference was estimated using the Student t-test (A) and ANOVA with Bonferroni *post hoc* test (B); bar graphs represent the data as mean ± standard deviation, including the distribution of individual data points; the figure represents the author's original work generated using Origin software [Origin(Pro), Version 2018. OriginLab Corporation, Northampton, MA, USA]; AALD – alcohol-associated liver disease; FLD – fatty liver disease (initial stage of alcohol-associated liver disease); ALC – alcoholic liver cirrhosis (end-stage alcohol-associated liver disease)

(3.3 ± 2.6/mm^3), but without reaching statistical significance ($p = 0.080$). As presented in Figure 3B, our data showed no significant difference in the density of trabecular microcalli in lumbar vertebrae collected from individuals with initial and end-stage AALD ($p > 0.05$).

The density of trabecular microcalli showed a strong positive correlation with age in all investigated individuals and controls, indicating an increasing trend in the density of trabecular microcalli with advanced age (Table 2). Furthermore, the density of the trabecular microcalli displayed a strong negative correlation with BV/TV and a moderate negative correlation with Tb.N in controls, suggesting that increased density of trabecular microcalli was noted in persons with lower BV/TV and Tb.N ($p < 0.05$). When analyzing samples from the AALD group only, our data indicated that density of trabecular microcalli displayed a strong positive correlation with BV/TV and mild positive correlation with Tb.N and Tb.Pf, implying that a trend toward a decrease in the density of trabecular microcalli in the AALD group was associated with deteriorated trabecular microarchitecture ($p < 0.05$, Table 2).

DISCUSSION

The concept of bone quality (features related to the bone material structure and composition) is increasingly considered important to heightened bone fragility without being noted in standard clinical tools [15]. Bone quality includes microdamage (micro-fractures, microcracks, and its healing events – microcalli), which accumulates due to physiological loading and mechanical stress occurring in daily life [21]. Numerous studies have focused on investigating the accumulation of ageing-related cortical bone microdamage, revealing that microcracks can dissipate energy and stimulate bone remodeling orchestrated by osteocyte mechanosensing mechanisms [21, 22]. Microdamage accumulation is more prevalent in interstitial bone, which is comparatively older than the surrounding tissues, and it is likely to have higher levels of non-enzymatic collagen cross-linking and lower water content [23]. Previous research devoted less attention to trabecular microdamage, but contemporary data suggest that if trabecular micro-fracture occurs extensively and if the micro-fracture healing rate is not adequate to repair the damage, the trabecular bone connectivity will be lost at the site of the micro-fracture, resulting in trabecular structural weakness, leaving a marked impact on bone strength and eventually leading to aging-related fragility fracture [17, 21]. However, it is essential to note that trabecular microdamage in patients with chronic diseases was rarely studied. To our knowledge, only one study revealed an increased density of vertebral trabecular microcalli in patients with chronic kidney disease [24]. The lack of microcalli research may have been due to the limited number of suitable techniques available for analyzing trabecular microcalli. These techniques include 2D histological assessment using optical microscopy or scanning electron microscopy, as well as 3D histomorphometric assessment using micro-CT (Figure 2). Since our previous studies revealed that the most prominent impairment in micro-scale trabecular vertebral features contributes to increased fracture susceptibility in AALD [4, 16, 25], we aimed to assess the density of trabecular microcalli in male individuals with pathohistological confirmation of AALD, using a high-resolution and non-destructive methodology.

Table 2. Correlation between density of trabecular microcalli and trabecular microarchitectural parameters in lumbar vertebrae

Parameters	Microcalli and age	Microcalli and BV/TV	Microcalli and Tb.Th	Microcalli and Tb.N	Microcalli and Tb.Sp	Microcalli and Tb.Pf
Total sample (n = 32)						
Correlation coefficient (r)	**0.455**	-0.060	-0.211	0.173	-0.277	0.164
p-value	**0.009**	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p > 0.05
AALD group (n = 13)						
Correlation coefficient (r)	0.410	**0.615**	-0.367	**0.578**	-0.147	**0.563**
p-value	p > 0.05	**0.049**	p > 0.05	**0.039**	p > 0.05	**0.045**
Control group (n = 19)						
Correlation coefficient (r)	**0.690**	**-0.634**	-0.327	**-0.400**	-0.128	0.205
p-value	**0.001**	**0.045**	p > 0.05	**0.035**	p > 0.05	p > 0.05

Pearson correlation was used to estimate the correlation between the density of trabecular microcalli and trabecular microarchitectural parameters in collected vertebral samples (significant correlations were presented in bold; AALD – alcohol-associated liver disease; BV/TV – bone volume ratio; Tb.Th – trabecular thickness; Tb.N – trabecular number; Tb.Sp – trabecular separation; Tb.Pf – trabecular pattern factor

Still, our data revealed only a nonsignificant declining trend in the density of trabecular microcalli in AALD (Figure 3), implying that AALD does not have a substantial impact on the healing process of trabecular micro-fractures and the formation of trabecular microcalli in the lumbar vertebrae. Considering that this result challenged our previous findings about the deteriorated vertebral bone quality in AALD, we relied on the Pearson correlation test to understand these puzzling findings [4]. In accordance with previous studies, our data revealed a strong positive correlation of microcalli density with age in all investigated individuals and control individuals, suggesting an increasing trend in the density of trabecular microcalli in advanced age (Table 2) [17, 21]. The density of the trabecular microcalli displayed a strong negative correlation with BV/TV and a moderate negative correlation with Tb.N in our control individuals (Table 2), implying that increased density of trabecular microcalli was associated with aging-related bone strength decline. Previous data suggested that neighboring microcalli are condensed near the endplates area, often appearing to form a line, corresponding to the mechanical loading pattern within the vertebral body [26, 27], while its mechanical characteristics were similar to that of a normal trabecular bone [28], indicating that microcalli might help maintain vertebral bone strength in the elderly [17]. Hahn et al. [26] reported that trabecular microcalli are most frequently found when vertebral BV/TV was less than 11% but without further correlation with declining BV/TV, indicating that this bone strength-maintaining mechanism is becoming ineffective in cases of severely declined microarchitecture. Since nine of our individuals with AALD (69.23%) had BV/TV lower than 11%, our data support this assumption (Table 2), implying that a trend toward a decrease in the density of trabecular microcalli in the AALD group was associated with the highly deteriorated trabecular microarchitecture [20, 29]. Subsequently, it is reasonable to assume that the decreased density of trabecular microcalli manifests reduced vertebral bone strength and subsequently increased vertebral fracture susceptibility in AALD [8].

Additionally, previous studies have not provided a clear answer regarding whether the stage of liver disease substantially impacts bone deterioration and, consequently, the healing process of micro-fractures. Some authors reported that skeletal damage was more pronounced in patients with advanced stages of liver disease and that the severity of skeletal changes is related to the disease stage [4, 20, 30]. Our data did not reveal a significant difference in the density of trabecular microcalli between individuals with initial and end-stage AALD (Figure 3), indicating that the AALD stage does not substantially affect the micro-fracture healing process in the lumbar vertebrae. These conflicting results may be due to differences in the study design (female subjects were predominantly examined in these previous studies), the type of chronic liver disease (cholestatic liver diseases were predominantly analyzed in previous studies), as well as the type of bone-assessing methodology (optic histomorphometry and peripheral quantitative CT were predominantly used in previous studies) or even interobserver differences when using the same methodological approach, which indicates that further research is necessary to elucidate this topic thoroughly [19].

Our study is limited by its cross-sectional cadaveric study design, which prevents us from following the progression of skeletal damage over time. Our findings may be biased due to the covariant effect of various confounding factors, which could not be avoided due to the scarcity of heteroanamnestic data. Although we utilized all available resources, the absence of significant inter-group differences in trabecular microcalli suggested that our assessment may have been underpowered due to the limited sample size. Our assessment may have limited informative value in evaluating bone metabolism, cellular indices, and mineral content of trabecular microcalli in AALD individuals, underscoring the need for further research. Lastly, this study exclusively focused on the density of trabecular microcalli in lumbar vertebrae of AALD individuals, thereby enabling only an indirect estimate of its effect on bone micro-fracture accumulation and healing.

CONCLUSION

Our data has revealed that the density of trabecular microcalli was not significantly altered in the lumbar vertebrae of men with different stages of AALD, suggesting that AALD does not have a substantial impact on the healing process of trabecular micro-fractures and the formation of trabecular microcalli in the lumbar vertebrae. Our data

implied that a trend toward a decrease in the density of trabecular microcalli in the AALD group was associated with the highly deteriorated trabecular vertebral microarchitecture. However, considering this study's limitations, further research is necessary to confirm our results.

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Трабекуларни микрокалуси слабинских кичмених пршљенова код одраслих мушкараца са алкохолном болешћу јетре – постмортална анализа методом микрокомпјутеризоване томографије

Урош Анђелић[1], Марко Узелац[1], Филип Филиповић[1], Михаило Илле[1,2], Марија Ђурић[3], Јелена Јаџић[3]

[1]Универзитет у Београду, Медицински факултет, Београд, Србија;
[2]Универзитетски клинички центар Србије, Клиника за ортопедску хирургију и трауматологију, Београд, Србија;
[3]Универзитет у Београду, Медицински факултет, Центар за биологију костију, Београд, Србија

САЖЕТАК

Увод/Циљ Претходне студије указале су на повећан ризик од прелома код особа са алкохолном болешћу јетре (АБД), али зарастање трабекуларних микропрелома (у виду микрокалуса) до сада није истраживано код ових болесника. Циљ наше студије био је да квантификује густину трабекуларних микрокалуса у слабинским кичменим пршљеновима мушких кадаверичних донора са патохистолошком потврдом различитих стадијума АБД-а.

Методе Користили смо снимке микрокомпјутеризоване томографије високе резолуције за процену густине трабекуларних микрокалуса у телима слабинских кичмених пршљенова прикупљених од 32 мушка одрасла кадаверична донора (старости између 33 и 75 година), који су били разврстани у две групе – АБД групу ($n = 19$) и контролну групу ($n = 13$). Патохистолошка анализа ткива јетре показала је да је седам особа имало почетни стадијум АБД-а (масна болест јетре), док је шест особа боловало од крајњег стадијума АБД-а (алкохолна цироза јетре).

Резултати У АБД групи уочен је тренд смањења густине трабекуларних микрокалуса ($1,8 \pm 1,7/mm^3$) у поређењу са контролном групом ($3,3 \pm 2,6/mm^3$), али без достизања статистичке значајности ($p = 0,080$; Студентов т-тест). Такође, није утврђена значајна разлика у густини трабекуларних микрокалуса код особа са различитим стадијумима АБД-а ($p > 0,05$; АНОВА, Бонферонијева *post hoc* анализа). Пирсонов тест корелације указује да је опадајући тренд густине трабекуларних микрокалуса повезан са пропадањем трабекуларне микроархитектуре уочене у АБД групи.

Закључак Наши резултати показују да густина трабекуларних микрокалуса није била значајно измењена код мушкараца са различитим стадијумима АБД-а, што указује да АБД нема значајан утицај на процес формирања трабекуларних микрокалуса слабинских кичмених пршљенова. Ипак, даља истраживања су неопходна како би потврдила ове резултате.

Кључне речи: алкохолна болест јетре; зарастање микропрелома; микрокалус; микрокомпјутеризована томографија; слабински кичмени пршљенови; мушкарци

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Efficacy of Xiaozhoutian fire dragon moxibustion combined with traditional Chinese medicine on polycystic ovary syndrome with kidney yang deficiency – a randomized controlled trial

Liuxiu Yu[1], Mingfeng Xiong[1], Xiaoyu Li[1], Chunhong Wu[2], Liqin Wang[1], Yao Gao[1], Minxia Long[1], Qin Gan[1]

[1]Nanchang University, Jiangxi Medical College, The First Affiliated Hospital, Preventive Center of Traditional Chinese Medicine, Nanchang, China;
[2]Nanchang University, Jiangxi Medical College, The First Affiliated Hospital, Outpatient department, Nanchang, China

SUMMARY

Introduction/Objective To investigate the efficacy of Xiaozhoutian fire dragon moxibustion combined with traditional Chinese medicine on polycystic ovary syndrome (PCOS) with kidney yang deficiency.

Methods 80 patients with kidney yang deficiency type PCOS admitted were selected and randomly divided into the control group and the intervention group, with 40 patients in each group. The control group was given routine nursing care and traditional Chinese medicine (TCM) treatment, and the intervention group was treated with Xiaozhoutian fire dragon moxibustion based on the control group. After three months, the therapeutic effects of the two groups were compared by TCM symptom score, menstrual condition and serum sex hormone level.

Results Patients in the intervention group showed a significant improvement in the TCM symptom score compared with the control group after treatment; menstrual conditions such as duration of menstrual period, menstrual flow, degree of dysmenorrhea, and blood clots, and serum sex hormone levels such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), luteinizing hormone/follicle-stimulating hormone ratio (LH/FSH), estradiol (E2), testosterone (T), and progesterone (P) were all better than those in the control group, and the difference was statistically significant ($p < 0.05$); the total effective rate of treatment for patients in the intervention group reached 92.50%, which was significantly higher than that of 72.5% of the control group ($p < 0.05$).

Conclusion Xiaozhoutian fire dragon moxibustion combined with traditional Chinese medicine has good clinical efficacy in treating PCOS with kidney yang deficiency, which can improve the symptoms and hormone level of patients and improve the quality of body.

Keywords: Xiaozhoutian fire dragon moxibustion; traditional Chinese medicine; kidney yang deficiency syndrome; polycystic ovary syndrome; randomized controlled study

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disease in gynecology, which is characterized by hyperandrogenemia, ovulatory dysfunction, insulin resistance and ovarian polycystic changes, with the main clinical manifestations of scanty menstruation or amenorrhea, infertility, obesity, and hirsuteness, etc. [1]. In China, the incidence of PCOS can reach 5–10%, and tends to increase year by year. Ovulatory dysfunction infertility due to PCOS accounts for about 75% of anovulatory infertility and is one of the most common causes of infertility in women of reproductive age [2]. Currently, ovulation promotion and blood androgen lowering are mostly used in clinical treatment, which have certain efficacy, but are often associated with adverse effects such as follicular developmental disorders, multiple pregnancies, high miscarriage rates, and long treatment cycles with poor patient compliance [3, 4]. Currently, the United States Food and Drug Administration has not approved any therapeutic drugs specifically for PCOS, but some drugs commonly used in clinical practice, such as oral contraceptives, anti-androgen drugs, insulin sensitizers, and ovulation-inducing drugs, are prescription drugs and usually require an endocrinologist or gynecologist to evaluate and prescribe according to the patient's specific situation [5, 6]. In addition to the need for improved development of new drug molecules and new drug discovery, new drugs can be found through drug repurposing methods [7]. Given that PCOS is a growing problem, unfortunately with many unwanted complications that follow, and that the available methods and drugs are not 100% effective, it is important to study its pathogenesis and carefully identify new pharmacological targets.

Fire dragon moxibustion is also called "long snake moxibustion" or "Du moxibustion." It is mostly performed on the Du channel and bladder meridian on the back of the human body. It is a large area moxibustion method [8].

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Correspondence to:
Qin GAN
Nanchang University
Jiangxi Medical College
The First Affiliated Hospital
Preventive Care Center,
No. 17 Yongwaizheng Street
Donghu District
Nanchang
330000 Jiangxi Province
China
ganq2024gq@163.com

The Du channel is the sea of yang channels. Fire dragon moxibustion performed on the sea of yang channels has a strong warming effect on the human body and is a "large moxibustion" method. Traditional fire dragon moxibustion generally selects the Du channel on the back and the bladder meridian on both sides. After a long period of clinical application and innovation, it has also been applied to the chest, abdomen, knee joints, and other body parts. Fire dragon moxibustion has the effects of local stimulation, regulating meridians, and improving immune function. The local stimulation effect is achieved through the warm stimulation generated by burning during moxibustion, which causes local skin congestion, capillary dilation, enhanced local blood circulation and lymphatic circulation, and enhanced metabolic capacity of local skin tissue, promoting the dissipation and absorption of pathological products such as inflammation, adhesion, exudate, and hematoma [9]; it can also cause the diffusion of inhibitory substances in the cerebral cortex, reduce the excitability of the nervous system, and exert a sedative and analgesic effect; the warming effect can promote the transdermal absorption of warming yang drugs, so that the warming and unblocking effects of moxa or powder can penetrate into the meridian acupoints of the Governor Vessel and the Bladder Meridian, which has the effect of invigorating yang; all the internal organs of the body have corresponding acupoints on the Governor Vessel. While moxibustion is performed, it can both control the yang of the whole body and regulate the internal organs of the whole body, with the effect of connecting the internal organs internally and connecting the limbs externally [10].

Based on the theory of traditional acupuncture and combining with the theory of Chinese medicine, Xiaozhoutian fire dragon moxibustion can treat any localized lesion from the overall qi to do the corresponding overall treatment and targeted lesion treatment. The overall qi treatment ensures the normal circulation of the body's overall qi, and the targeted lesion treatment ensures the healing of specific lesions. Xiaozhoutian fire dragon moxibustion is applied to the Du and Ren veins in the back and abdomen of the human body through the warming effect of burning moxa and the efficacy of herbal medicine and wine, which has the effect of relaxing the tendons and activating the collaterals, warming yang and tonifying the kidneys, invigorating the spleen and nourishing the stomach, and regulating the yin and yang [11]. Its clinical application is mostly based on the syndrome differentiation and classification of the patient's symptoms. Different drugs are selected and matched, and with the help of the warming power of moxa and the thermal effect of fire, the "four-in-one" combination of acupuncture points, meridians, moxibustion, and drugs is achieved. It stimulates the body's healthy energy and drives away disease and pathogenic factors [8]. It is currently mainly used in lung diseases, joint diseases, gynecological diseases, spleen and stomach diseases and other systems [10]. For example, Chen et al. [12] used a clinical randomized controlled study to compare fire dragon moxibustion with traditional Chinese medicine encapsulated hot compress in the treatment of ankylosing spondylitis with kidney deficiency and Du Cold type. Afterwards, the patient's pain, morning stiffness, symptoms and signs were significantly improved; Wen [13] used Fire Dragon Moxibustion combined with Hot Ampoule to treat COPD patients compared with conventional breathing. COPD patients treated with the disease not only improved their clinical symptoms but also improved their lung function; Yue et al. [14] performed on 60 patients with primary dysmenorrhea. Fire dragon moxibustion has been found to improve the quality of life of sub-healthy women while treating diseases and has the effect of improving the patient's physical condition. According to the clinical manifestations of PCOS, it belongs to the categories of "late menstruation," "amenorrhea" and "infertility" in Chinese medicine [15]. A study by Huang and Wang [16] evaluated the clinical effects of acupuncture combined with Western medicines and herbs that warm the yang and tonify the kidneys, strengthen the spleen and activate the blood in the treatment of PCOS. The results showed that the combined treatment approach was more effective in lowering serum testosterone, fasting insulin levels, luteinizing hormone/follicle-stimulating hormone ratios, and in increasing ovulation rates than Western medicine alone.

According to the theories of "the liver and kidney have the same origin" and "the liver stores blood" [17], the traditional Chinese medicine formula used in this study was Shugan Yanggan Fuyang Tang, which is effective in warming yang, dispersing cold, nourishing the liver, and warming the kidneys. Tang is commonly used in the clinical treatment of PCOS in traditional Chinese medicine (TCM), but few studies have used it together with Xiaozhoutian fire dragon moxibustion as a treatment program for PCOS patients with kidney-yang deficiency. Internal and external medications often complement each other, and combining Xiaozhoutian fire dragon moxibustion therapy with tonics can lead to better drug absorption and greater effectiveness. Therefore, this study takes this therapy as an entry point and adopts the research method of randomized controlled trial to explore the overall efficacy of Xiaozhoutian fire dragon moxibustion combined with traditional Chinese medicine in the treatment of kidney-yang-deficient PCOS patients and the differences in the changes of relevant indicators. This provides a relevant basis for future clinical treatment and research, with a view to providing a novel and effective treatment option for this disease and diversified treatment choices for the broader kidney-yang deficiency PCOS group.

METHODS

Participants

This randomized controlled trial (RCT) was conducted from October 2022 to February 2024 at the First Affiliated Hospital of Nanchang University. Convenience sampling method was used to recruit PCOS patients. This study was approved by the ethics committee of the First Affiliated Hospital of Nanchang University (Approval number: (2024)CDYFYYLK(12-241). Written informed consent

was obtained from all participants. In addition, voluntary participation and confidentiality of responses were assured.

Randomization and blinding

Random numbers were generated by IBM SPSS Statistics Version 26.0 (IBM Corp, Armonk, NY, USA). The corresponding allocation schemes were saved by the researchers into opaque airtight envelopes. Participants entering the trial opened the envelopes in the order of enrollment and received treatment in the corresponding group according to the allocation scheme in the envelopes. 80 PCOS patients with kidney yang deficiency who met the inclusion criteria were divided into control group and intervention group, 40 patients in each group.

Diagnostic criteria

Western medical diagnostic criteria: refer to the diagnostic criteria of the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine [18]: (a) scanty menstruation or amenorrhea or irregular uterine bleeding; (b) clinical hyperandrogenism or hyperandrogenemia, such as hirsutism, obesity, etc; (c) ultrasonography suggests that the ovary is polycystic changes. The diagnosis can be confirmed if (a) and any one of (b) and (c) are met and other diseases related to the disease are excluded.

Chinese medicine identification criteria: referring to the "Chinese Medicine Gynecology" [19] and the "Chinese Medicine Diagnostic Efficacy Criteria" [20] published by the State Administration of Traditional Chinese Medicine to develop the diagnostic criteria for kidney yang deficiency. Primary symptoms: menstrual cycle disorders or amenorrhea, cold limbs and cold feet, lower libido, lumbar and knee pain and weakness. Secondary symptoms: excessive amount of discharge, tinnitus and dizziness, pale face, clear and long urine, frequent nocturnal enuresis, light and fat tongue, white moss, and a sunken and weak pulse. Diagnostic requirements: the primary symptom has two of them at the same time, and the secondary symptom has two of them, and the tongue and pulse coincide, then the diagnosis can be confirmed.

Inclusion and exclusion criteria

The inclusion criteria were listed as follows: (a) meeting the above western medical diagnostic criteria of PCOS and Chinese medicine identification criteria; (b) aged 18-50 years old; (c) normal comprehension ability and fluent verbal communication; (d) voluntarily participating in this study and signing the informed consent form; and (e) no history of western medicine treatment for PCOS.

The exclusion criteria were as follows: (a) patients with comorbid psychiatric diseases or serious cognitive deficiencies; (b) patients with comorbid serious medical and surgical diseases, hematologic diseases, cardiovascular and cerebrovascular diseases, immunodeficiencies, etc. who were not suitable to participate in the study; (c) patients who might have other causative factors, such as premature ovarian failure, adrenocortical hyperplasia, Cushing's syndrome, etc.; (d) patients who were taking part in other therapeutic research studies; and (e) pregnant women.

Interventions

Control group

In the control group, in addition to the routine care in the Chinese medicine department, traditional Chinese medicine prescriptions were taken orally for treatment. Based on the theories of "liver and kidney homology" and "liver storing blood" [17], the traditional Chinese medicine prescription adopts Shugan Yanggan Fuyang Tang. The specific drug composition includes: Radix Pseudostellariae, Poria, Atractylodes, Radix Paeoniae Rubra, Radix Paeoniae Alba, Radix Astragali seu Hedysari, Radix Angelicae Sinensis, Rhizoma Cyperi, Fructus Amomi, Radix Curcumae, Radix Puerariae, Herba Artemisiae Scopariae, Rhizoma Imperatae, Pyrrosia, Herba Lysimachiae, and Radix Glycyrrhizae. Patients start to take Chinese herbal medicine orally on the third day of menstruation every month, decocting with water, taking 200 ml each in two doses in the morning and evening, one dose/day, 28 days as a course of treatment, and taking three consecutive courses of treatment.

Intervention group

The intervention group was treated with Xiaozhoutian fire dragon moxibustion combined with traditional Chinese medicine [21]. The traditional Chinese medicine, the method of administration and the course of treatment were the same as that of the control group. On its basis, the combination of Xiaozhoutian fire dragon moxibustion was treated for three courses (starting from the third day of the end of menstruation, three times a month for 1 course of treatment, excluding menstruation) for a total of three months. The operation method of Xiaozhoutian fire dragon moxibustion is as follows. Do the moxibustion of the Du vessel first, and then the Ren vessel. After the patient exposes the moxibustion site, lay a warm yang medicinal wine gauze block, lay two layers of large towels on top of it, and then lay a layer of warm wet small towels. Mugwort was laid flat on the towels, 95% alcohol was sprinkled on top of the moxa, and the fire was lit and burned. When the patient complains of tolerable warm spots, use a small wet towel (two overlapping) to cover the moxibustion site to extinguish the fire, and press the moxa part with both hands using the vertical method, the tremor method, the patting method, and other nudging techniques. Until the heat subsides, spray alcohol again to repeat the above operation. Flip the moxa after three times. Apply moxibustion a total of 3–5 times until the skin is evenly red, and blistering is not necessary. Each treatment time 30~40 minutes, the treatment process at any time to ask the patient's feelings and adjust the heat to avoid burns.

Measurements

TCM symptom score

Referring to the scoring criteria developed by the State Administration of Traditional Chinese Medicine's "TCM Diagnosis and Treatment Programs for 104 Diseases in 24 Specialties (Trial)" [22], the patients' main symptoms were recorded one by one before and after treatment. These included (a) cold hands and feet, (b) fear of cold in the stomach and epigastric region, back, or waist and knees, (c) fear of cold and wearing more clothes than others, (d) intolerance of cold (cold in winter, air-conditioning fan in summer, etc.) than others, (e) catching a cold more easily than others, and (f) feeling uncomfortable or fear of eating (and drinking) cold things. All symptoms were scored on a five-point Likert scale from 1 to 5, with 1 being the absence of the symptom and 5 being the presence of the symptom all the time. The total TCM Symptom Score was summed up from all the symptom scores, and the total score ranged from 5 to 30. The higher the score, the more severe the symptoms are.

Menstrual situation

Changes in the duration of the menstrual period and menstrual flow, as well as the scoring of symptoms accompanying menstruation, such as dysmenorrhea and the number of blood clots, were recorded before and after the treatment of the patients, and scored with reference to the scoring criteria of TCM drawn up by the "Guidelines for the Clinical Research of New Traditional Chinese Medicines (Trial Implementation)" [23]. (i) Duration of menstrual period: 4–7 days as 0 points, 1–3 days as 1 point, 8–15 days as 2 points. (ii) menstrual flow: 50~80 mL is normal, 0 points; less than normal is 1 point; more than normal is 2 points. (iii) Degree of dysmenorrhea: a visual analogue scale (VAS) [24] was used, using a 10 cm long ruler marked with a scale of 0 to 10 points, with 0 points representing no pain felt and 10 points representing the strongest pain. The score of pain intensity was derived directly from the values marked by the patient on the scale. (iiii) Clot status: no clot was rated as 0, a small number of clots was rated as 1, and many clots was rated as 2.

Serum sex hormone level

Early morning fasting venous blood was drawn from patients before and after treatment, and serum was cryo-separated using the SteroIDQ kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) to measure serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), luteinizing hormone/follicle-stimulating hormone ratio (LH/FSH), estradiol (E2), testosterone (T), and progesterone (P) levels [25].

Efficacy criteria

Evaluate the efficacy of patients after treatment with reference to the PCOS-related standards in Gynecological Endocrinology [26]. (a) Cured: menstrual cycle returns to normal, symptoms accompanying menstruation disappears, menstrual volume, color and quality basically return to normal, and sex hormone level returns to normal. (b) Obvious effect: the menstrual cycle is basically back to normal (the interval between two menstrual periods is within 30–35 days), the symptoms accompanying menstruation have been significantly relieved, menstrual flow, color and quality have been greatly improved compared with the pre-treatment period, and the sex hormone levels have been improved to a certain extent compared with the previous period. (c) Effective: the menstrual cycle returns to normal to a certain extent (the interval between two menstrual periods is within 34–40 days), the accompanying symptoms of menstruation are mildly relieved, the menstrual volume, color and quality are slightly improved, and the sex hormone level is slightly improved compared with the previous one. (d) Ineffective: no significant improvement in menstrual cycle with treatment, no improvement in symptoms accompanying menstruation, no significant change in menstrual volume, color and quality, and no improvement in sex hormone level. Total effective rate = (cured + obvious effect + effective) number of cases / total number × 100%.

Statistical analysis

IBM SPSS Statistics Version 26.0 (IBM Corp, Armonk, NY, USA) software was used for statistical analysis. Measurement information was compared between groups using t-test and expressed as mean ± standard deviation. Comparison of count data between groups was performed using the χ^2 test test and expressed as frequency and percentage; $p < 0.05$ indicated that the difference was statistically significant.

Technical route

The study of technical route consult Figure 1.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the First Affiliated Hospital of Nanchang University [(2024) CDYFYYLK(12-241)]. Written informed consent was obtained from all participants.

RESULTS

Patients' characteristics

A total of 80 PCOS patients with kidney-yang deficiency were included in this study, and they were divided into the control group and the intervention group according to the principle of randomized control, and 40 subjects were included in each of the two groups. Both groups of participants completed the study, and no one withdrew (Figure 2).

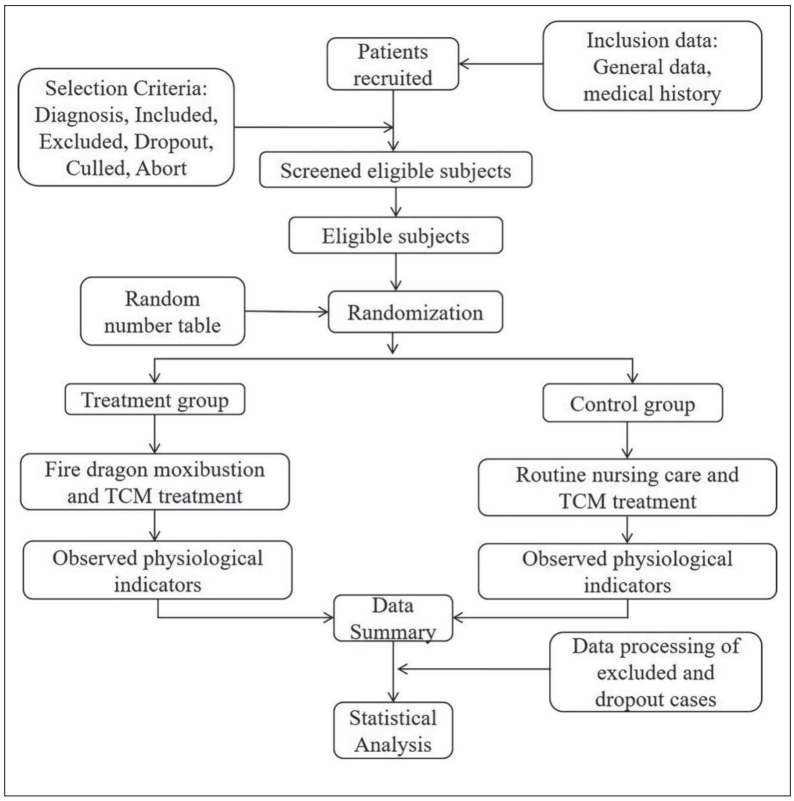

Figure 1. Technical route; TCM – traditional Chinese medicine

Demographic and disease information

The minimum age of the participants in the control group was 19 years and the maximum age was 44 years with a mean age of (32.63 ± 5.53) years. The age range of the participants in the intervention group was 19–43 years old, with a mean age of (31.48 ± 5.22) years. There was no statistically significant difference in the age of the patients in the two groups ($p > 0.05$) and they were comparable. The mean duration of the disease in the control group was (3.37 ± 1.68) years, of which 1(3%) had a duration of less than 12 months, 4(10%) from 12 to 24 months, 7(17%) from 25 to 48 months and 28(70%) from more than 48 months. The mean duration of the disease in the intervention group was (3.43 ± 1.96) years, of which three (7%) had a duration of less than 12 months, 0 (0%) had a duration of 12–24 months, 13 (33%) had a duration of 25–48 months, and 24 (60%) had a duration of

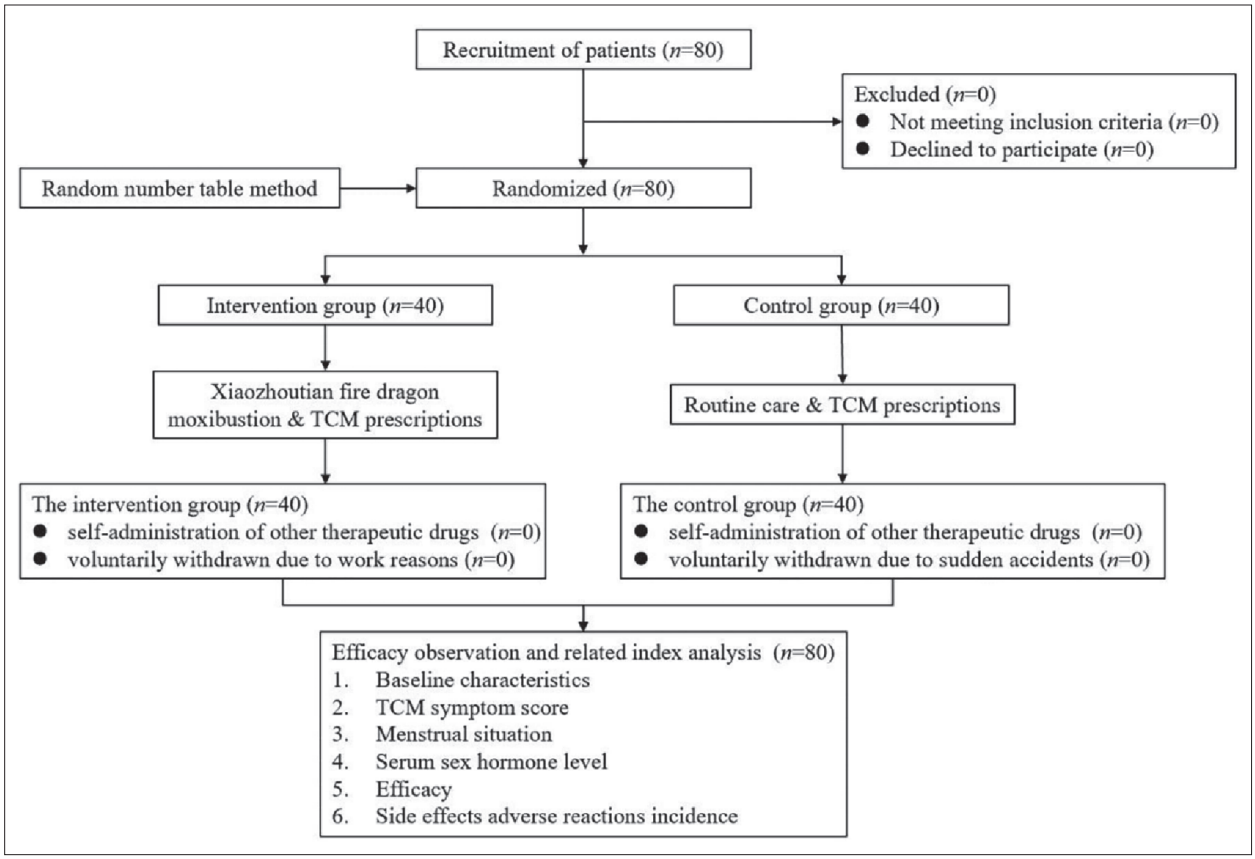

Figure 2. Flow of participants through the trial; TCM – traditional Chinese medicine

more than 48 months. There was no statistically significant difference in the duration of PCOS between the two groups ($p > 0.05$), and they were comparable. See Table 1 for details.

Table 1. Baseline profile of participants (age, disease duration) in both groups

Groups	n	Mean age (years old)	Mean duration of polycystic ovarian syndrome (years)
Control group	40	32.63 ± 5.53	3.37 ± 1.68
Intervention group	40	31.48 ± 5.22	3.43 ± 1.96
t-value		-0.127	-0.113
p-value		0.803	0.795

*$p < 0.05$

Ultrasound monitoring status before treatment

Table 2 shows that there was no statistically significant difference ($p > 0.05$) in ovarian volume, number of follicles, and endometrial thickness between the two groups of participants compared to the pre-treatment period, and the baseline balance was comparable.

Table 2. Comparison of pre-treatment ultrasound monitoring between the two groups of participants

Groups	*n*	Ovarian volume (M^3)	Number of follicles	Endometrial thickness (mm)
Control group	40	12.68 ± 1.73	13.12 ± 1.82	16.75 ± 2.31
Intervention group	40	12.67 ± 1.70	13.14 ± 1.81	16.61 ± 2.34
t-value		5.010	2.768	2.332
p-value		0.853	0.876	0.890

*$p < 0.05$

Comparison of pain scores before treatment

Table 3 shows that there was no statistically significant difference ($p > 0.05$) in the pre-treatment pain scores of the two groups of participants, and they were comparable.

Table 3. Comparison of pre-treatment pain scores between the two groups of participants

Groups	n	Pain scores
Control group	40	1.96 ± 0.45
Intervention group	40	1.93 ± 0.44
t value		5.210
p-value		0.764

*$p < 0.05$

Comparison of TCM symptom scores before and after treatment between the two groups of patients

Comparison of the TCM symptom scores of the two groups of patients before treatment, the difference was not statistically significant ($p > 0.05$). After treatment, the TCM evidence scores of patients in both groups improved, and the improvement in the intervention group was more obvious than that in the control group, and the difference was statistically significant ($p < 0.05$). See Table 4 for details.

Table 4. Comparison of traditional Chinese medicine symptom scores before and after treatment between the two groups

Groups	n	Pre-treatment	Post-treatment
Control group	40	25.45 ± 2.83	21.78 ± 2.71
Intervention group	40	25.53 ± 3.03	18.58 ± 4.05
t-value		-0.114	4.152
p-value		0.909	< 0.001*

*$p < 0.05$

Comparison of menstrual situation before and after treatment in the two groups

Before treatment, there was no statistically significant difference in the comparison of menstrual conditions between the two groups ($p > 0.05$). After treatment, the duration of menstrual period, menstrual flow, degree of dysmenorrhea, and blood clots in both groups decreased compared with the pre-treatment period, and the decrease in the intervention group was more obvious than that in the control group, and the difference was statistically significant ($p < 0.05$). See Table 5 for details.

Comparison of serum sex hormone levels before and after treatment between the two groups of patients

As can be seen from Table 6, before treatment, there was no statistical difference between the serum sex hormone levels of the two groups of patients ($p > 0.05$). After treatment, the serum sex hormone levels of both groups of patients showed significant improvements compared with the pre-treatment period, with more pronounced improvements in the intervention group. Specifically, the levels of luteinizing hormone (LH) decreased from 22.76 ± 15.32 mIU/ml to 17.62 ± 13.50 mIU/ml in the control group and from 21.54 ± 14.65 mIU/ml to 11.55 ± 8.30 mIU/ml in the intervention group, with the intervention group exhibiting a greater reduction. The follicle-stimulating hormone (FSH) levels also decreased, from 6.04 ± 2.51 mIU/ml to 5.41 ± 2.25 mIU/ml in the control group and from 5.97 ± 2.49 mIU/ml to 4.48 ± 1.79 mIU/ml in the intervention group. The LH/FSH ratio, which is an important indicator of PCOS, decreased from 3.58 ± 0.97 to 3.07 ± 1.12 in the control group and from 3.46 ± 0.88 to 2.47 ± 0.55 in the intervention group, indicating a more significant improvement in the intervention group. Additionally, the E2 levels increased from 50.50 ± 12.09 pg/ml to 43.43 ± 10.52 pg/ml in the control group and from 52.25 ± 10.80 pg/ml to 37.85 ± 6.24 pg/ml in the intervention group, while the testosterone (T) levels decreased from 82.40 ± 15.12 ng/dL to 67.75 ± 15 ng/dL in the control group and from 81.89 ± 14.45 ng/dL to 49.25 ± 11.55 ng/dL in the intervention group, with the intervention group showing a more substantial decrease. Finally, the P levels increased from 1.38 ± 0.97 ng/dL to 1.65 ± 1.01 ng/dL in the control group and from 1.26 ± 0.91 ng/dL to 2.17 ± 1.10 ng/dL

Table 5. Comparison of menstrual situation before and after treatment in the two groups

Menstrual situation	Time	Control group (n = 40)	Intervention group (n = 40)	t value	p-value
Duration of menstrual period	Pre-treatment	1.15 ± 0.74	1.25 ± 0.74	-0.605	0.547
	Post-treatment	0.45 ± 0.71	0.13 ± 0.40	2.504	0.015*
Menstrual flow	Pre-treatment	1.35 ± 0.62	1.33 ± 0.66	0.175	0.862
	Post-treatment	0.68 ± 0.80	0.28 ± 0.55	2.606	0.011*
Degree of dysmenorrhea	Pre-treatment	3.73 ± 2.45	4 ± 2.36	-0.511	0.611
	Post-treatment	1.70 ± 1.27	0.98 ± 1.12	2.713	0.008*
Blood clots	Pre-treatment	1.55 ± 0.50	1.65 ± 0.48	-0.906	0.368
	Post-treatment	1 ± 0.64	0.43 ± 0.55	4.309	< 0.001*

*p < 0.05

Table 6. Comparison of serum sex hormone levels before and after treatment between the two groups of patients

Serum sex hormone	Time	Control group (n = 40)	Intervention group (n = 40)	t value	p-value
LH (mIU/ml)	Pre-treatment	22.76 ± 15.32	21.54 ± 14.65	0.363	0.717
	Post-treatment	17.62 ± 13.50	11.55 ± 8.30	2.422	0.018*
FSH (mIU/ml)	Pre-treatment	6.04 ± 2.51	5.97 ± 2.49	0.12	0.905
	Post-treatment	5.41 ± 2.25	4.48 ± 1.79	2.055	0.043*
LH/FSH	Pre-treatment	3.58 ± 0.97	3.46 ± 0.88	0.574	0.568
	Post-treatment	3.07 ± 1.12	2.47 ± 0.55	3.038	0.004*
E_2(pg/ml)	Pre-treatment	50.50 ± 12.09	52.25 ± 10.80	-0.683	0.497
	Post-treatment	43.43 ± 10.52	37.85 ± 6.24	2.884	0.005*
T (ng/dL)	Pre-treatment	82.40 ± 15.12	81.89 ± 14.45	0.154	0.878
	Post-treatment	67.75 ± 15	49.25 ± 11.55	6.181	< 0.001*
P (ng/dL)	Pre-treatment	1.38 ± 0.97	1.26 ± 0.91	0.584	0.561
	Post-treatment	1.65 ± 1.01	2.17 ± 1.10	-2.234	0.028*

LH – luteinizing hormone; FSH – follicle-stimulating hormone; E2 – estradiol; T – testosterone; P – progesterone
*p < 0.05

in the intervention group. All these changes in serum sex hormone levels were statistically significant ($p < 0.05$), with the intervention group demonstrating more pronounced improvements compared to the control group.

Comparison of the efficacy of the two groups of patients

As shown in Table 7, after treatment, the total effective rate of treatment of patients in the intervention group reached 92.5%, which was higher than that of 72.50% in the control group ($p < 0.05$).

Table 7. Comparison of efficacy between the two groups (cases, %)

Groups	n	Cured	Obvious effect	Effective	Ineffective	Total effective rate
Control group	40	2	18	9	11	29 (72.50)
Intervention group	40	6	26	5	3	37 (92.50)
c						8.966
p-value						0.032*

*p < 0.05

DISCUSSION

The aim of this study was to investigate the clinical efficacy of Xiaozhoutian fire dragon moxibustion combined with traditional Chinese medicine in the treatment of PCOS with kidney yang deficiency. The results of the study showed that the intervention group demonstrated significant improvements in TCM symptom scores, menstrual conditions, and serum sex hormone levels. Specifically, the TCM symptom scores of patients in the intervention group improved significantly compared with those of the control group after treatment, and the menstrual situation, including the duration of the menstrual period, the amount of menstrual flow, the degree of dysmenorrhea, and blood clots, also improved significantly, while the serum sex hormone levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), luteinizing hormone/follicle-stimulating hormone ratio (LH/FSH), estradiol (E_2), T and P were improved, and the total effective rate of the intervention group was also significantly increased.

The results of this study echo previous studies that have shown that TCM treatment has potential effects in regulating endocrine and improving PCOS symptoms. For instance, a study by Li et al. [27] found that acupuncture combined with moxibustion, as an adjunct to the basic treatment, could improve pregnancy, ovulation, and miscarriage rates, as well as certain sex hormone levels and metabolic indicators in patients with PCOS, with good safety. A meta-analysis by Kwon et al. [28] explored the efficacy and safety of Oriental Herbal Medicine (OHM) combined with moxibustion in the treatment of PCOS. The study found that compared with the use of Western medicine (WM) alone, OHM combined with moxibustion significantly increased pregnancy rates,

normal biphasic basal body temperature rates, and total effective rate (TER) in patients with PCOS [28]. When OHM combined with moxibustion was used as an adjunct to WM, it also showed significant improvements in pregnancy rates and TER. Deng et al. [29] studied the efficacy of combined traditional Chinese medicine therapies in the treatment of infertility associated with PCOS. The study found that compared with traditional single therapies, the combined Chinese medicine therapy showed better efficacy in treating PCOS-related infertility, with moxibustion combined with Chinese herbs, fire needle combined with Chinese herbs, and acupuncture combined with Chinese herbs being the three most effective therapies in improving clinical pregnancy rates. Moini Jazani et al. [30] reviewed the herbs used globally for the treatment of PCOS. It was found that herbs such as cinnamon, fenugreek, and chaste tree may be beneficial in ameliorating different aspects of PCOS by significantly reducing serum FBS, insulin and insulin resistance levels, as well as cholesterol, triglyceride, and low-density lipoprotein (LDL) levels. This is corroborated with the composition of the formula used in this study.

The results of this study not only confirm the potential of traditional Chinese medicine in treating PCOS but also reveal the possible mechanism of action of this comprehensive treatment method. Firstly, based on the TCM theory that "the kidney is the master of reproduction," we selected the herbal formula Shu Gan Wen Yang Tang, which aims to warm the kidney yang, disperse cold, nourish the liver, and warm the kidneys. These effects are consistent with the observed improvements in menstrual conditions, reduced serum sex hormone levels, and increased ovulation rates. We found that the intervention group showed a significant improvement in TCM symptom scores after treatment, which may be related to the warming and cold-dispersing effects of the herbal formula, helping to adjust and improve the overall endocrine status of the patients. Secondly, the application of Xiaozhoutian fire dragon moxibustion, through its warming effect and the efficacy of herbs and alcohol, impacts the Du and Ren meridians, which aligns with the TCM concept of regulating yin and yang as well as qi and blood. The warm stimulation of the fire dragon moxibustion may promote local blood and lymphatic circulation, enhance the metabolic capacity of the local skin, which is consistent with our observed improvements in menstrual conditions and changes in serum sex hormone levels. Furthermore, the local stimulation effect of the fire dragon moxibustion may diffuse inhibitory substances in the cerebral cortex, reduce the excitability of the nervous system, and exert sedative and analgesic effects. This may explain why the intervention group showed more significant improvements in the degree of dysmenorrhea and the number of blood clots. Lastly, our study also emphasizes the importance of complementary internal and external medications. The combination of Xiaozhoutian fire dragon moxibustion and tonic therapy may improve drug absorption and efficacy, which is consistent with our observation that the total effective rate of treatment in the intervention group was significantly higher than that in the control group.

However, it should be noted that this study still has some shortcomings, including small sample size, simple study design, and lack of international diagnostic standards. Therefore, when further similar studies are conducted in the future, it is necessary to standardize the study design, increase the sample size, fully consider potential interfering factors, and optimize the treatment protocols to further validate the reliability of the results of this study and the prospect of clinical application. Meanwhile, future studies should also incorporate broader expert knowledge and introduce a more rigorous randomized controlled design to improve the credibility and scientific validity of the study.

CONCLUSION

In conclusion, TCM combination therapy has shown certain advantages in the treatment of PCOS, but more high-quality and multilevel studies are still needed to verify its clinical efficacy and mechanism to further promote the application and development of TCM in the treatment of reproductive endocrine diseases.

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Ефикасност моксибустије Сјаоџоутијан ватреног змаја у комбинацији са традиционалном кинеском медицином на синдром полицистичних јајника са недостатком јанга бубрега – рандомизовано контролисано испитивање

Љусју Ју[1], Мингфенг Сјунг[1], Сјаоју Ли[1], Чунгхонг Ву[2], Лићин Ванг[1], Јао Гао[1], Минсја Лонг[1], Ћин Ган[1]

[1]Универзитет у Нанчангу, Медицински факултет Ђангшиа, Прва придружена болница, Превентивни центар за традиционалну кинеску медицину, Нанчанг провинција, Кина;
[2]Универзитет у Нанчангу, Медицински факултет Ђангшиа, Прва придружена болница, Одељење за амбулантну негу, Нанчанг провинција, Кина

САЖЕТАК

Увод/Циљ Циљ овог рада био је да истражи ефикасност моксибустије Сјаоџоутијан ватреног змаја у комбинацији са традиционалном кинеском медицином код синдрома полицистичних јајника са недостатком јанга бубрега.

Методе Одабрано је 80 пацијената са синдромом полицистичних јајника уз недостатак јанга бубрега, који су насумично подељени у контролну и интервентну групу, са по 40 пацијената у свакој групи. Контролна група је добила рутинску негу и третман традиционалне кинеске медицине, а интервентна група је третирана моксибустијом Сјаоџоутијан ватреног змаја. После три месеца, терапијски ефекти у обе групе упоређени су на основу симптома традиционалне кинеске медицине, менструалног стања и нивоа полних хормона у серуму.

Резултати Пацијенти у интервентној групи показали су значајно побољшање у скору симптома традиционалне кинеске медицине у поређењу са контролном групом након третмана ($p < 0,05$). Менструални услови, као што су трајање менструалног периода, менструални ток, степен дисменореје и присуство крвних угрушака, као и нивои полних хормона у серуму (лутеинизирајући хормон, фоликулостимулирајући хормон, однос лутеинизирајућег и фоликулостимулирајућег хормона, естрадиол, тестостерон и прогестерон) били су бољи од оних у контролној групи, а разлика је била статистички значајна ($p < 0,05$). Укупна ефикасност лечења пацијената у интервентној групи достигла је 92,50%, што је више од оне од 72,50% у контролној групи ($p < 0,05$).

Закључак Моксибустија Сјаоџоутијан ватреног змаја у комбинацији са традиционалном кинеском медицином има добру клиничку ефикасност у лечењу синдрома полицистичних јајника са недостатком јанга бубрега, што може побољшати симптоме и ниво хормона пацијената и побољшати опште здравствено стање.

Кључне речи: моксибустија Сјаоџоутијан ватреног змаја; традиционална кинеска медицина; синдром недостатка јанг бубрега; синдром полицистичних јајника; рандомизована контролисана студија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Morphometric characteristics of the great saphenous vein as graft for surgical myocardial revascularization in relation to sex

Saša S. Živić[1], Ivan Nikolić[2], Marko Jović[2], Vladimir Petrović[2], Dragan C. Bogdanović[3], Vladimir Stojiljković[1], Aleksandar Kamenov[1]

[1]University Clinical Centre of Niš, Clinic for Cardiac Surgery, Niš, Serbia;
[2]University of Niš, Faculty of Medicine, Department of Histology and Embryology, Niš, Serbia;
[3]Niš Institute of Public Health, Niš, Serbia

SUMMARY

Introduction/Objective The significant difference in the patency of venous coronary grafts in relation to sex still does not have a clearly defined cause. Our research determined the existence of morphometric differences in the wall of the great saphenous vein, in relation to sex.

Methods The research included 268 patients classified by sex in five age groups, who underwent morphometric measurement of the wall of the great saphenous vein.

Results In our research, no significant difference was found in the total thickness of the vein wall between the sexes ($p = 0.111$), nor was significant association found between wall thickness, age, and sex. The average thickness of the intimal layer of the vein wall was significantly higher in male subjects ($p = 0.005$), and multivariate regression analysis found a significant correlation between intimal thickness, age and sex ($p < 0.001$). The medial layer of the vein in women was significantly larger ($p < 0.001$), both overall and in all age groups. Multivariate regression analysis confirmed a significant association between media thickness, age and sex ($p < 0.001$). The thickness of the adventitial layer was significantly higher in men ($p = 0.031$) and a significant association between the thickness of the adventitia, age and sex was also determined ($p < 0.001$).

Conclusion Our results indicate significant morphometric differences in the wall of the great saphenous vein in relation to sex and age of the patients, which can be related to significant difference in the flow rate of vein grafts in coronary surgery in relation to sex.

Keywords: cardiac surgery; vein morphometry; patency

INTRODUCTION

The most common treatment for multiple coronary artery disease (CAD) is coronary artery bypass grafting (CABG), and the great saphenous vein (GSV) is the most commonly used conduit. Data indicate that in as many as 89.3% of cases GSV is used during CABG [1], although all analyses of CABG results show significant differences in the patency of arterial *vs.* venous grafts [2, 3]. Also, the available data indicate the existence of a significant difference in the early and late patency of vein grafts in relation to sex.

By analyzing the first 2054 patients who underwent CABG at the Cleveland Clinic (USA), it can be concluded that the female sex is the third most important operative risk factor in coronary surgery, and two years after CABG women have significantly lower patency of vein grafts than men [4].

In a Dutch multicenter study, after one year of follow-up from coronary revascularization, vein graft occlusion was 16.7% in women and 12.4% in men [5].

In the "Reduction in Graft Occlusion Rates (RIGOR) Study," analyzing 611 grafts, vein graft occlusion was found much more often in women ($p = 0.03$) and as one of the main significant risk factors for occlusion, independent of others, they state female sex ($p = 0.01$) [6].

There are numerous authors who state that female sex is one of the risk factors for poor results after CABG as well as for vein graft occlusion [7, 8, 9], although those who deny it are not rare [10, 11].

The main cause of intermediate and late reduction of vein graft patency in coronary surgery is neointimal hyperplasia of the intimal layer. The main pathophysiological mechanism of intimal hyperplasia is the migration of smooth muscle cells (SMC) from the medial to the intimal layer of the vein, their proliferation, and accumulation of extracellular matrix [12, 13]. This process begins immediately after implanting of the vein into the arterial system, so that as soon as 4-6 weeks later most veins develop intimal thickening by migration and proliferation of SMC from the media and reduction of the venous lumen [14].

We analyzed the presence of differences in the histological morphometry of the GSV wall used for CABG in relation to sex and their association with the level of patency.

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Correspondence to:
Saša S. ŽIVIĆ
University Clinical Centre of Niš
Clinic for Cardiac Surgery
Bul. Dr Z. Đinđića 48
18000 Niš, Serbia
sasa.dr.zivic@gmail.com

METHODS

The study included 268 patients at the Clinic for Cardiac Surgery, Niš University Clinical Center, 134 women and 134 men, randomized by sex into five age groups: Group 1 (up to 39 years of age); Group 2 (40–49 years old); Group 3 (50–59 years old); Group 4 (60–70 years old) and Group 5 (70 years and over). There were 27 men and women in each age group, except in Group 1, where there were 26 men and 26 women.

The number of study participants was determined using the G*Power 3.1.6 program (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany).

Patients who were scheduled for at least one venous coronary bypass were included in the study. Exclusion criteria were patients who were treated with venous drug therapy or chemical vein ablation due to venous disease, and those with a history of thrombosis in the superficial and deep venous system.

Patients included in the study were divided into groups according to age and sex, and simple randomization was achieved using computer-generated random numbers.

A 1 cm-long GSV sample was taken through a longitudinal skin and subcutaneous incision using a conventional technique at the level of the medial malleolus, without prior dilatation and lavage, and the perivascular tissue and fascia were sharply dissected with scissors.

Immediately after collection, the GSV samples were fixed in a 4% buffered formalin solution (via immersion fixation) and embedded in paraffin. The paraffin blocks were sectioned at a thickness of 4 μm, then deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E), following standard protocols.

Analysis, measurements, and photography of venous preparations were performed by histologists on an Olympus BX50 microscope (Olympus, Tokyo, Japan) equipped with a Leica DFC295 digital camera (Leica Microsystems, Wetzlar, Germany). Morphometric analysis was performed using the "ImageJ" program (LOCI, University of Wisconsin, Madison, WI, USA).

Determination of the thickness of the individual layers of the vein wall and the total thickness of the vein wall was performed by measuring each preparation at three equal distances (0°, 120°, and 240°) due to marked differences in the thickness of the individual layers of the same vein. The mean value of three measurements for each preparation was used for statistical processing.

The results of the statistical analysis are presented in tabular and graphical form. Calculations were performed using PASW Statistics, Version 18.0 (SPSS Inc., Chicago, IL, USA). In all analyses, the default error of estimation was less than 0.05 (5%) as the limit of statistical significance. The Shapiro–Wilk test was used to test the normality of the distribution of numerical values. The comparison of the thickness of the tunica intima, media, adventitia, as well as the total thickness of the vein wall between men and women was performed by the Student's t-test for two independent samples. The comparison of the mean values of the mentioned characteristics between the five age groups was performed by one-way ANOVA, and subsequent Tukey post-hoc test. Multivariate linear regression analysis using the enter method was used to assess the association of age and sex with the thickness of the tunica

Table 1. Thickness of tunica intima (μm) by sex and age

Age group	Parameter	Sex		Total	Comparison between men and women p-value
		Male	Female		
Group 1	X	153.85	96.04	124.95	< 0.001
	SD	58.67	38.75	57.23	
	Med	150.7	93.37	106.43	
	Min	30.92	24.87	24.87	
	Max	288.97	236.24	288.97	
Group 2	X	110.97	151.74	131.36	0.002
	SD	41.22	49.77	49.72	
	Med	102.13	159.41	124.25	
	Min	14.19	43.65	14.19	
	Max	193.88	248.87	248.87	
Group 3	X	148.95	152.63	150.79	0.818
	SD	59.51	57.07	57.78	
	Med	138.59	139.54	139.23	
	Min	59.32	73.90	59.32	
	Max	354.05	297.64	354.05	
Group 4	X	208.4	129.81	169.1	< 0.001
	SD	50.53	46.6	62.38	
	Med	200.36	113.31	177.82	
	Min	84.35	63.12	63.12	
	Max	314.95	214.54	314.95	
Group 5	X	164.7	153.45	159.08	0.500
	SD	48.32	71.27	60.58	
	Med	164.42	146.8	157.52	
	Min	76.69	12.62	12.62	
	Max	278.82	383.79	383.79	
Total	X	157.4	137.04	147.22	0.005
	SD	60.17	57.51	59.63	
	Med	154.61	126.94	139.23	
	Min	14.19	12.62	12.62	
	Max	354.05	383.79	383.79	
Comparison between age groups		A*, C†, F‡, G†, H‡, J*	A†, B†, D†	C†, D*, F*	

X – arithmetic mean; SD – standard deviation; Med – median; Min – minimum; Max –maximum; A – Group 1 *vs.* Group 2; B – Group 1 *vs.* Group 3; C – Group 1 *vs.* Group 4; D – Group 1 *vs.* Group 5; E – Group 2 *vs.* Group 3; F – Group 2 *vs.* Group 4; G – Group 2 *vs.* Group 5; H – Group 3 *vs.* Group 4; I – Group 3 *vs.* Group 5; J – Group 4 *vs.* Group 5;
*p < 0.05
†p < 0.01;
‡p < 0.001

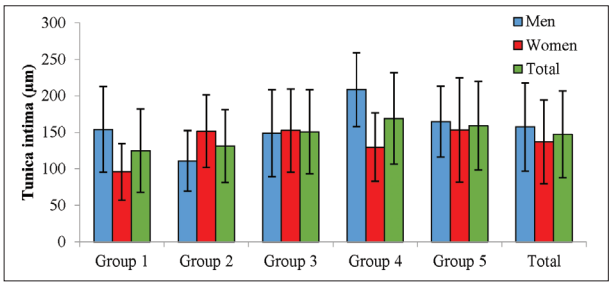

Figure 1. Comparison of intimal thickness (μm) by sex and age

intima, media, adventitia, as well as the total thickness of the vein wall.

The study was carried out with the consent of the Ethics Committee of the Faculty of Medicine of the University of Niš, No. 12-10580-2/5 from October 9, 2018, and the Ethical Committee of the Niš Clinical Center, No. 35933/8 from November 6, 2018. Prior to engaging in the research, all participants signed informed consent according to the Declaration of Helsinki.

RESULTS

The average age of all subjects in the study was 54.42 ± 14.45 years. The average age of examined women was higher than that of men, but without statistical significance. Also, there was no statistically significant difference in the average age between sexes within the same age groups.

The total average thickness of the tunica intima was significantly greater in men (Table 1; Figure 1). The overall average thickness of the intima irrespective of sex was the highest in Group 4, and it is a significantly higher value compared to Group 1 and Group 2. The total average thickness of the intima in Group 5 is significantly higher compared to Group 1.

In the examined men, the average thickness of the intima was significantly higher in Group 4 compared to all other age groups.

In the examined women, the total average thickness of the intima was highest in Group 5 and lowest in Group 1. The thickness of the intimal layer in women in Group 1 was significantly lower compared to Group 2, 3, and 5.

Regression analysis confirmed a significant association between intimal thickness, age, and sex. Each year of age was associated with an increase in intimal thickness of 1.059 μm ($p < 0.001$).

In Figure 2, the trend of an increase in the thickness of the intimal layer with aging can be clearly seen, which is somewhat more pronounced in men than in women.

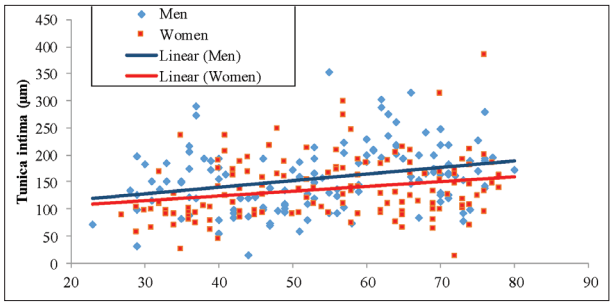

Figure 2. Correlation between the thickness of the intimal layer and age

The total average thickness of the tunica media of all study participants was 394.82 ± 104.10 μm (Table 2; Figure 3). The total average thickness of the medial layer in women was significantly higher than in men ($p < 0.001$). Comparing the average thickness of the medial layer in relation to sex, it was significantly higher in women in all age groups compared to the same male age groups.

Table 2. Thickness of tunica media (μm) by sex and age

Age group	Parameter	Sex		Total	Comparison between men and women p-value
		Male	Female		
Group 1	X	319.7	430.36	375.03	< 0.001
	SD	52.74	104.85	99.37	
	Med	314.88	413.79	365.42	
	Min	191.45	164.71	164.71	
	Max	422.86	721.67	721.67	
Group 2	X	361.34	448.82	405.08	0.005
	SD	132.01	79.73	116.69	
	Med	376.4	435.52	409.67	
	Min	126.93	265.3	126.93	
	Max	781.07	593.51	781.07	
Group 3	X	328.02	398.32	363.17	0.003
	SD	91.91	75.92	90.72	
	Med	319.21	384.19	369.44	
	Min	178.64	221.48	178.64	
	Max	512.99	601.65	601.65	
Group 4	X	291.88	361.96	326.92	0.006
	SD	81.99	98.62	96.54	
	Med	275.51	361.22	317.61	
	Min	164.89	147.33	147.33	
	Max	503.79	546.61	546.61	
Group 5	X	255.5	304.17	279.84	0.006
	SD	48.64	74.52	66.99	
	Med	267.36	300.84	278.9	
	Min	164.04	150.32	150.32	
	Max	332.89	448.96	448.96	
Total	X	311.22	388.42	349.82	< 0.001
	SD	92.98	100.55	104.1	
	Med	300.61	388.25	344.64	
	Min	126.93	147.33	126.93	
	Max	781.07	721.67	781.07	
Comparison between age groups		F*, G‡, I*	C*, D‡, F†, G‡, I†	D‡, F‡, G‡, I‡	

X – arithmetic mean; SD – standard deviation; Med – median; Min – minimum; Max – maximum; E – Group 2 *vs.* Group 3; F – Group 2 *vs.* Group 4; G – Group 2 *vs.* Group 5; H – Group 3 *vs.* Group 4; I – Group 3 *vs.* Group 5; J – Group 4 *vs.* Group 5; C – Group 1 *vs.* Group 4; D – Group 1 *vs.* Group 5;
*$p < 0.05$;
†$p < 0.01$;
‡$p < 0.001$

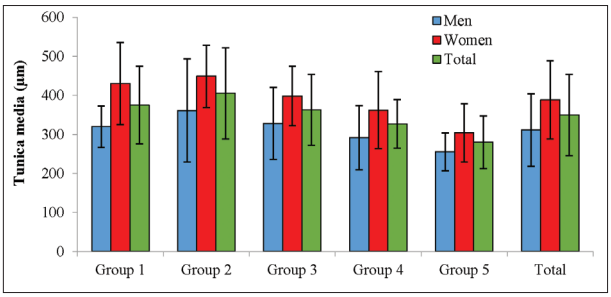

Figure 3. Comparison of tunica media thickness (μm) by sex and age

In men, the average thickness of the medial layer was the highest in Group 2, and significantly higher compared to Group 4 and 5.

In the examined women, the average thickness of the media was the highest in Group 2 and the lowest in Group

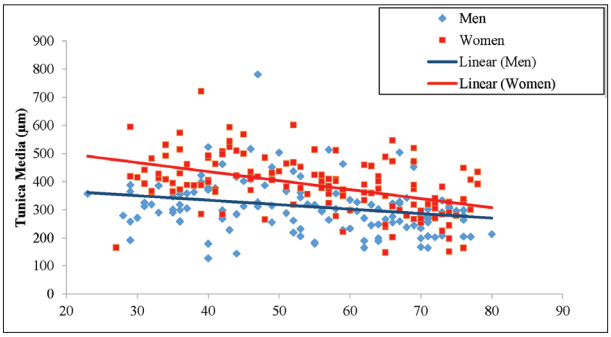

Figure 4. Correlation between the thickness of the medial layer and age

Table 3. Thickness of tunica adventitia (μm) by sex and age

Age group	Parameter	Sex		Total	Comparison between men and women p-value
		Male	Female		
Group 1	X	241.99	206.98	224.48	0.014
	SD	47.55	51.82	52.32	
	Med	264.66	201.82	236.05	
	Min	143.54	104.85	104.85	
	Max	303.25	316.08	316.08	
Group 2	X	330.75	309.5	320.13	0.553
	SD	159.14	94.42	130.05	
	Med	309.52	307.81	308.67	
	Min	167.89	157.14	157.14	
	Max	951.18	475.75	951.18	
Group 3	X	364.6	344.09	354.34	0.403
	SD	91.46	87.17	89.1	
	Med	367.96	354.86	367.42	
	Min	168.65	203.04	168.65	
	Max	511.92	560.21	560.21	
Group 4	X	360.35	276.60	318.48	0.001
	SD	62.28	104.65	95.19	
	Med	377.64	291.47	337.74	
	Min	216.45	48.19	48.19	
	Max	473.56	482.64	482.64	
Group 5	X	315.23	337.29	326.26	0.403
	SD	92.69	99.27	95.78	
	Med	301.82	347.22	328.22	
	Min	178.54	156.29	156.29	
	Max	516.01	473.42	516.01	
Total	X	323.19	295.55	309.37	0.031
	SD	106.73	101.38	104.81	
	Med	309.73	291.36	300.80	
	Min	143.54	48.19	48.19	
	Max	951.18	560.21	951.18	
Comparison between age groups		A*, B‡, C‡	A†, B‡, C*, D‡	A‡, B‡, C‡, D‡,	

X – arithmetic mean; SD – standard deviation; Med – median; Min – minimum; Max – maximum; A – Group 1 *vs.* Group 2; B – Group 1 *vs.* Group 3; C – Group 1 *vs.* Group 4; D – Group 1 *vs.* Group 5;
*p < 0.05;
†p < 0.01;
‡p < 0.001

5. Media thickness in Group 5 was significantly lower than that in Groups 1, 2, and 3. Media thickness in Group 4 was significantly less than that in Groups 1 and 2.

Regression analysis confirmed a significant association between the thickness of the medial layer of the vein wall, age, and sex. Each year of age was associated with a

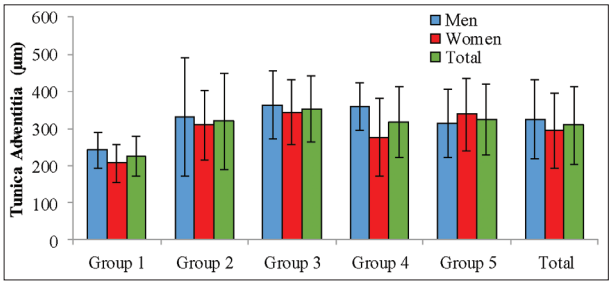

Figure 5. Comparison of tunica adventitia thickness (μm) by sex and age

decrease in media thickness of 2.411 μm (p < 0.001). In examined women, the thickness of the medial layer was 78.217 μm (p < 0.001) higher than in men.

Figure 4 clearly shows the existence of a trend of medial thickness decline with aging, and that this decline is more pronounced in women than in men.

The total average thickness of the tunica adventitia in all subjects was 309.37 ± 104.81 μm (Table 3; Figure 5). The average thickness of the adventitia was significantly higher in all examined men than in women (p = 0.031). Compared with age groups, the thickness of the adventitia in men compared to women was significantly higher in Group 1 and Group 4. The overall average thickness of the adventitia of all subjects in Group 1 was significantly lower than in all other age groups. In the examined men, the average thickness of the adventitia in Group 1 was significantly lower than in Groups 2, 3, and 4. In women, the thickness of the adventitia in Group 1 was significantly lower than in all other age groups. There were no significant differences between the other age groups of women.

Regression analysis confirmed a significant association between adventitia thickness, age, and sex. Each year of age was associated with an increase in adventitia thickness of 1.882 μm (p < 0.001). In examined women, the thickness of the adventitia was 28.441 μm (p = 0.022) lower than in men. In Figure 6, it can be seen that the trend of increasing the thickness of the adventitia with aging is somewhat more pronounced in women than in men.

The average total wall thickness in all subjects was

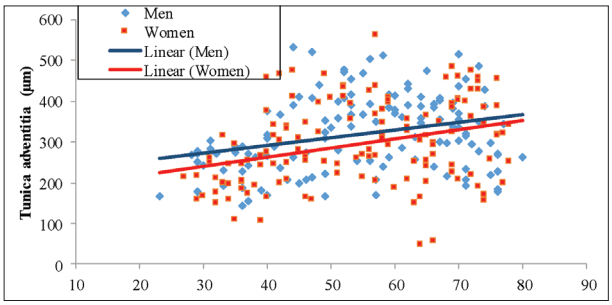

Figure 6. Correlation between the thickness of the tunica adventitia and age

805.28 ± 138.31 μm (Table 4, Figures 7 and 8). The average total thickness of the wall in all examined women was higher than in men, but not statistically significant (p = 0.111). The total thickness of the wall was significantly higher in men than in women in Group 4 (p = 0.002),

Table 4. Thickness of total vein wall (μm) by sex and age

Age group	Parameter	Sex		Total	Comparison between men and women p-value
		Male	Female		
Group 1	Xsr	715.56	733.38	724.47	0.559
	SD	80.2	131.75	108.37	
	Med	708.52	717.49	714.52	
	Min	579.38	450.14	450.14	
	Max	890.08	1066.58	1066.58	
Group 2	X	803.02	898.95	850.99	0.033
	SD	185.19	131.53	166.3	
	Med	812.27	901.08	849.56	
	Min	419.98	512.95	419.98	
	Max	1346.72	1180.01	1346.72	
Group 3	X	841.57	895.03	868.3	0.054
	SD	89.87	108.49	102.3	
	Med	823.97	872.68	854.71	
	Min	675.63	716.25	675.63	
	Max	1023.05	1157.85	1157.85	
Group 4	X	860.63	768.37	814.5	0.002
	SD	89.53	115.82	112.61	
	Med	850.93	785.24	816.58	
	Min	724.99	497.65	497.65	
	Max	1059.6	950.56	1059.6	
Group 5	X	735.44	794.91	765.17	0.123
	SD	98.27	170.83	141.26	
	Med	734.68	807.99	763.24	
	Min	525.11	389	389	
	Max	910.32	1219.98	1219.98	
Total	X	791.81	818.76	805.28	0.111
	SD	127.35	147.7	138.31	
	Med	792.3	826.98	812.04	
	Min	419.98	389	389	
	Max	1346.72	1219.98	1346.72	
Comparison between age groups		B†, C‡, I†, J†	A‡, B‡, F†, G*, H†	A‡, B‡, C†, G†, I‡	

X – arithmetic mean; SD – standard deviation; Med – median; Min – minimum; Max – maximum; A – Group 1 *vs.* Group 2; B – Group 1 *vs.* Group 3; C – Group 1 *vs.* Group 4; F – Group 2 *vs.* Group 4; G – Group 2 *vs.* Group 5; H – Group 3 *vs.* Group 4; I – Group 3 *vs.* Group 5; J – Group 4 *vs.* Group 5;
*p < 0.05;
†p < 0.01;
‡p < 0.001

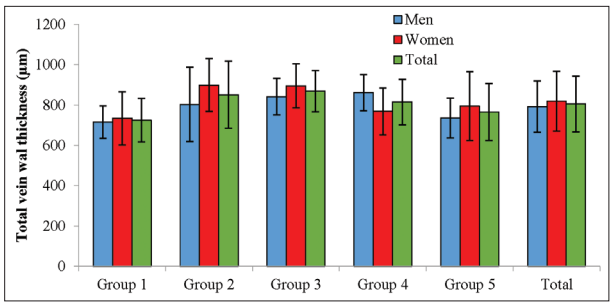

Figure 7. Comparison of total vein wall thickness (μm) by sex and age

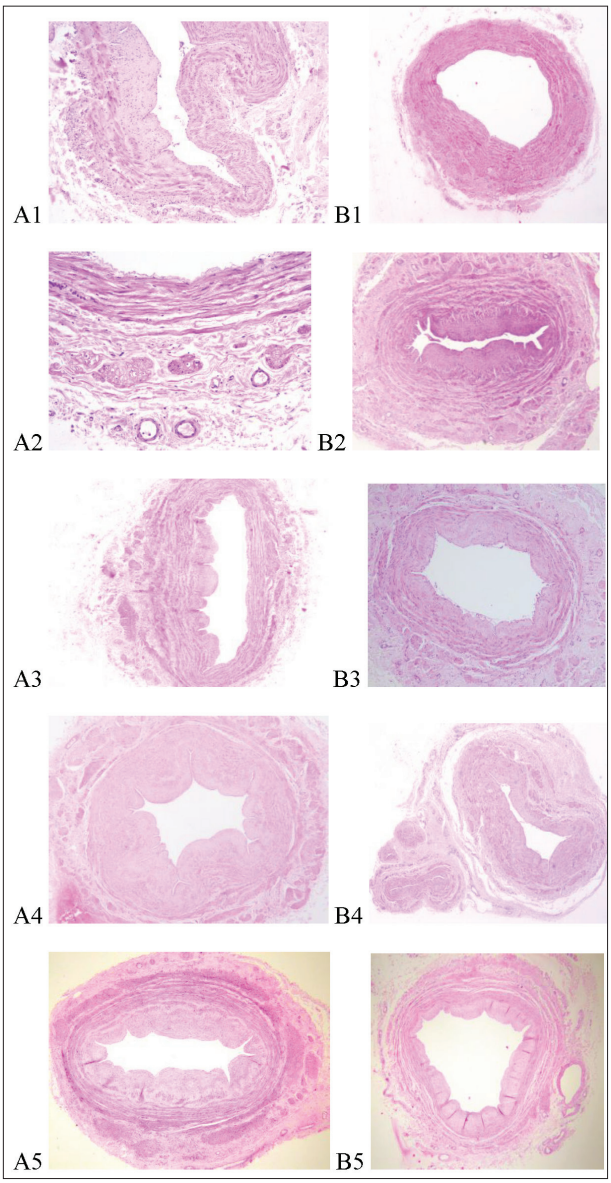

Figure 8. Cross sections of veins by sex and age groups, stained with the H&E method; A – male group [A1 – Group 1 (magnification: × 100); A2 – Group 2 (magnification: × 200); A3 – Group 3 (magnification: × 40); A4 – Group 4 (magnification: × 40); A5 – Group 5 (magnification: × 64)]; B – female group [B1 – Group 1(magnification: × 40); B2 – Group 2 (magnification: × 50); B3 – Group 3 (magnification: × 40); B4 – Group 4 (magnification: × 40); B5 – Group 5 (magnification: × 40)]

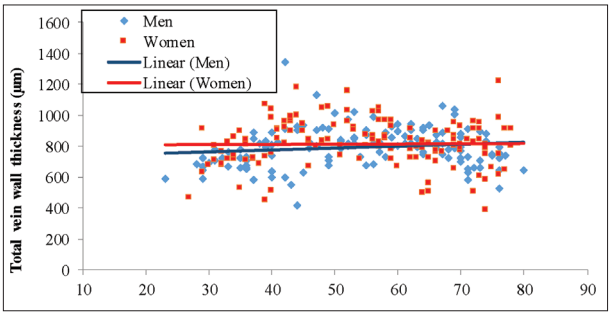

Figure 9. Correlation between total vein wall thickness and age

while the total thickness of the wall was significantly higher in women than in men in Group 2 (p = 0.033). Differences between total wall thickness in men and women in other age groups are not statistically significant.

Regression analysis did not confirm a significant relationship between total wall thickness, age, and sex, and Figure 9 shows that there is no trend in total wall thickness changes associated with aging, both in men and in women.

DISCUSSION

In general, a very small number of studies which, in an attempt to explain the difference in the flow rate of vein grafts in relation to sex, analyzed the morphometric structure of the vein wall, especially microscopically analyzing the morphometry of the wall and its elements.

Numerous authors in earlier studies explained the significant difference in the flow rate of vein grafts in women compared to men by the smaller average diameter of the recipient coronary arteries [4, 7]. However, comparing body surface area and body mass index (BMI) with the diameter of recipient coronary arteries, it was determined that there is no difference in relation to sex, meaning BMI is proportional to the diameter of coronary blood vessels regardless of sex [14].

In our study, the average age of the patients is significantly lower (54.42 years) compared to the analyzed studies that examined the morphometry of the venous conduit (60 years – Human et al. [15], and 62.9 years – Perek et al. [16]), but this finding cannot be taken into account because we are dealing with a selected and not a total group of patients who were operated on. Otherwise, the average age of those operated on in our region does not deviate significantly from the data in the literature [17].

In the morphometric analyses of veins used as coronary conduits, the transverse diameter of the veins, wall diameter, and lumen diameter were most often measured, and the results were presented descriptively, and poor postoperative results were associated with veins with a thick wall and a larger lumen [7, 18]. Studies that histomorphometrically determine the diameter of the vein wall and its layers are extremely rare, especially those that compare the structure of the veins in relation to sex.

Unlike us, Human et al. [15] found a significant difference in the average thickness of the vein wall in men (476.7 μm) compared to women (396.2 μm). In the study by Perek et al. [16], a significantly greater thickness of the vein wall was observed in women (359.1 μm *vs.* 469.3 μm). The significant difference in the thickness of the vein wall obtained in our study compared to earlier research can be explained by the difference in the vein treatment methodology when taking the sample. Namely, in our study, a native preparation was taken, without prior dilation and stretching, unlike other studies where the veins were dilated using different techniques before fixation, and it should be noted that due to the elastic properties of the vein wall, even very small differences in pressure can result in significant differences in diameters of both the lumen and the thickness of the vein wall.

By measuring the intimal layer of the vein wall, a significant difference was found in favor of men ($p = 0.005$), but, analyzing age groups in relation to sex, this difference is not linear. Regression analysis revealed a significant relationship between the thickness of the intimal layer, age, and sex. Our results correlate with those of Human et al. [15], which also found a significantly thicker intima in men.

The average thickness of the medial layer of the vein wall of all participants was 394.82 ± 104.10 μm, with a significantly higher thickness found in women, both in total (388.42 ± 100.55 μm *vs.* 311.22 ± 92.98 μm; $p < 0.001$), and in relation to all age groups. Regression analysis determined a significant association between medial thickness, age, and sex, where each year of age is associated with a decrease in medial thickness by 2.411 μm ($p < 0.001$), and the trend of decreasing medial layer thickness is more pronounced in women. In the mentioned study by Perek et al. [16], a significantly thicker medial layer of the vein wall was observed in the group of patients with an occluded or severely damaged venous conduit (257.2 μm *vs.* 211.5 μm; $p < 0.001$), and the logistic regression model indicates that hypertrophy of the medial layer represents one of the independent risk factors for the development of severe graft disease and poor outcome after coronary surgery in patients with venous aortocoronary bypass.

All previous findings have confirmed that the main reservoir of SMC is precisely in the medial layer of the vein wall. Proliferation and migration into the intimal layer of these SMC is the main cause of intermediate and late reduced flow or occlusion of the vein graft in coronary surgery [12, 13]. Comparing our results and those of Perek et al. [16], as well as the mentioned facts, it can be concluded that there is a direct correlation of the thickness of the medial layer with the results in coronary surgery, i.e., considering the results of our research, the greater thickness of the medial layer of the vein wall found in women can be the cause of significant difference in patency of vein grafts in women. From both public health and clinical perspective, our results underscore the importance of promoting timely cardiovascular prevention in women who – regrettably – as in other more developed countries present themselves later for any kind of revascularization, with a greater burden of disease and are often misdiagnosed due to our cultural and regional habits [19].

CONCLUSION

Unfortunately, there are very few histological morphometric studies of venous conduits in coronary surgery, especially those comparing in relation to sex, which we could compare with our results. More research is needed to determine the true causes of the different patency of vein grafts in relation to sex.

Our results may point to the conclusion that in women, especially younger and middle-aged women, due to a significantly thicker tunica media, the use of a venous conduit in coronary surgery should be avoided due to better medium-term and long-term postoperative results.

ACKNOWLEDGMENT

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Conflict of interest: None declared.

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Морфометријске карактеристике велике сафенске вене као графта за хируршку реваскуларизацију миокарда у односу на пол

Саша С. Живић[1], Иван Николић[2], Марко Јовић[2], Владимир Петровић[2], Драган Ц. Богдановић[3], Владимир Стојиљковић[1], Александар Каменов[1]

[1]Универзитетски клинички центар „Ниш", Клиника за кардиохирургију, Ниш, Србија;
[2]Универзитет у Нишу, Медицински факултет, Катедра за хистологију и ембриологију, Ниш, Србија;
[3]Институт за јавно здравље „Ниш", Ниш, Србија

САЖЕТАК

Увод/Циљ Значајна разлика у проходности венских коронарних графтова у односу на пол још увек нема јасно дефинисан узрок. Наше истраживање има за циљ да утврди евентуално постојање морфометријских разлика у зиду велике сафенске вене у односу на пол.

Методе Истраживањем је обухваћено 268 пацијената класификованих по полу у пет старосних група, којима је вршено морфометријско мерење зида велике сафенске вене.

Резултати У нашем истраживању није утврђена значајна разлика укупне дебљине зида вене између полова ($p = 0,11$), нити значајна повезаност између укупне дебљине зида, старости и пола. Просечна дебљина интималног слоја венског зида била је значајно већа код мушких испитаника ($p = 0,005$) а мултиваријантном регресионом анализом утврђена је значајна повезаност између дебљине интиме, старости и пола ($p < 0,001$). Медијални слој вене код жена био је значајно већи ($p < 0,001$), како укупно тако и у свим старосним групама. Мутиваријантна регресиона анализа је потврдила значајну повезаност између дебљине медије, старости и пола ($p < 0,001$). Дебљина адвентицијалног слоја била је значајно већа код мушкараца ($p = 0,031$), а утврђена је и значајна повезаност између дебљине адвентиције, старости и пола ($p < 0,001$).

Закључак Резултати нашег испитивања указују на постојање значајне разлике у зиду велике сафенске вене у односу на пол и старост испитиваних пацијената, што се може довести у везу са значајном разликом у проточности венских графтова у коронарној хирургији у односу на пол.

Кључне речи: кардиохирургија; венска морфометрија; проточност

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The impact of certain anti-seizure medications on cognitive status, behavior, anxiety, and depression in school-aged children with newly diagnosed epilepsy – a six-month follow-up study

Željka Rogač[1,2], Aleksandar Dimitrijević[3,4], Ivana Andrić[5], Vesna Miranović[1,2], Dejan Stevanović[6]

[1]Clinical Center of Montenegro, Institute for Children's Diseases, Podgorica, Montenegro;
[2]University of Montenegro, Faculty of Medicine, Podgorica, Montenegro;
[3]University of Belgrade, University Children's Hospital, Belgrade, Serbia;
[4]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[5]University of Kragujevac, Faculty of Medical Sciences, Kragujevac Serbia;
[6]Clinic for Neurology and Psychiatry for Children and Youth, Belgrade, Serbia

SUMMARY

Introduction/Objective Previously, we have shown that six months after initiating monotherapy in school-age children with new-onset uncomplicated epilepsy, minimal changes in cognition and significant symptoms of anxiety, depression, and behavioral changes were observed.
In the same group of children, we aimed to show and compare the effects of the most commonly used anti-seizure medications (ASMs) on cognition, psychopathological symptoms, and behavior, to provide guidance in selecting appropriate ASMs.

Methods Children with newly diagnosed epilepsy completed the Revised Wechsler Intelligence Scale for Children in Serbian (REVISK), the Revised Child Anxiety and Depression Scale (RCADS), and the Nisonger Child Behavior Rating Form (NCBRF), immediately after initiating therapy and six months later, at the University Children's Clinic in Belgrade.

Results Scores on the social phobia subscale increased significantly in children on lamotrigine monotherapy compared to other ASMs, as well as on the separation anxiety disorder subscale and total internalizing symptoms in patients on ethosuximide ($p < 0.05$). The scores on the depressive disorder subscale increased significantly in those on ethosuximide, followed by levetiracetam ($p < 0.05$). There is no statistically significant difference in the change of other RCADS scores and REVISK and NCBRF scores between different types of ASMs during the six months ($p < 0.05$).

Conclusion The subtle influence of the tested ASMs was already present during the first six months of treatment. Valproate led to a trend of improved cognition, while ethosuximide and levetiracetam contributed to worsening internalizing symptoms during the first six months.

Keywords: cognition; anxiety; depression; behavior; ASMs

INTRODUCTION

Children with epilepsy experience challenges in behavioral, cognitive, psychological, and emotional functioning. It has been shown that anti-seizure medications (ASMs) may contribute to these issues in different ways [1].

Thus, topiramate (TPM), valproate (VPA), and carbamazepine (CBZ) can significantly adversely affect cognitive status, while the adverse impact of ethosuximide (ESM), levetiracetam (LEV), and lamotrigine (LTG) is minimal, although there are other findings [2, 3].

Some studies have suggested that VPA, LTG, and CBZ may lead to a mood-stabilizing effect in children with anxiety, depression, and bipolar disorder [3, 4, 5]. On the other hand, the same drugs have also been linked to increased anxiety and symptoms of depression in some patients [6]. LEV may also induce anxiety, depression, emotional lability, reversible psychotic symptoms, and behavioral disorders, particularly in predisposed individuals, although there are also other findings [7, 8, 9]. Moreover, after six months of treatment with TPM, children may exhibit varying emotional improvement or deterioration [10].

Previously, we have shown that six months after initiating monotherapy, minimal changes in cognitive functioning and significant symptoms of anxiety, depression, and attention-deficit/hyperactivity disorder (ADHD) were observed [11]. Adverse effects of ASMs significantly contributed only to depressive symptoms (Table 1) [11].

In some cases, the impact of ASMs during the initial months of treatment may be subtle and hard to notice and, in fact, can be a prelude to more serious damage [1]. Therefore, the question remains: What is the subtle influence of antiepileptic drugs on anxiety, depression, behavior, and cognition?

On those grounds, we aimed to evaluate the effects of the most commonly used ASMs on cognition, psychopathology, and behavior in school-aged children with newly diagnosed

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Correspondence to:
Željka ROGAČ
G. Washington Street
81000 Podgorica, Montenegro
zeljka.rogac89@gmail.com

Table 1. Summarized predictors of cognitive status, anxiety, depressive and behavioral disorder symptoms

Predictors		VIQ	PIQ	Anxiety symptoms	Depressive symptoms	ADHD symptoms	Behavior disorder
VIQ	before	▲					
	after						
PIQ	before		▲				
	after						
Anxiety symptoms	before			▲	▲		
	after			▲	▲	▲	
Depressive symptoms	before				▲		
	after		▲	▲	▲		
ADHD symptoms	before					▲	▲
	after				▲		▲
Behavior disorder	before					▲	▲
	after					▲	
Type of ASM					▲		

VIQ – verbal IQ; PIQ – performance IQ; ADHD – attention-deficit/hyperactivity disorder

epilepsy, as well as to determine which antiepileptic drugs contributed most significantly to depressive symptoms. Here, we present the individual effects of these medications during the first six months of treatment to guide the selection of appropriate ASMs.

METHODS

Study design and methodology

The study was designed as a segment of a more extensive prospective study investigating the impact of ASM monotherapy on cognition, behavior, and psychopathological symptoms in school-aged children with newly diagnosed epilepsy. The diagnosis of epilepsy was made based on the definition of the International League Against Epilepsy (ILAE) [12]. It was conducted during two research visits, immediately after initiating therapy and six months later, at the University Children's Clinic in Belgrade in 2020. The selection of ASM was determined independently of the researcher, based on ILAE guidelines [13].

Inclusion criteria were regular psychomotor development, an intelligence quotient (IQ) > 80, normal physiological and neurological status, normal brain MRI, absence of comorbid conditions, and no concurrent therapy. Exclusion criteria included the need to switch the prescribed ASM, the addition of another ASM to therapy (polytherapy), poor compliance, a subsequently discovered structural lesion on the MRI, or an IQ lower than 80 in children whose test results were received after the start of treatment.

Testing and follow-up procedures

After obtaining consent for participation, participants completed a set of questionnaires. During the two research visits, children and/or their parents completed the following questionnaires, and psychological testing was conducted: Revised Wechsler Intelligence Scale for Children in Serbian (REVISK), Revised Child Anxiety and Depression Scale (RCADS), and the Nisonger Child Behavior Rating Form (NCBRF) for typically developing children and adolescents in Serbian.

Questionnaires

Revised Wechsler Intelligence Scale for Children in Serbian (REVISK)

This instrument was used to assess the cognitive status in patients [11, 14]. REVISK is a standardized battery of Wechsler tests tailored to evaluate intelligence and cognitive functioning in children aged 5–15 years, culturally adapted for the Serbian population [14]. REVISK is based on the WISC-R (Wechsler Intelligence Scale for Children) standardization and is psychometrically closest to the WISC-III [15]. It consists of 11 subtests, and scores are calculated relative to age norms and expressed as scaled scores ranging 1–19 [14]. Total scores are reported as verbal IQ (VIQ), performance IQ (PIQ), and total IQ (TIQ). In this study, internal consistency reliability measured by Cronbach's α coefficient was 0.77, 0.86, and 0.88 for VIQ, PIQ, and TIQ scores respectively [11].

Revised Child Anxiety and Depression Scale (RCADS)

RCADS was used to assess anxiety and depressive symptoms [11, 16]. It includes both a self-report and a parent-report version, each containing 47 questions addressing anxiety symptoms (31 questions), depressive symptoms (10 questions), and obsessive-compulsive disorder (OCD; six questions). Higher scores indicate greater presence of global and specific anxious, depression, and OCD symptoms. Psychometric studies have demonstrated reliable and valid measurements in the Serbian version applied in this study [17, 18]. Cronbach's α coefficients for the self-report version were ≥ 0.70 for all scores except for the depression subscale (0.50) [11]. For the parent-report version, the social phobia and OCD subscales had α coefficients of 0.57 and 0.41, respectively, while all other subscale scores had α ≥ 0.78**15** [11].

Nisonger Child Behavior Rating Form TIQ Version (NCBRF)

This rating form was used to evaluate behavior [11]. The questionnaire, completed by parents only, consists of 64 questions rated on a Likert scale from 0 (never) to 3 (always). Scores are calculated by summing item responses. ADHD symptoms are assessed through the hyperactivity and inattention subscales, disruptive behavior disorder symptoms through conduct and compliance subscales, and total externalizing symptoms through the sum of the previous scores. Higher scores indicate greater behavioral difficulties. The questionnaire has demonstrated reliability and validity. In this study, internal consistency reliability measured by Cronbach's α was ≥ 0.76 for all scores except for the hyperactivity subscale (0.56) [11].

Statistical analysis

In this study, the type of ASM was analyzed as an independent variable. The dependent variables included total scores from the REVISK, RCADS, and NCBRF scales. Only adequately completed data from filled questionnaires and tests were included in the analysis.

Descriptive statistical methods used included absolute values, percentages, mean values (M), and measures of dispersion (standard deviation – SD and standard error – SE). Analytical statistical methods included the following tests and analyses: paired t-tests were conducted to assess differences in participants' questionnaire scores at the beginning of treatment (baseline) and after six months of follow-up. For statistically significant changes, the effect size of the score differences was expressed using Cohen's d coefficient, interpreted as small (< 0.5), medium (0.5–0.8), or large (> 0.8) [11]. Analysis of variance (ANOVA) for repeated measures was used to examine the magnitude of score changes in questionnaires over time (baseline and after six months) regarding the type of ASM. All analyses were performed using the PASW Statistics, Version 18.0 (SPSS Inc., Chicago, IL, USA), with a significance threshold of $p < 0.05$.

The study was conducted following Good Clinical Practice guidelines, the Declaration of Helsinki, and applicable local and regional regulations, following approval by the Ethics Committee of the University Children's Clinic (UDK) in Belgrade, number 13/208. It was designed as an academic, non-profit, non-interventional clinical study.

RESULTS

The study included 69 school-aged children who were treated at the University Children's Hospital in Belgrade in 2020 who met the inclusion criteria. Nine patients were lost to the six-month follow-up due to poor compliance and necessary polytherapy. The demographic and clinical data of the subjects are presented in Table 2.

Table 2. Clinical data of the subjects

Age (SD), span	All included, n = 68	Followed for 6 months, n = 60
	12.32 (3.34), 7–18	12.45 (3.25), 7–18
Male/female, n (%)	38 (55.9) / 30 (44.1)	34 (56.7) / 26 (43.3)
Antiepileptic, n (%)		
VPA	23 (33.8)	18 (30)
LEV	16 (23.5)	15 (25)
CBZ	14 (20.6)	13 (21.7)
LTG	8 (11.8)	7 (11.7)
ESM	6 (8.8)	6 (10)
TPM	1 (1.5)	1 (1.7)

TPM – topiramate; VPA – valproate; CBZ – carbamazepine; ESM – ethosuximide; LEV – levetiracetam; LTG – lamotrigine

Table 3 shows the mean values (SD) of the REVISK scores for the type of ASM. There is no statistically significant difference in the change in scores between different types of ASM during the six months.

Table 4 shows the mean values (SD) of the subjects' RCADS scores for the type of ASM. Scores on the social phobia subscale increased significantly less than those on the separation anxiety disorder subscale and total internalizing symptoms compared to lamotrigine. The scores on the depressive disorder subscale increased significantly less than those on ethosuximide. There is no statistically significant difference in the change of other scores between different types of ASM during the six months.

Finally, there was no statistically significant difference in the change in NCBRF scores between different types of ASM over the six months (Table 5).

DISCUSSION

The impact of ASMs on cognitive status

Although it was not clinically significant, subtle effects of ASMs on specific cognitive domains were observed.

In our study, VPA demonstrated a positive impact on cognitive status in the first six months. Children receiving VPA therapy showed increased verbal, nonverbal, and overall intelligence quotients. However, the overall effect of VPA did not differ significantly from other ASMs.

VPA, like ESM, is commonly used as a first-line treatment for absence epilepsy. Prior research reported that ESM is more favorable than VPA for cognitive outcomes [19]. However, in our study, during the first six months of treatment, children treated with ESM exhibited a trend of decline in VIQ, PIQ, and overall IQ. Due to the small sample size, this negative impact of ESM on cognition was not statistically significant and does not warrant changes in clinical guidelines for treating absence epilepsy. Nevertheless, our findings suggest that in children with absence epilepsy who present with cognitive deficits at baseline, VPA may be a preferable treatment option.

We have shown that LEV is associated with a trend of decreasing nonverbal IQ, which is novel. However, consistent with earlier observations, LEV was linked to mild cognitive improvement in verbal IQ, attention, and overall cognitive status [20]. While most studies report cognitive abatement following CBZ use [21], our findings indicate mild improvement in VIQ despite a trend of decline in nonverbal IQ domains. It would be useful to see what happens to our subjects later, considering recent studies showing significant cognitive improvement over one year in children treated with LEV and LTG compared to school-aged children treated with CBZ [21, 22]. Of course, this remains a preliminary observation; no significant differences between these drugs were found.

The subtle trend of adverse effects of ESM, LTG, and CBZ on cognitive status during the first six months, though unexpected, highlights the need for further investigation into the cognitive impacts of ASMs. Therefore, we

Table 3. Distribution of REVISK scores with regard to the type of ASM*

IQ	VPA n = 18		LEV n = 15		CBZ n = 13		LTG n = 7		ESM n = 6		Significant differences between ASMs
	M	SD	M	SD	M	SD	M	SD	M	SD	
VIQ before	92.8	11.3	92.1	11.6	95	12.9	98.7	26.4	101.5	20.5	No
VIQ after	93	13.51	86.7	9.2	99.2	13.11	94.1	21.7	96.2	17.2	
PIQ before	93.8	13.2	97	15	108.7	15.11	105.5	17.8	104.3	15.3	No
PIQ after	98.3	17.2	86.7	10.7	105.9	16.3	104.3	16.1	97.51	11	
TIQ before	93.1	10.2	94.1	10.4	101.7	12.5	104	17.5	97.5	11.1	No
TIQ after	95.8	13.9	86.9	9.5	101.9	12.9	100.9	13	97	14	

ASMs – anti-seizure medications; VPA – valproate; CBZ – carbamazepine; ESM – ethosuximide; LEV – levetiracetam; LTG – lamotrigine; VIQ – verbal IQ; PIQ – performance IQ; TIQ – total IQ;
*ANOVA for repeated measurements, Bonferoni corrected, $p < 0.05$

Table 4. Distribution of RCADS scores about the type of ASM*

Parameter	VPA n = 18		LEV n = 15		CBZ n = 13		LTG n = 7		ESM n = 6		Significant differences between ASM
	M	SD	M	SD	M	SD	M	SD	M	SD	
TotAbefore	10.3	9.4	10.3	5.9	10.5	7.2	17.2	11	14.5	9	VPA < ESM
TotA after	16.1	8	26.9	12.9	20.1	13.6	25	12.2	29.8	10.8	
TotD before	2.7	1.9	1.8	2	2.7	1.5	3.9	2.9	2.2	1.5	No
TotD after	5.7	4.5	7.5	4.3	6.9	3.7	5.1	3.1	10.9	7.1	
Sph before	4.2	3.8	4.7	2.7	3.8	2.9	8.1	4.3	6.3	3.8	VPA < LTG
Sph afer	6.9	2.6	10.9	4.8	7.3	4.9	11.6	5.1	12.8	4.4	
OCD before	1.72	1.82	0.91	0.9	2.2	1.8	2.62	2.2	0.3	0.5	No
OCD after	2.2	2.6	1.9	2	2.5	2.6	3.1	2.6	2.5	2.4	
PD before	1.22	0.8	1.3	1.2	1.3	1.4	2.7	2.1	1.3	0.5	No
PD after	2.7	2.4	3.9	3.6	2.3	2.91	3.3	2.8	5.8	5.6	
SAD before	1.6	2.8	1.5	1.6	1.9	1.9	3.1	3.9	3.7	3	No
SAD after	2.5	3.8	2.7	2.4	1.71	2.2	3.4	3.5	7.8	5.6	
GAD before	2.7	1.9	2.4	1.7	1.8	1.7	2.4	2.1	2.7	1.6	No
GAD after	3.1	1.9	4.8	2.7	2.5	1.7	4.4	3.7	6.7	3.6	
TotINbefore	13	10	12.1	7.3	13.2	8.2	21.1	13.9	16.7	9.8	VPA < ESM
TotINafter	921.8	11.9	34.4	16.7	26.9	16.2	30.1	14.8	50.7	16.7	

ASMs – anti-seizure medications; VPA – valproate; CBZ – carbamazepine; ESM – ethosuximide; LEV – levetiracetam; LTG – lamotrigine; TotA – total score for anxiety; TotD – total score for depression; Sph – social phobia; OCD – obsessive-compulsive disorder; PD – panic disorder; SAD – separation anxiety disorder; GAD – generalized anxiety disorder; TotIN – internalizing symptoms total score;
*ANOVA for repeated measurements, Bonferroni corrected, $p < 0.05$

Table 5. Distribution of NCBRF scores about the type of ASM*

Parameter	VPA n = 18		LEV n = 15		CBZ n = 13		LTG n = 7		ESM n = 6		Significant differences between ASM
	M	SD	M	SD	M	SD	M	SD	M	SD	
ADHD before	5.4	4.7	4.5	3.2	7.3	4.8	7.7	3.8	6.2	4.3	No
ADHD after	10.3	7.1	11	6.7	11.7	7.2	11.7	5.3	15.3	7.7	
TE before	11.6	10	8.8	4.3	15.9	13.2	18.4	10.2	3.3	9.8	No
TE after	22.5	17.7	18.6	16.8	24.5	18.2	16.61	13	31.5	14.3	
DBD before	6.1	5.6	4.31	2.6	8.6	9	10.7	6.6	7.2	5.8	No
DBD after	12.1	10.9	17.7	12.5	12.9	12.5	14.9	8.8	16.2	7.1	

ADHD – attention-deficit/hyperactivity disorder; DBD – disruptive behavior disorder; TE – total externalizing score;
*ANOVA for repeated measurements, Bonferroni corrected, $p < 0.05$

underscore the necessity of individualized approaches to ASM selection and emphasize the importance of monitoring cognitive changes in children undergoing antiepileptic treatment.

The impact of ASMs on anxiety, depression, and behavioral problems

Although antiepileptic treatment did not significantly affect the presence of anxiety symptoms after six months [11], some ASMs were more likely to contribute to anxiety than others. Participants treated with ESM had the highest anxiety scores, followed by those on LEV, LTG, CBZ, and finally VPA, which demonstrated the lowest average anxiety scores.

Among all the ASMs evaluated, VPA was the only one associated with the trend of positive effects on symptoms of social phobia and generalized anxiety disorder. It suggests that VPA has the most favorable effect on anxiety symptoms and, if possible, should be the first-line choice in children with seizures and anxiety. Nevertheless, LTG

and VPA demonstrated favorable effects on obsessive-compulsive disorder symptoms after six months, supporting earlier evidence [23].

It has already been said that this research is part of a larger project in which we showed that ASMs, during the first six months, only contribute to the significant occurrence of internalizing symptoms [11]. Based on these findings, LEV stood out for its effect on the occurrence of depressive symptoms, compared to other ASMs. There is a clinically significant negative effect of LEV on internalizing symptoms, including anxiety and depression, which was recently demonstrated and explained in the population of adult patients with epilepsy [24].

In contrast to previous studies [25], our findings suggest that, like other drugs, LEV did not clinically significantly influence behavioral disorders within the first six months of treatment. However, children on LEV exhibited the most pronounced difficulties with conduct, attention, and social competence, alongside increased hypersensitivity, hyperactivity, and ADHD symptoms. Monitoring these trends over time is essential to determine whether LEV's impact on behavioral issues may become clinically significant in the long term.

According to earlier findings of favorable or neutral effects of LTG and CBZ on ADHD symptoms [26, 27], our study showed their less negative, although not clinically significant, impact on behavioral aspects than other drugs, in the following order: ESM > LEV > VPA > CBZ > LTG.

CONCLUSION

Our study is the first to compare the effects of the most commonly used ASMs on specific domains in cognition (verbal/nonverbal), behavior, anxiety, and depression in the first six months, in one act, in children with new-onset uncomplicated epilepsy.

Considering the subtle improvement in PIQ and VIQ, VPA seems like a good option. Given that we have previously shown that the side effects of antiepileptic therapy can significantly contribute to the appearance of internalizing symptoms after six months [11], the present study suggests that the negative impact of LEV and ESM should be considered in children who develop internalizing symptoms after six months. Regardless, this study compared antiepileptic drugs in a gradational way, so certain conclusions can still be drawn. In children who are on ESM and LEV therapy, the epileptologist should be careful in the event of early signs of behavioral disorder symptoms.

However, our research has several limitations. We did not analyze patients regarding epileptic syndromes, seizure type, the impact of epileptogenesis, and epileptiform discharges on the EEG. Also, it would be useful to continue our research so that the trend of the influence of certain antiepileptic drugs would be statistically more significant and could contribute to recommendations for clinical practice.

Conflict of interest: None declared.

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Утицај појединих антиепилептичких лекова на когнитивни статус, понашање, анксиозност и депресију код деце школског узраста са новодијагностикованом епилепсијом – студија шестомесечног праћења

Жељка Рогач[1,2], Александар Димитријевић[3,4], Ивана Андрић[5], Весна Мирановић[1,2], Дејан Стевановић[6]

[1]Клинички центар Црне Горе, Институт за болести деце, Подгорица, Црна Гора;
[2]Универзитет Црне Горе, Медицински факултет, Подгорица, Црна Гора;
[3]Универзитет у Београду, Универзитетска дечја клиника, Београд, Србија;
[4]Универзитет у Београду, Медицински факултет, Београд, Србија;
[5]Универзитет у Крагујевцу, Факултет медицинских наука, Крагујевац, Србија;
[6]Клиника за неурологију и психијатрију деце и омладине, Београд, Србија

САЖЕТАК

Увод/Циљ Претходно смо показали да се шест месеци након почетка лечења деце школског узраста са новодијагностикованом некомпликованом епилепсијом јављају минималне промене у когнитивном функционисању, али и значајни симптоми анксиозности, депресије и поремећаја понашања. У поменутој групи деце процењивали смо и упоређивали ефекте најчешће коришћених антиепилептичких лекова (АЕЛ) на когницију, симптоме психопатологије и поремећај понашања шест месеци након почетка лечења, са циљем да допринесемо смерницама за избор одговарајућег АЕЛ.

Методе Деца са новодијагностикованом епилепсијом тестирана су Ревидираном Вешлеровом скалом за интелигенцију на српском језику, Ревидираном скалом за анксиозност и депресију код деце и Нисонгеровим обрасцем за процену понашања деце, одмах по увођењу терапије и након шест месеци, на Универзитетској дечјој клиници у Београду.

Резултати Резултати на супскали социјалне фобије били су статистички значајно виши код деце на монотерапији ламотригином у поређењу са другим АЕЛ, као и на супскали поремећаја сепарације и укупних интернализацијских симптома код деце на етосуксимиду ($p < 0,05$). Резултати на супскали депресивног поремећаја значајно су се повећали код оних на терапији етосуксимидом, а потом леветирацетамом ($p < 0,05$). Нема статистички значајне разлике у променама осталих резултата на Ревидираној скали за анксиозност и депресију код деце, као ни у резултатима Ревидиране Вешлерове скале интелигенције за децу и Нисонгеровог обрасца за процену понашања деце између различитих типова АЕЛ током првих шест месеци ($p < 0,05$).

Закључак Суптилан утицај испитиваних АЕЛ уочен је већ током првих шест месеци лечења. Валпроат је показао тренд побољшања когнитивних функција, док су етосуксимид и леветирацетам у највећој мери допринели погоршању интернализујућих симптома у том периоду.

Кључне речи: когнитивне функције; анксиозност; депресија; понашање; антиепилептички лекови

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Renal injury in children with a congenital solitary kidney – a single center experience

Jera Grabnar[1], Rina R. Rus[2,3]

[1]University Medical Centre Ljubljana, Children's Hospital, Ljubljana, Slovenia;
[2]University Medical Centre Ljubljana, Children's Hospital, Department of Pediatric Nephrology, Ljubljana, Slovenia;
[3]University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia

SUMMARY

Introduction/Objective Reduced kidney length, low birth weight, obesity, and ipsilateral congenital anomalies of the kidney and urinary tract (CAKUT) are risk factors for renal injury (hypertension, proteinuria, and chronic kidney disease) in single-functioning kidneys. Our study aimed to investigate the risk factors for renal injury and outcome in children with congenital solitary kidney (CSK).

Methods We collected data from the medical records of 95 children with CSK.

Results Children with CSK were predominantly male (61%). An abnormal ultrasound (US) view of a solitary kidney was found in nine (9%) and renal length below the 75th percentile in eight (11%) children. Seven (7%) children had low birth weight, 18 (20%) were obese, 26 (28%) had urinary tract infections, 24 (25%) had CAKUT and 28 (29%) were treated with an angiotensin-converting enzyme (ACE) inhibitor. Decreased glomerular filtration rate was found in three, proteinuria in 14 (15%), and arterial hypertension in 10 (11%) children. A total of 23 (24%) children met the criteria for renal injury. In multiple logistic regression, only US abnormalities approached significance (OR 5.6, p = 0.08). Compared to other studies, we had a higher percentage of an ACE inhibitor prescribed for renal protection. This could be the reason for the low percentage of renal injuries in our study.

Conclusion Monitoring blood pressure, proteinuria, and renal function might be of utmost importance, especially in children with CSK and abnormal US appearance. Additionally, further studies are needed to confirm the possible beneficial effect of renoprotective treatment in patients with CSK.

Keywords: solitary kidney; renal injury; renoprotective treatment; ACE inhibitor; children; abnormal ultrasound appearance

INTRODUCTION

Congenital solitary kidney (CSK) is a kidney's anatomical or functional absence from birth. It results from abnormal or incomplete kidney development *in utero* leading to a non-functioning kidney, as in multicystic dysplastic kidney and renal aplasia, or from unilateral renal agenesis [1]. Dysplastic kidneys can regress spontaneously either prenatally or within the first few years of life. Additionally, other congenital anomalies of the kidney and urinary tract (CAKUT), in particular vesicoureteral reflux (VUR), are often associated with CSK [1].

Unilateral renal agenesis is adequately confirmed in most cases with a neonatal abdominal ultrasound (US) performed by a pediatric radiologist. Further imaging with renal scintigraphy with dimercaptosuccinic acid (DMSA) or dimercaptoacetyltriglycine (MAG3) is only recommended if the diagnosis is uncertain (e.g., in the absence of compensatory renal hypertrophy) [2].

In the majority of CSK, there is increased renal growth, which is initiated prenatally. A reduced number of nephrons leads to glomerular hyperfiltration, which results in renal injury, such as high blood pressure, proteinuria, and chronic kidney disease [3]. In a large group of children with a single functioning kidney (SFK), the median age at which renal injury occurred was around 15 years [4]. Risk factors for renal injury in SFK were found to be ipsilateral CAKUT and insufficient kidney length, obesity, and low birth weight [4, 5, 6]. The best indicator of renal function in children and adolescents is the glomerular filtration rate (GFR) and as such is used as the most reliable marker of a functioning kidney mass [7]. Angiotensin-converting enzyme (ACE) inhibitors slow the progression of chronic kidney disease in children with renal hypodysplasia [8].

In this study, we sought to evaluate the risk factors for renal injury and outcome in children with SFK compared to other similar studies.

METHODS

This is a retrospective study, conducted at the University Children's Hospital in Ljubljana, Slovenia.

The patients

We reviewed the medical records of 310 children diagnosed with solitary kidney disease between January 1980 and December 2017.

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Correspondence to:
Rina R. RUS
University Medical Centre Ljubljana
Children's Hospital
Bohoriceva 20
1000 Ljubljana
Slovenia
rina.rus@kclj.si

Following the inclusion criteria [e.g., an abdominopelvic US examination without evidence of renal tissue on one side (empty renal fossa, no renal tissue in the retroperitoneum or pelvis) and confirmatory renal scintigraphy] and the exclusion of children with a surgically removed kidney, 95 children were finally included in the study.

We collected the following data: sex, reasons for first US examination, appearance of kidneys at US examination, measurement of kidney length, duration of follow-up, additional risk factors (low birth weight, obesity, urinary tract infections (UTI), CAKUT (other than CSK)), use of renoprotective medications (ACE inhibitors), GFR, proteinuria, and blood pressure.

US

US examinations of the abdomen and pelvis were performed by various examiners with children in the supine position using a 3.5 to 5 MHz probe, usually with the Toshiba US devices (Eccocce, Ecusson, SSA 140A or Power Vision 6000, Toshiba Corporation, Minato, Tokyo, Japan). Compensatory hypertrophy of the CSK was defined in children in the prone position as kidney length in the maximal renal longitudinal section exceeding the 95th percentile value for normal kidney length, as described by Akhavan et al. [9]. The US appearance was considered normal if echogenicity, structure, and thickness of the kidney parenchyma were normal, normal corticomedullary differentiation without calyceal dilatation was present and 10 mm was considered the upper limit of normal anterior-posterior renal pelvis diameter [10], otherwise, it was considered abnormal.

Scintigraphy

Renal scintigraphy with DMSA (57/95; 60%) or MAG3 (38/95; 40%) was performed in all children to confirm CSK and exclude obstruction or parenchymal scarring.

Additional risk factors

Low birth weight is defined as ≤ 2500 g and obesity is diagnosed with a BMI ≥ 95th percentile for age and sex.

CAKUT

Because children with CAKUT had a higher proportion of renal injury on the CSK side [11], we divided the children into those with and without CAKUT. CAKUT was identified by renal US and scintigraphy (performed in all patients) and by a cystourethrogram performed in 56/95 patients (58.9%) when VUR was suspected, mainly in children with recurrent UTI.

Renoprotective treatment

In our study, ACE inhibitor treatment was prescribed not only to children with proteinuria and hypertension, but also to some children with prehypertension, CAKUT, and kidney length below the 75th percentile for renoprotection. Therefore, we did not include the ACE inhibitor as a possible marker of renal injury as was the case in the KIMONO study [4].

Renal injury

Renal injury was defined as the persistent presence of one or more of the following: significantly impaired GFR, proteinuria, and hypertension.

The Schwartz formula with adjustment to the Slovenian population (k = 40 children ≤ 3 years; k = 48 girls > 3 years; k = 58 boys > 3 years) was used to calculate the GFR [12]. According to Hellerstein et al. [13], the threshold for significantly impaired GFR in solitary kidney was 78 mL / min / 1.73 m^2 in 1–2-year-old children, 73 mL / min / 1.73 m^2 in girls over two years and boys 2–13 years, and 70 mL / min / 1.73 m^2 in boys over 13 years. Proteinuria was defined as urinary protein excretion > 100 mg / m^2 / 24 hours or spot urine protein/creatinine ratio > 0.2 g/g Cr [14]. Blood pressure was determined with a 24-hour or 48-hour ambulatory blood pressure measurement. A blood pressure ≥ 95th percentile for sex, age, and height was defined as arterial hypertension [15].

Statistical analysis

Statistical analysis was performed using the SPSS package for IBM SPSS Statistics for Windows, Version 29.0. (IBM Corp., Armonk, NY, USA). Numeric variables are expressed as median or mean with standard deviation (SD), depending on the distribution of the data. Descriptive variables are expressed as percentages. Multiple logistic regression analysis was performed to determine the predictive risk factors for renal injury. The results are expressed as odds ratios (OR) with 95% confidence intervals (CI). A p-value of less than 0.05 was considered statistically significant.

Ethical approval

The study was approved by the National Medical Ethics Committee of the Republic of Slovenia (0120-374/2017/7). It complies with the 1964 Helsinki Declaration and its subsequent amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

RESULTS

Patient characteristics

Among the children with CSK, there were more boys (61%), and CSK was predominantly right-sided (56%).

The solitary kidney was detected prenatally in 10/95 (10.5%), immediately after birth during newborn screening in 31/95 (32.6%) children, by US examination due to UTI in 8/95 (8.4%), and by US examination during screening

for other congenital malformations, enuresis, urinary incontinence or abdominal pain in 31/95 (32.6%) children. For the remaining 15/95 (15.8%), no data was found at the time of diagnosis.

The last US examination showed an abnormal kidney appearance in nine (10%) children (Table 1).

CAKUT (other than CSK) was detected in 24 (25%) children, among those more than one anomaly was found in 8/24 (33%). The most common anomaly was VUR, followed by hydronephrosis and/or hydroureter with or without ureterovesical junction obstruction (Table 2).

Impaired GFR for solitary kidney according to Hellerstein et al. [13] was found in three patients. In 14/91 (15%) children proteinuria was present and in 10/95 (11%) arterial hypertension. The mean age of children with a diagnosis of hypertension was 12.4 ± 2 years. A total of 28 (29%) children were treated with an ACE inhibitor for renal injury or for renal protection itself (Table 3). All children with renal injury were recommended to receive treatment with an ACE inhibitor; however, only nine of them were taking the medication. The mean age at the start of treatment with an ACE inhibitor was 10.2 years (SD 3.2 years).

Table 1. Patient characteristics

Characteristic	N of patients (%)
Male gender	58/95(61.1)
Right side CSK	53/95 (55.8)
Abnormal kidney appearance (last US)	9/93* (9.7)
Kidney length (last US) according to Akhavan et al. [9]	
< 75%	8/74* (10.8)
75–95%	22/74* (29.7)
> 95%	44/74* (59.5)
Low birth weight	7/95 (7.4)
Obesity	18/89* (20.2)
UTIs	26/93* (28)
CAKUT	24/95 (25.3)
Extrarenal anomalies	23/95 (24.2)
Median age at first diagnosis, months	2 (2–61)**
Median follow-up, months	106 (3–230)**

CSK – congenital solitary kidney; US – ultrasound; UTI – urinary tract infection; CAKUT – congenital anomalies of the kidney and urinary tract;
*there are a few missing data results due to retrospective studies;
**range

Table 2. Congenital anomalies of the kidney and urinary tract (CAKUT)

Parameters	No. of patients (%)
CAKUT (total)	24/95 (25.3)
VUR	14/95 (14.7)
Hydronephrosis and/or hydroureter with or without ureterovesical junction obstruction	9/95 (9.5)
Pyelon duplex	4/95 (4.2)
Ectopic CSK	2/95 (2.1)
Cystic dysplasia of CSK	1/95 (1.1)
Hypospadias	1/95 (1.1)

VUR – vesicoureteral reflux; CSK – congenital solitary kidney

Table 3. Renal injury and the use of ACE inhibitor

Parameters	No. of patients (%)
Renal injury (total)	23/95 (24.2)
Impaired GFR according to Hellerstein	3/91* (3.3)
Proteinuria	14/91* (15.4)
Arterial hypertension	10/95 (10.6)
ACE inhibitor (total)	28/95 (29.5)
for Renal injury	9/95 (9.5)
for Renoprotection	19/95 (20)

ACE – angiotensin-converting enzyme; GFR – glomerular filtration rate;
*there are a few missing data results due to retrospective studies

Table 4. Multiple logistic regression analysis of risk factors

Risk Factor	OR (95% CI)	p-value
CAKUT	0.40 (0.08–1.94)	0.254
Low birth weight	0.64 (0.06–5.62)	0.637
UTI	0.50 (0.10–2.43)	0.394
Obesity	1.44 (0.36–5.75)	0.604
Renal length < 95%	1.33 (0.39–4.56)	0.647
Abnormal US appearance	5.57 (0.81–38.04)	0.080

CAKUT – congenital anomalies of the kidney and urinary tract; UTI – urinary tract infection; US – ultrasound

Renal injury risk factors

A total of 23 (24%) patients met the criteria for renal injury, defined as significantly impaired GFR and/or the presence of proteinuria and/or hypertension. In the multiple logistic regression analysis (Table 4), an abnormal US appearance is almost significant (OR 5.6).

DISCUSSION

In our retrospective unicentric study, 95 children with CSK were evaluated. The median age at diagnosis was two months, with most cases diagnosed either prenatally or immediately after birth, like in the KIMONO study (both 43%) [4]. We had a higher proportion of males like the study by Jørgensen et al. [16] (61% *vs*. 67%). When considering abnormal kidney appearance, our data is also like the study by Jørgensen et al. [16] (10% *vs*. 8%). In addition, 25% of our children were found to have CAKUT, mainly VUR, which occurred in 15% of cases. This number is lower than the 24% of patients with VUR in the meta-analysis by Westland et al. [1]. Siomou et al. [17], on the other hand, report an even lower number of VUR. Since low-grade VUR can be self-limiting, but high-grade VUR may severely impair renal function, it is important to be aware of the latter. As noted in the Westland et al. [1] meta-analysis, these findings highlight the need for further validation to avoid routinely using voiding cystourethrography in children with a normal sonographic appearance of CSK and no recurrent UTIs, especially given the increasing availability of non-invasive methods for detecting VUR.

In our study, a significantly higher percentage of children were treated with an ACE inhibitor compared to the KIMONO study (29% *vs*. 17%, respectively). In the majority of our children, treatment with an ACE inhibitor was started due to renal protection [19/28 (68%)], whereas in the KIMONO study, the indications for an ACE inhibitor were mostly proteinuria or hypertension. The mean age

at treatment initiation is approximately the same in both our and the KIMONO study [10.2 years (SD 3.2 years) and 9.8 years (SD 5.5 years), respectively]. However, the main difference between these two studies was that the KIMONO study included not only children with CSK but also those with acquired SFK [4].

Impaired GFR, proteinuria, and hypertension were found in 3%, 15%, and 11%, respectively. In comparison, Westland et al. [1] found impaired GFR in 10%, microalbuminuria in 21%, and hypertension in 16% in their meta-analysis of more than 2500 unilateral renal agenesis patients. In contrast, in the KIMONO study of more than 400 patients with SFK (congenital and acquired), 4% had impaired GFR, 13% proteinuria, and 22% hypertension [4]. We can speculate that the lower prevalence of hypertension among CSK patients in our study, compared to other studies, may be attributed to the use of ACE inhibitors as renoprotective agents, rather than solely being a consequence of the smaller sample size or the exclusive inclusion of CSK patients in our cohort.

In our study, 23 patients (24%) met the criteria for renal injury, defined as significantly impaired GFR, and/or the presence of proteinuria, and/or hypertension. This is lower than the KIMONO study, where renal injury was observed in 37% of patients [4]. Although in the KIMONO study, ACE inhibitor was used as a criterion for renal injury, we can speculate that the renoprotective use of the ACE inhibitor in our study could reduce the percentage of patients with proteinuria and hypertension.

According to our data, no statistical significance of risk factors for renal injury was found, in contrast to other previous studies that found ipsilateral CAKUT, insufficient kidney length [4], obesity [5], and low birth weight [6] as risks factors for renal injury in SFK. In multiple logistic regression, only the US abnormalities approached significance (OR 5.6, p = 0.08). The reason for this discrepancy between our study and other studies could be the smaller number of patients included in our study compared to other studies [4, 18], although a possible positive effect of an ACE inhibitor as a renoprotective medication cannot be completely excluded, especially in children with prehypertension and US abnormalities. To date, there is limited data on this topic. In 2017, a systematic literature review was conducted, concluding that anti-RAAS drugs (renin-angiotensin-aldosterone system inhibitors) may also provide renoprotective benefits in patients with an SFK. The use of direct renin inhibitors and angiotensin receptor blockers appears to be particularly suitable, especially in children [18]. Additionally, studies in animal models suggest that early administration of ACE inhibitors in cases of SFK is associated with impaired glomerular hyperfiltration-mediated kidney disease [19]. However, further studies are needed to draw more definitive conclusions.

Most studies highlight the importance of lifelong, regular follow-up for CSK patients, focusing on monitoring proteinuria, blood pressure, and kidney function. The KIMONO study recommended at least annual follow-up for children with CSK until adulthood [4, 15]. However, some studies suggest a less intensive follow-up program for children without associated CAKUT and with adequate compensatory renal hypertrophy [3, 4]. Our study demonstrates that patients with CSK and abnormal US appearances require close monitoring.

Limitations of the study

An important obstacle in a retrospective study is missing data, a large difference in follow-up time, the number of outpatient visits, and data that was not collected systematically and longitudinally. Due to the retrospective study, albuminuria was not routinely measured and the results found were far too low to include the parameter in the study. In our study, we used proteinuria as a parameter, even though urinary albumin measurement is recognized as a more specific and sensitive indicator of changes in glomerular permeability compared to total urinary protein [20]. On the other hand, the protein-to-creatinine ratio has been demonstrated to be a relevant diagnostic biomarker in clinical trials involving children with glomerular diseases [21].

CONCLUSION

According to our results, a significant percentage of children with CSK and an abnormal US appearance exhibit renal injury compared to those with a normal US. Therefore, close monitoring of blood pressure, protein levels, and kidney function in these children is critically important. Moreover, further research is needed to explore the benefits of renoprotective treatments for individuals with CSK.

Conflict of interest: None declared.

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Оштећење бубрега код деце са урођеним солитарним бубрегом – искуство једног центра

Јера Грабнар[1], Рина Р. Рус[2,3]

[1]Универзитетски медицински центар у Љубљани, Дечја болница, Љубљана, Словенија;
[2]Универзитетски медицински центар у Љубљани, Дечја болница, Одељење за педијатријску нефрологију, Љубљана, Словенија;
[3]Универзитет у Љубљани, Медицински факултет, Љубљана, Словенија

САЖЕТАК

Увод/Циљ Смањена дужина бубрега, ниска порођајна тежина, гојазност и урођене аномалије бубрега и мокраћних путева на истој страни представљају факторе ризика за оштећење бубрега (хипертензију, протеинуријe и хроничну болест бубрега) код пацијената са једним функционалним бубрегом. Циљ наше студије био је да истражи факторе ризика за оштећење бубрега и исход лечења код деце са урођеним солитарним бубрегом (*CSK*).

Методе Податке смо прикупили из медицинске документације 95 деце са *CSK*.

Резултати Деца са *CSK* била су претежно мушког пола (61%). Абнормалан ултразвучни налаз солитарног бубрега забележен је код деветоро (9%) деце, а дужина бубрега испод 75. перцентила код осморо (11%) деце. Седморо (7%) деце је имало ниску порођајну тежину, 18 (20%) било је гојазно, 26 (28%) имало је инфекције уринарног тракта, 24 (25%) имало је урођене аномалије бубрега и мокраћних путева, а 28 (29%) било је лечено инхибитором ангиотензин-конвертујућег ензима (*ACE*). Смањена брзина гломеруларне филтрације забележена је код троје деце, протеинурија код 14 (15%), а артеријска хипертензија код 10 (11%) деце. Укупно 23 (24%) детета испунила су критеријуме за оштећење бубрега. У вишеструкој логистичкој регресији само је абнормалан ултразвучни налаз показао тренд статистичке значајности (*OR* 5,6; $p = 0,08$). У поређењу са другим студијама, имали смо већи проценат деце којој је прописан *ACE* инхибитор за заштиту бубрега. То би могао бити разлог за мањи проценат оштећења бубрега у нашој студији.

Закључак Праћење крвног притиска, протеинурије и бубрежне функције може бити од пресудног значаја, посебно код деце са *CSK* и абнормалним ултразвучним налазом. Поред тога, потребне су даље студије како би се потврдио могући повољан ефекат ренопротективног третмана код пацијената са *CSK*.

Кључне речи: солитарни бубрег; оштећење бубрега; ренопротективни третман; *ACE* инхибитор; деца; абнормалан ултразвучни налаз

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Relation of oncogenic microRNA-10b and microRNA-21 to glioblastoma size and localization

Marina Nikitović[1,2], Aleksandar Stepanović[1,2], Tatjana Arsenijević[1,2], Nina Petrović[3,4]

[1]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[2]Institute for Oncology and Radiology of Serbia, Department of Radiation Oncology, Belgrade, Serbia;
[3]Institute for Oncology and Radiology of Serbia, Department of Experimental Oncology, Belgrade, Serbia;
[4]University of Belgrade, Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, Belgrade, Serbia

SUMMARY

Introduction/Objective In glioblastoma, upregulation of oncogenic microRNA-10b (miR-10b) and microRNA-21 (miR-21) is often found. Our study aimed to investigate whether there is a link between miR-10b and miR-21 expression levels and tumor size and tumor localization.

Methods The research involved 43 patients diagnosed with glioblastoma. We analyzed the expression levels of miR-10b and miR-21 post-surgery. The data on tumor size and tumor localization were obtained from magnetic resonance imaging.

Results The median expression level of miR-10b in patients with tumors < 4 cm was 214.86 (min–max: 2.13–816.89), while in patients with tumors ≥ 4 cm, the median expression level was 92.99 (min–max: 19.24–491.82). The median expression level of miR-21 in patients with tumors < 4 cm was 81.69 (min–max: 11.39–825.43), whereas in patients with tumors ≥ 4 cm, the median expression level was 40.84 (min–max: 2.68–278.98). For both miR-10b and miR-21, a statistically significant difference was found for tumors < 4 cm ($p = 0.027$ and $p = 0.047$, respectively) compared to those ≥ 4 cm. There was no statistically significant difference in the expression levels of miR-10b ($p = 0.675$) and miR-21 ($p = 0.183$) in relation to tumor localization.

Conclusion Glioblastomas smaller than 4 cm have statistically significantly higher expression levels of miR-10b and miR-21 compared to glioblastomas equal to or larger than 4 cm. Although this result is unexpected, it could mean that miR expression levels dynamically change after surgery and according to the altered microenvironment.

Keywords: glioblastoma; microRNA; tumor size

INTRODUCTION

Tumor histology, tumor size, grade, vascular invasion, stage and other clinicopathological and molecular features are often described as prognostic factors in patients with extracranial cancer [1, 2]. For patients diagnosed with malignant intracranial tumors such as glioblastoma, the most reliable prognostic factors are age, O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation, isocitrate dehydrogenase (IDH) mutation, extent of surgical resection and tumor location [3, 4]. The size of the tumor in patients with glioblastoma has also been shown to be a possible prognostic factor for survival [3, 5, 6].

MicroRNA-10b (miR-10b) and microRNA-21 (miR-21) are amongst the most researched microRNAs in human oncology. They are often overexpressed in a spectrum of human cancers. MiR-21 is frequently overexpressed in various cancers, including glioblastoma [7]. Due to the hypoxic conditions present in glioblastoma cells, an upregulation of miR-10b/miR-21 can be observed [8].

miR-10b is recognized as a potent oncogenic microRNA (oncomiR) involved in the regulation of the cell cycle. Upregulation of miR-10b can promote tumor growth, invasion and migration [9]. Through diversiform gene regulation and signaling pathways, overexpression of miR-21 is shown to play an important role in oncogenesis and tumor metastasis, as well as in resistance to oncologic treatment [10]. In glioblastoma, miR-21 is upregulated and thus, often linked with tumor pathogenesis, as well as radioresistance and chemotherapy resistance [7]. Both miR-10b and miR-21 are shown to be associated with clinical and pathologic features such as tumor size [11], disease stage, and metastatic lymph nodes in extracranial tumors [12].

Inhibition of tumor growth and glioblastoma cell proliferation has been demonstrated in *in vitro* and *in vivo* experiments in gliomas [13] and glioblastoma [14] by knocking down microRNAs, such as miR-10b [15] and miR-21 [13, 14]. However, the level of their expression in body fluids and the possible association with glioblastoma features, such as tumor size and tumor location, remains less clear.

Our study aimed to investigate whether there is an association between the expression levels of miR-10b and miR-21 and the tumor

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Correspondence to:
Marina NIKITOVIĆ
Pasterova 14
11000 Belgrade, Serbia
marina.nikitovic@ncrc.ac.rs

size and its localization in patients diagnosed with glioblastoma.

METHODS

This prospective cohort study followed forty-three glioblastoma patients who had undergone surgery and were about to start treatment with Stupp regimen [16]. The research focused on the levels of two specific miRNAs, miR-10b and miR-21, extracted from the patients' peripheral blood mononuclear cells (PBMCs). These patients were treated at the Clinic of Neurosurgery and the Neuro-Oncology Department at the University Clinical Center of Serbia, as well as the Institute for Oncology and Radiology of Serbia, starting in October 2017.

The study gathered clinical parameters, focusing on tumor size (< 4 cm and ≥ 4 cm) and tumor location (frontal lobe, temporal lobe, parietal lobe, occipital lobe, thalamus, or multilobar). Clinical data on tumor size and location were obtained from medical records, specifically magnetic resonance imaging (MRI).

PBMCs were isolated from heparinized whole blood through centrifugation at 4°C using Histopaque-1077, following the manufacturer's instructions. miRNA molecules were extracted and purified from PBMCs using TRI Reagent, as per the manufacturer's protocol. The RNA samples were quantified using a BioSpec-nano spectrophotometer (Shimadzu Corporation, Kyoto, Japan), with samples having an A260/280 ratio between 1.7 and 2.1 considered suitable for further analysis. To analyze miR-10b and miR-21, specific TaqMan® MicroRNA assays and the TaqMan® MicroRNA Reverse Transcription Kit were used. Starting with 10 ng of total RNA for reverse transcription, the cDNA was then amplified using TaqMan™ Universal Master Mix II on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Relative quantities (RQ) were calculated using the comparative delta-delta Ct method, normalizing all samples to the endogenous control RNU6B and calibrating to the sample with the lowest RQ value.

Statistical analysis

To compare differences between two separate groups, we utilized the Mann-Whitney U test. For analyzing the correlation between miRNA expression levels and clinical parameters, we employed both Pearson and Spearman tests. The Kruskal Wallis test was used for comparing three groups. The Log-Rank (Mantel–Cox) test was used to determine the significance of differences. All statistical analyses were performed using IBM SPSS Statistics, Version 22.0 (IBM Corp., Armonk, NY, USA).

Ethics

All participants gave their informed consent, and the study complied with the ethical guidelines of the Declaration of Helsinki. The Ethical Research Committee at the Faculty of Medicine, University of Belgrade, reviewed and approved the study protocol under the reference number 1322/X-39.

RESULTS

In this study, we examined the correlation between the expression levels of miR-10b and miR-21 and the data on tumor size and tumor location in 43 patients with glioblastoma, using 43 samples for each microRNA molecule.

Most patients had a tumor located in the temporal lobe (34.9%), while both multifocal and thalamic tumor were present in 2.3% of the patients. Tumor ≥ 4 cm was observed in 62.8% of the patients. The clinical features are presented in Table 1.

Table 1. Data on tumor location and tumor size of patients with glioblastoma

Tumor location	No (%)
Frontal lobe	14 (32.6%)
Temporal lobe	15 (34.9%)
Parietal lobe	10 (23.3%)
Occipital lobe	2 (4.7%)
Multifocal tumor	1 (2.3%)
Thalamic tumor	1 (2.3%)
Tumor size	No (%)
< 4 cm	16 (37.2%)
≥ 4 cm	27 (62.8%)

The association between miR-10b and miR-21 expression levels and tumor size (< 4 cm and ≥ 4 cm) was investigated. The median expression level of miR-10b in patients with tumors smaller than 4 cm was 214.86 (range: 2.13–816.89), while in patients with tumors ≥ 4 cm, the median expression level was 92.99 (range: 19.24–491.82). For miR-10b, a statistically significant difference was found for tumors smaller than 4 cm ($p = 0.027$). The median expression level of miR-21 in patients with tumors smaller than 4 cm was 81.69 (range: 11.39–825.43), whereas in patients with tumors ≥ 4 cm, the median expression level was 40.84 (range: 2.68–278.98). A statistically significant difference was also found for miR-21 in tumors smaller than 4 cm ($p = 0.047$) compared to tumors ≥ 4 cm.

Due to the unfavorable ratio of the number of different tumor localizations (frontal lobe – 14 patients, temporal lobe – 15 patients, parietal lobe – 10 patients, occipital lobe – two patients, multilobar tumor – one patient, thalamus – one patient) and potential predictors, it was not possible to make a comparison for each localization separately. Instead, according to data from the literature, three groups were created to compare tumor localization with miR-10b and miR-21 expression levels: tumors in the frontal lobe (frontal), tumors in the temporal lobe (temporal), and tumors in other lobes (Figures 1 and 2). The results showed that there was no statistically significant difference in the expression levels of miR-10b ($p = 0.675$) and miR-21 ($p = 0.183$) in relation to tumor localization (Table 2) (Figures 1 and 2).

Table 2. MicroRNA-10b and microRNA-21 expression levels and tumor localization

MicroRNA	Median (range)	p-value
microRNA-10b		
Frontal lobe	153.23 (2.13–622.53)	0.675
Temporal lobe	92.99 (41.18–816.89)	
Other lobes	102.18 (30.09–465.62)	
microRNA-21		
Frontal lobe	81.69 (11.42–825.43)	0.183
Temporal lobe	53.48 (2.68–706.23)	
Other lobes	35.70 (5.82–278.98)	

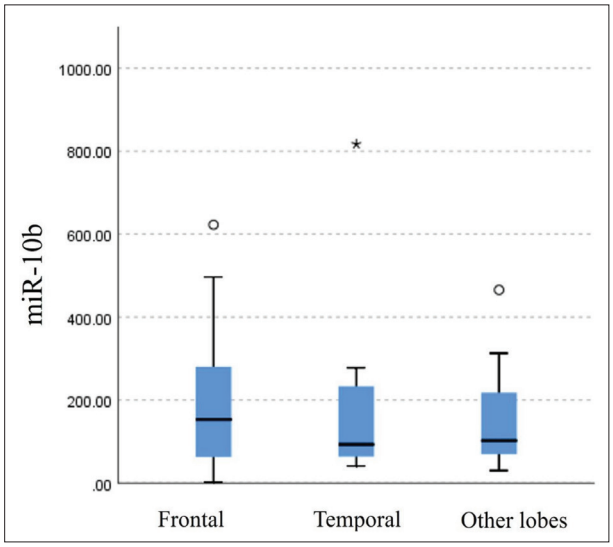

Figure 1. Comparison of median expression levels of microRNA-10b in the frontal, temporal, and other lobes where glioblastoma is diagnosed

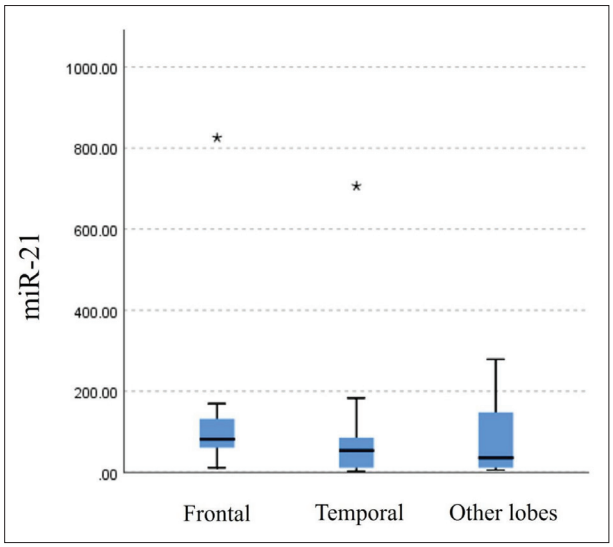

Figure 2. Comparison of median expression levels of microRNA-21 in the frontal, temporal, and other lobes where glioblastoma is diagnosed

DISCUSSION

Our study aimed to determine whether there is a link between the expression levels of miR-10b and miR-21 and clinicopathological factors such as tumor size and localization.

Scientific studies on glioblastoma often classify tumors into two categories based on size: those measuring < 4 cm and those measuring ≥ 4 cm. However, the rationale for selecting this specific size as a benchmark is not clearly established. The debate on this matter frequently involves questions about whether 4 cm represents the size at which tumors are most commonly diagnosed – namely, when patients exhibit symptoms prompting diagnostic procedures – or whether this threshold impacts the extent of surgical resection, the success of adjuvant therapies, and the overall prognosis of the disease. Upon review of the literature and the data presented, we decided to utilize a tumor size of 4 cm as the reference value in this study. Statistical significance was found only for the expression levels of miR-10b and miR-21 in tumors smaller than 4 cm, which was unexpected. We had formed a hypothesis that the expression levels of miR-10b and miR-21 would be statistically significant in tumors larger than 4 cm.

MiR-21 is recognized as one of the most potent oncogenes, playing a pivotal role in carcinogenesis, metastatic potential, and disease relapse [17]. This observation led us to hypothesize that larger tumors at the time of disease presentation may exhibit higher expression levels of miR-21. Supporting this hypothesis, we noted that overexpression of miR-21 was identified in breast tumor tissue, and using a miR-21 inhibitor, known as antimiR-21, inhibited tumor cell growth both *in vitro* and *in vivo* [18]. Furthermore, miR-21 is also one of the most upregulated microRNAs in glioblastoma, and studies demonstrated that knocking down miR-21 in glioblastoma cells resulted in reduced cell growth [19]. On the other hand, miR-10b is regarded as a highly oncogenic microRNA, with its overexpression observed in glioblastoma, influencing tumorigenesis, or gliomagenesis [20]. Ji et al. [21] investigated the association between miR-10b expression levels and prognosis in patients with glioma. They found that there is a higher expression level of miR-10b in glioma patients compared to normal brain parenchyma. Also, upregulation of miR-10b in glioma was correlated with higher glioma grade and larger tumor size [21]. Regarding glioblastoma size and microRNA expression, Siegal et al. [22] reported that in the group of patients with glioblastoma who were treated with bevacizumab, there was a significant negative correlation between miR-10b and miR-21 levels and changes in tumor diameters. The authors revealed that they used serum for the determination of expression levels of specific microRNAs. Moderately analogous to the previously mentioned study, in our study, we investigated microRNA expression levels from patients' plasma and measured tumor size by MRI, as well.

Glioblastoma is an infiltrative tumor, and glioma stem cells could be in the area of the cavity or in the remaining tumor after partial resection, which could be responsible for different microRNA expression levels. Nevertheless, for accurate interpretation of microRNA expression levels and their association with tumor size, it could be essential to measure microRNA levels before any surgical treatment and compare them not only to the MRI size of the tumor, but to the actual size of the tumor after surgery. In our

study, we measured these levels after surgery and before the start of radiotherapy with temozolomide, which could affect the results. However, when it comes to glioblastomas, which are known for their heterogeneous pathological features, including varying foci and sizes of necrosis, smaller tumors might show less pronounced necrotic areas. This means that the microenvironment of glioblastoma and the surrounding tissue, as well as intracellular and intercellular communication, might not be completely disrupted. That might imply that even smaller tumors could show microRNA overexpression and exhibit a higher proliferative capacity. In most cases, for extracranial tumors, the proportion of tumor necrosis is often associated with tumor size [23]. Regarding glioblastoma, the interpretation of the connection between tumor size and tumor necrosis size may be difficult. Some studies reveal that even a small glioblastoma can have various extents of necrosis, and conversely [24]. Moreover, due to the complexity of glioblastoma microenvironment in terms of the possibility of glioblastoma and glioblastoma stem cells reprogramming their microenvironment [25], different microenvironments may affect microRNA expression, especially after surgery. When interpreting microRNA expression levels, it's important to consider how microRNAs are released, the ability of cells to release them, and what tissue is used for the determination of their expression levels. Although the exact mechanism of microRNA release is not fully understood, data suggests that microRNAs are released from both apoptotic bodies and viable tumor cells. Intercellular communication involving microRNAs occurs through exosomes, extracellular vesicles, and other pathways [26]. It also should be borne in mind that in the human brain, the only cells that could express miR-10b are human brain microvascular endothelial cells [27], which could affect the results as well.

Bearing in mind data from the literature on the different expressions of specific microRNAs in the corresponding parts of the brain, we investigated whether there is a difference between the expression level of miR-10b and miR-21 in relation to the localization of the tumor. However, we did not find statistical significance in the expression level of miR-10b and miR-21 between tumors in the frontal lobe, temporal lobe, and tumors in other localizations. To the best of our knowledge, there is not a study that compares the expression level of miR-10b and miR-21 in relation to the localization of glioblastoma in different brain lobes. However, we found another research on a similar topic. Among other results, Ozdogan et al. [28] did not find a significant correlation between the expression level of miR-221 and glioma localization in the brain.

We considered that the main limitations of our study were (1) collecting samples after surgery and (2) tumor size measurements that were noted only in the MRI. On the other hand, these limitations could also lead to more research on the different dynamic changes in microRNA profiles after surgery and the possible influence of the microenvironment or treatment on tumor size and microRNA expression profiles, respectively.

Given that PBMCs are a minimally invasive and easily accessible source of microRNAs, we isolated miRNAs from patients' PBMCs with glioblastoma in our study. Nevertheless, some studies acknowledge that miRNA values may differ between PBMCs and whole blood [29]. The results of our study would be even more precise if the miRNA values isolated from PBMCs were compared with those from whole blood or even tumor tissue.

Results of this paper are part of the doctoral dissertation of the second author of the paper, and are part of the translational research from the radiobiology team of our institute [30].

CONCLUSION

Tumors smaller than 4 cm have statistically significantly higher expression levels of miR-10b and miR-21 compared to glioblastomas equal to or larger than 4 cm. Although this result is unexpected, it could mean that microRNA expression levels dynamically change after surgery and with the altered microenvironment. There was no statistical significance in the expression levels of miR-10b and miR-21 between tumors in the frontal lobe, temporal lobe, and tumors in other localizations.

Considering all the functions microRNAs possess in normal cells and tumor cells, further research on glioblastoma microRNA profiles is needed. Elucidation of the mechanisms of gliomagenesis and tumor growth in relation to the expression profile of specific microRNAs is important in the future for developing potential diagnostic methods using liquid biopsies or new therapeutic strategies.

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Only part of the results was presented in poster form at the 1st Net4Brain Annual Meeting: Closing the translational gap in brain cancer treatment, Ljubljana, Slovenia, 4th–6th September 2024, under the title "Correlation of microRNAs-10b/21 expression levels and tumor size in patients with glioblastoma."

Conflict of interest: None declared.

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Повезаност онкогених микроРНК-10б и микроРНК-21 са величином и локализацијом глиобластома

Марина Никитовић[1,2], Александар Степановић[1,2], Татјана Арсенијевић[1,2], Нина Петровић[3,4]

[1]Универзитет у Београду, Медицински факултет, Београд, Србија;
[2]Институт за онкологију и радиологију Србије, Клиника за радијациону онкологију, Београд, Србија;
[3]Институт за онкологију и радиологију Србије, Одељење за експерименталну онкологију, Београд, Србија;
[4]Универзитет у Београду, Институт за нуклеарне науке „Винча", Београд, Србија

САЖЕТАК

Увод/Циљ Код глиобластома често се уочава појачана експресија онкогених микроРНК-10б и микроРНК-21. Циљ наше студије је био да истражи да ли постоји повезаност између нивоа експресије микроРНК-10б/21 и величине и локализације глиобластома.

Методе У истраживању су учествовала 43 болесника са дијагнозом глиобластома. Анализирали смо нивое експресије микроРНК-10б/21 након оперативног лечења. Подаци о величини и локализацији глиобластома добијени на основу налаза магнетне резонанце.

Резултати Медијана експресије микроРНК-10б код болесника са туморима < 4 *cm* износила је 214,86 (опсег: 2,13–816,89), док је код болесника са туморима ≥ 4 *cm* била 92,99 (опсег: 19,24–491,82). Медијана експресије микроРНК-21 код болесника са туморима < 4 *cm* била је 81,69 (опсег: 11,39–825,43), док је код болесника са туморима ≥ 4 *cm* била 40,84 (опсег: 2,68–278,98). За микроРНК-10б и за микроРНК-21 нађена је статистички значајна разлика за туморе < 4 *cm* ($p = 0,027$, односно $p = 0,047$) у поређењу са туморима ≥ 4 *cm*. Није било статистички значајне разлике у нивоима експресије микроРНК-10б ($p = 0,675$) и микроРНК-21 ($p = 0,183$) у односу на локализацију тумора.

Закључак Глиобластоми мањи од 4 *cm* имају статистички значајно веће нивое експресије микроРНК-10б и микроРНК-21 у поређењу са глиобластомима једнаким или већим од 4 *cm*. Иако је овај резултат неочекиван, он може упућивати на то да се нивои експресије микро РНК динамички мењају након операције и у складу са измењеним микроокружењем глиобластома.

Кључне речи: глиобластом; микроРНК; величина тумора

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Effect of auricular point acupressure combined with three-step analgesic therapy on cancer pain

Xiaomei Huang[1], Hanxue Cai[1], Wen Zeng[2], Xueqin Zeng[1], Xinhong Xiao[1], Yi Liu[1], Yanhong Xu[1], Xiaomei Liu[2], Qinghua Zhong[2]

[1]Ganzhou Cancer Hospital, Department of Nursing, Ganzhou, China;
[2]Ganzhou Cancer Hospital, Department of Head and Neck Surgery, Ganzhou, China

SUMMARY

Introduction/Objective The objective was to evaluate the effect of auricular point pressing with beans combined with three-step analgesic therapy on cancer pain.

Methods Sixty patients with cancer admitted to the Ganzhou Cancer Hospital from January to December 2021 were selected and randomly divided into experimental and control groups. The control group received three-step pain relief and routine care, while the experimental group was treated with auricular point acupressure combined with three-step analgesic therapy. The pain intensity was assessed by a numerical rating scale (NRS) at 0, 24, 48, and 72 h after treatment, and the incidence of adverse reactions was recorded.

Results The NRS score of the experimental group was lower than that of the control group ($F_{treatment} = 105.521$, $p = 0.001$). The difference in NRS scores before and 24, 48, and 72 h after treatment was statistically significant ($F_{time} = 335.521$, $p = 0.001$). The number of eruption pain cases in the experimental group and the control group was found to be statistically significant ($\chi^2 = 10.767$, $p = 0.001$), and the occurrence of eruption pain in the control group was more severe than that in the experimental group ($Z = -4.472$, $p = 0.001$). The incidence of adverse reactions in the experimental and control groups was 3.33% and 30%, respectively, and the difference was statistically significant ($\chi^2 = 12.738$, $p = 0.001$).

Conclusion The combination of auricular point pressing and three-step ladder analgesic therapy can significantly improve the pain of cancer patients.

Keywords: cancer pain; pain relief; auricular point pressing; three-step analgesic therapy

INTRODUCTION

Cancer pain is pain caused by cancer, cancer-related lesions, and anticancer treatment; it can occur at all tumor stages [1]. According to statistics, half of the 10 million new patients with cancer worldwide each year will experience painful reactions. Also, up to 70% of patients in advanced stages will experience painful symptoms [2, 3]. As one of the most common symptoms in these patients with cancer, pain leads to various physical and psychological problems, seriously affecting the quality of life [4]. Currently, the treatment of cancer pain is based on the 'three-step analgesic therapy' (TSAT) recommended by the World Health Organization, in which strong opioids are the main drugs used to treat moderate to severe cancer pain. Although the pain can be controlled to a certain extent, analgesics can produce obvious adverse reactions, drug resistance, or addiction that can sometimes necessitate discontinuation or adjustment of the original therapy [5, 6].

It has been shown that the incidence of constipation in patients receiving oral opioids for chronic cancer pain ranges 40–70%, with two-thirds of patients experiencing nausea and vomiting [7]. Therefore, taking appropriate nursing measures to effectively relieve the pain of patients with cancer is the focus of our attention.

Ear acupoint embedding is a traditional Chinese medicine (TCM) technique that stimulates ear acupoints, unblocks meridians, and regulates the qi and blood of the internal organs [8]. As one of the common techniques in TCM, it is easy to operate, inexpensive, noninvasive, and popular with the public [9]. Auricular point acupressure has positive effects in controlling multiple mechanisms of pain [10, 11]. A study demonstrated that integrating auricular point acupressure into nursing care for patients with cancer-related pain led to a 38% reduction in pain, as well as improvements in fatigue and sleep disturbance [10]. Stimulation of ear points may cause a broad spectrum of systemic effects, such as modulation of inflammatory cytokine levels, which may explain pain relief [12]. Stimulating auricular acupoints can regulate the secretion of central neurotransmitters, thereby activating the analgesic system in vivo [13]. In other words, it exerts a pain-relieving effect by activating the downward pain inhibitory pathway and thus inhibiting the upward pain pathway in the brainstem [14]. The effectiveness of auricular point pressing in preventing symptoms caused by opioid drugs remains unclear. However, auricular point acupressure has demonstrated efficacy, leading to a 24% reduction in any type of pain medication usage and a 19% decrease in opioid usage [10].

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Correspondence to:
Qinghua ZHONG
Department of Head and Neck Surgery
Ganzhou Cancer Hospital
19 Huayuan qian Road
Shuidong Town
Zhanggong District
341000 Ganzhou, China
ping2023liu@163.com

Because it is simple, inexpensive, and has no negative side effects, auricular point acupressure can be widely disseminated as an alternative to opioids. Therefore, this study aimed to evaluate the effect of auricular point pressing with beans combined with TSAT on cancer-related pain.

METHODS

Study subjects

Sixty patients who met the criteria as inpatients of the Ganzhou Cancer Hospital from January to December 2021 were selected as study subjects. This study used a simple randomization method. Participants were divided into 30 cases each in a control group and an experimental group, according to the random number table method.

Inclusion criteria: (1) patients who were clinically diagnosed with malignancy and met the diagnosis of cancer pain; (2) patients who were able to cooperate with pain scoring; (3) age ≥ 18 years and ≤ 70 years; (4) patients with a survival period of ≥ 3 months; (5) those with tumor staging system stage III–IV; (6) the study subjects and their families gave their informed consent to participate in this study and signed an informed consent form.

Exclusion criteria: (1) pain not caused by cancer after examination; (2) allergy or other serious adverse reactions to analgesic drugs; (3) gastrointestinal bleeding, pregnancy, acute abdominal conditions, and individuals with severe cardiovascular disease; (4) non-cooperative individuals, such as patients with psychiatric conditions and those with communication difficulties.

Withdrawal criteria: (1) patients with cancer pain who were unwilling or unable to continue to cooperate with the study for various reasons; (2) patients with unexpected death unrelated to their condition.

Study tools

A numerical rating scale (NRS) was used to assess the pain intensity of patients before and after treatment. The NRS is a method of assessing pain by dividing a straight line into 10 equal parts, each with a number of 0 to 10 indicating the degree of pain in increasing order; 0 was classified as no pain and 10 as the most severe pain imaginable. The pain level was classified as follows: 1–3 as mild pain, 4–6 as moderate pain, and 7–10 as severe pain. Breakthrough cancer pain was determined by referring to the diagnostic criteria in the Experts' Consensus on Breakthrough Cancer Pain in 2019 [15], and patients were considered to have had a single episode of explosive pain when their pain score exceeded 4.

Treatment methods

Control group

Patients received TSAT and routine care according to WHO Guidelines [16]. Patients were classified into mild (1–3), moderate (4–6), and severe (7–10) pain according to the pain assessment at the time of admission, and a three-step analgesic principle was adopted. Patients with mild pain were mainly given non-steroidal anti-inflammatory drugs, such as ibuprofen and acetaminophen. Patients with moderate pain were given weak opioids, mainly oral tramadol hydrochloride or oxycodone tablets. Patients with severe pain were given strong opioids, mainly morphine hydrochloride extended-release tablets, and the application of painkillers was increased from weak to strong.

Experimental group

Under the guidance of the TCM physician and on the basis of the TSAT combined with the auricular acupuncture-point bean burial method, a nurse performed auricular acupuncture-point bean burial on the patients. The main acupuncture points were Shenmen and Jiaoshen, and the supporting acupuncture point was the Ashi point, which is the mapping position of the painful area in the ear's acupuncture point. Once the patient was placed in a comfortable position, the nurse held a probe, found the corresponding sensitive point in one ear, and located the acupuncture point; then, they selected the Jiaoshen, Shenmen, and Ashi point, disinfected the skin of the auricle with 75% ethanol and used forceps to uncover Vaccariae Semen seed, which they pasted onto the corresponding acupuncture point. Using the thumb and index finger to pinch and rotate the seed, it was pressed for 5–10 minutes each time so that there was localized soreness and swelling or slight pain. It was pressed 3–5 times a day for three days. If the patient felt increased pain, the number of presses was increased.

Observation indicators

Pain level: Immediately after admission, the responsible nurse conducted pain assessments for all patients, assessing whether the patients were in pain, the nature, location, and intensity of pain and whether pain medication was applied. The nurse in charge assessed and recorded the pain 0, 24, 48, and 72 h after admission.

Patients with breakthrough pain

Breakthrough cancer pain typically refers to a transitory flare of pain in the setting of chronic pain managed with opioid drugs [17]. Pain management was given once according to the location and nature of the pain, and pain medication was reassessed, including oral pain medication for 60 min, intramuscular or subcutaneous pain medication for 30 minutes, and intravenous pain medication for 15 minutes. The number of breakthrough pains 72 hours after admission was recorded using a patient's diary card.

Occurrence of adverse reactions

The occurrence of adverse reactions, including drowsiness, constipation, vomiting, and dizziness, was recorded for both groups. The incidence of adverse reactions = the

number of cases of adverse reactions / total cases × 100%. The occurrence of adverse reactions 72 hours after admission was recorded using a patient's diary card.

Statistical analysis

Data were statistically analyzed using IBM SPSS Statistics, Version 22.0 (IBM Corp., Armonk, NY, USA). The measurement data were expressed as x ± s, and an independent-samples t-test was used to compare the two groups. Repeated-measures data were analyzed using a repeated-measures variance analysis (ANOVA). Count data were expressed as the number of cases and percentages, and a χ^2 test and a rank-sum test were used for comparisons between the groups. All differences were considered statistically significant at $p < 0.05$.

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Ganzhou Cancer Hospital. The ethical number is (2023) kelunshen (No. 8). Written informed consent was obtained from all participants.

RESULTS

General information

There were 30 cases in the control group, of which 12 were male, and 18 were female, aged 54.87 ± 8.5; and 30 cases in the experimental group, of which 14 were male, and 16 were female, with a mean age of 52.53 ± 9.9 years. The differences in age (t = 1.086, p = 0.286), sex (Z = -0.471, p = 0.637), tumor site (Z = -0.000, p = 1.000), tumor staging (Z = -0.805, p = 0.421), and usage of analgesics (Z = -0.753, p = 0.451) between the two groups were not statistically significant and were comparable (see Table 1).

Comparison of pain scores between the two groups of patients 24, 48, and 72 hours after treatment

A one-way repeated-measures ANOVA was used to determine the effect of applying auricular acupuncture bean burial combined with TSAT on the NRS scores of patients with cancer pain after three days. In summary, there was no interaction effect between the treatment and time factors ($F_{interaction} = 2.231$, $p = 0.450$), so the main effect was analyzed directly. Different treatments had different effects on the NRS scores of the patients with cancer pain, and the NRS scores of the experimental group were lower than those of the control group ($F_{treatment} = 105.521$, $p = 0.001$). The differences in NRS scores before treatment and at 24, 48, and 72 h after treatment were statistically significant ($F_{time} = 335.521$, $p = 0.001$). The NRS scores in the experimental group were (4.7 ± 1.7) before treatment and (2 ± 0.26) 24 hours after treatment, with a statistically significant difference of 2.33 (95% CI: 2.15–2.61) points lower than before treatment (t = 5.596, p = 0.000). The NRS score at 48 and 72 hours post-treatment was (1.9 ± 0.3), 2.76 (95% CI: 2.54–2.88) points lower than before treatment and 0.13 (95% CI: 1.86–2.07) points lower than 24 hours post-treatment; both values showed statistically significant differences (t = 1.884, p = 0.045) (see Table 2).

Comparison of the number of pain episodes between the two groups

The results showed that the cases of pain episodes in the experimental group were significantly less than those of the control group ($\chi^2 = 10.767$, $p = 0.001$). Additionally, the number of pain episodes in the control group was significantly more than in the experimental group (Z = -4.472, p = 0.001), indicating that auricular point pressing combined with TSAT could effectively relieve the pain levels of patients with cancer pain (see Table 3).

Table 1. General comparison of patients in both groups (x ± s)

Group		Control group	Experimental group	t/Z value	p-value
Case		30	30		
Age		54.87 ± 8.5	52.53 ± 9.9	t = 1.086	0.286
Sex	Male	12	14	Z = -0.471	0.637
	Female	18	16		
Tumor site	Head and neck	4	4	Z = 0.000	1.000
	Chest	6	10		
	Abdomen	19	11		
	Other	1	5		
Tumor staging	III	9	12	Z = -0.805	0.421
	IV	21	18		
Analgesics	Yes	27	25	Z = -0.753	0.451
	No	3	5		

Table 3. Comparison of breakthrough cancer pain in the two groups (n)

Group	No. of breakthrough pain	Occurrence of breakthrough cancer pain (time)		
		1	2–3	> 4
Control group	3	6	11	10
Experimental group	23	4	2	1
χ^2 value	10.767			
p-value	0.001			
Z-value	-4.472			
p-value	0.001			

Table 2. Comparison of pain scores between the two groups (x ± s)

Group	Pre-treatment	Post-treatment			$F_{interaction}$	p	F_{time}	P	$F_{treatment}$	p
		24 h	48 h	72 h						
Control group	3.6 ± 1.6	2 ± 0.4	2.3 ± 0.8	2.2 ± 0.7	2.231	0.450	335.521	0.001	105.521	0.001
Experimental group	4.7 ± 1.7	2 ± 0.26	1.9 ± 0.3	1.9 ± 0.3						

Table 4. Comparison of the occurrence of adverse reactions during the treatment of cancer pain patients in the two groups

Group	Case	Sleepiness	Constipation	Vomiting	Dizziness
Control group	30	3	3	2	1
Experimental group	30	1	0	0	0
χ^2 value		12.738			
p-value		0.001			

Comparative analysis of the occurrence of adverse reactions in the treatment process between the two groups of patients

The incidence of adverse reactions in the experimental group and the control group was 3.33% and 30%, respectively, and the difference was statistically significant ($\chi^2 = 12.738$, $p = 0.001$) (see Table 4).

DISCUSSION

Cancer pain is one of the main causes of suffering for patients with mid- to late-stage cancer. Most of the pain is caused by the continuous pressure of the tumor on the surrounding organs and nerves, and some of the pain is caused by the adverse effects of cancer treatment [18]. Currently, the use of TSAT for cancer pain relief is a medical consensus at home and abroad. Still, strong opioids are prone to adverse effects, such as dizziness, constipation, nausea and vomiting. Additionally, 50% of patients with cancer pain are not treated [19]. Ear acupuncture is a TCM acupuncture-point therapy that stimulates the ear points corresponding to the internal organs to unblock the meridians, thus balancing yin and yang, regulating the internal organs, activating blood, and relieving pain. It has positive effects in controlling the pain of multiple mechanisms [20, 21]. Auricular point acupressure showed great promise to reverse chronic pain through an inflammatory mechanism, i.e., it exhibits anti-inflammatory efficacy by blocking pro-inflammatory cytokines or releasing anti-inflammatory cytokines or β-endorphins [14]. It was an ideal tool for training healthcare professionals to provide treatments for patients with pain [10].

Therefore, in this study, the patients were given analgesia using the auricular acupuncture method on the basis of TSAT. By comparing the pain intensity of the two groups at different times after treatment, it was found that the NRS scores of the experimental group were lower than those of the control group at different times after intervention. The patients' pain scores were more stable, and the number of cases and times of breakthrough pain were significantly reduced. This indicates that auricular bean burial can effectively relieve patients' pain. The results of this study demonstrate the potential of auricular point acupressure in pain management, which is consistent with previous research findings, which showed that auricular point acupressure was a feasible and effective self-management tool [22].

Our findings are consistent with the findings of Li et al. [23], whose intervention with systematic nursing combined with auricular bean for cancer pain significantly reduces the sense of pain, relieves negative emotions and improved quality of life in patients with advanced gastric cancer.

Auricular point acupressure has certain potential in alleviating medication side effects such as gastrointestinal reactions and fatigue [24]. Research has confirmed that auricular point acupressure regulates digestive system function and can regulate constipation and diarrhea through different acupoint combinations [25]. Auricular point acupressure has an improving effect on the side effects of chemotherapy [26]. This is consistent with the results of this study. In this study, the incidence of adverse reactions in the experimental group was better than in the control group. The reason for this is that the non-pharmacological analgesic method can relieve both pain and the discomfort reactions brought about by oral analgesics, which can enable patients to reduce the number of times they take medication, avoid irregularities in the use of medication, prevent situations where medication fails to relieve pain or the overflow of medication, and effectively control pain [27]. On the basis of TCM theory, auricular point acupressure has the potential to regulate the balance of qi and blood. This regulation enhances physiological function, modulates neurotransmitter production, and decreases nerve sensitivity [28]. Numerous studies have proved that TCM can expedite the secretion of diverse mediators and opioid peptides from peripheral nerves to the central nervous system, collectively constituting an "anti-pain system" [29].

When looking at the changes in pain scores before and after the intervention, it was found that the location of the pain varied from patient to patient, depending on the location of the tumor. Also, the presence of distant metastases had an impact on the cancer pain scores. However, this study did not examine multiple sites of pain. Cancer pain is mostly chronic pain with a long duration of illness, and different levels of pain can affect patients' comfort and influence their prognosis and quality of life [30]. Considering the limitations, such as small sample size, single-center design, and potential observer bias in this study, further research is necessary.

Additionally, due to various limitations, such as human and material resources, this study initially compared only the analgesic effects of auricular acupuncture bean burial combined with TSAT for patients in a tertiary hospital without comparing drug dosage, economics, or drug combinations. As each person's degree of pain perception is different, this has certain limitations in the study and needs to be further explored. Hence, further research is needed in future studies to provide better ideas for clinical work.

CONCLUSION

The combination of TSAT and auricular point acupressure in cancer pain can effectively reduce patients' pain and reduce the occurrence of adverse reactions, such as nausea, vomiting, and constipation, caused by opioids. Thus, it improves the quality of life of patients with cancer pain and is worth promoting in subsequent clinical care.

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Ефекат комбинације акупресуре аурикуларних тачака и тростепене аналгетске терапије на бол код оболелих од карцинома

Сјаомеј Хуанг[1], Ханшуе Цај[1], Вен Ценг[2], Сјуецин Ценг[1], Сјинхонг Сјао[1], Ји Љу[1], Јанхонг Сју[1], Сјаомеј Љу[2], Ћингхуа Џонг[2]

[1]Болница за лечење рака у Ганџоуу, Одељење за негу, Ганџоу, Кина;
[2]Болница за лечење рака у Ганџоуу, Одељење за хирургију главе и врата, Ганџоу, Кина

САЖЕТАК

Увод/Циљ Циљ овог истраживања је био да се процени ефекат акупресуре аурикуларних тачака уз примену зрна, у комбинацији са тростепеном аналгетском терапијом, на бол код болесника оболелих од карцинома.

Методе Шездесет болесника са карциномом, примљених у Болницу за лечење рака у Ганџоуу од јануара до децембра 2021. године, насумично је распоређено у експерименталну и контролну групу. Контролна група добијала је тростепену аналгезију и уобичајену негу, док је експериментална група лечена акупресуром аурикуларних тачака у комбинацији са тростепеном аналгетском терапијом. Интензитет бола процењиван је нумеричком скалом за оцену бола (*NRS*) 0, 24, 48 и 72 сата након почетка лечења, а бележена је и учесталост нежељених реакција.

Резултати *NRS* скор у експерименталној групи био је нижи него у контролној групи ($F_{treatment}$ = 105,521; p = 0,001). Разлика у *NRS* скору пре и 24, 48 и 72 сата након третмана била је статистички значајна (F_{time} = 335,521; p = 0,001). Број случајева тзв. еруптираног бола (енг. *eruption pain*) био је статистички значајно различит између експерименталне и контролне групе (χ^2 = 10,767; p = 0,001), а појава еруптираног бола била је већа у контролној групи него у експерименталној (Z = –4,472; p = 0,001). Учесталост нежељених реакција износила је 3,33% у експерименталној групи и 30% у контролној групи, што представља статистички значајну разлику (χ^2 = 12,738; p = 0,001).

Закључак Комбинација акупресуре аурикуларних тачака и тростепене аналгетске терапије може значајно побољшати контролу бола код болесника са карциномом.

Кључне речи: карциномски бол; ублажавање бола; акупресура аурикуларних тачака; тростепена аналгетска терапија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Familial adenomatous polyposis and colorectal cancer – how sensitive is computed tomography in detecting the underlying disease?

Sofija Jovanović[1], Tijana Tomić[1], Nemanja Bidžić[2,3], Miloš Raspopović[4], Dragan Vasin[1,5]

[1]University Clinical Centre of Serbia, Center for Radiology and Magnetic Resonance Imaging, Belgrade, Serbia;
[2]University Clinical Centre of Serbia, Clinic for Digestive Surgery, Department for HPB Surgery, Belgrade, Serbia;
[3]University of Belgrade, Faculty of Medicine, Department for Surgery, Belgrade, Serbia;
[4]University of Clinical Center of Serbia, Clinic for Emergency Surgery, Belgrade, Serbia;
[5]University of Belgrade, Department for Radiology, Faculty of Medicine, Belgrade, Serbia

SUMMARY

Introduction Familial adenomatous polyposis (FAP) is an autosomal dominant disorder characterized by the presence of 100 or more adenomatous polyps in the mucosal lining of the large intestine, with a significant risk of colorectal cancer development.

Case outline This article presents a case report of previously undiagnosed FAP in a patient admitted to the surgical emergency department with suspected sigmoid carcinoma. On computed tomography (CT), the findings of the colonic mucosa were inconclusive due to inadequate distension of the bowel lumen and insufficient preparation. Edema of the bowel wall was clearly observed, a CT characteristic of the carcinoma that had formed at the level of the sigmoid colon, while the two other foci of malignant transformation were obscured by a diffuse, uniform thickening of the wall, which was clearly diagnosed as FAP on subsequent colonoscopy. The patient underwent a total proctocolectomy, after which he continued his oncological treatment.

Conclusion Computed tomography is inadequate for the diagnosis of diffuse polyposis of the colonic mucosa, especially in emergency situations when patients are not prepared for the examination, i.e., without sufficient dilation of the bowel lumen. Since the underlying disease in this patient masked two of the three malignant lesions of the colon, we point out the diagnostic inferiority of the CT examination in the regular emergency settings, compared to CT and MR colonography and especially to colonoscopy as the gold standard in the detection of colorectal cancer.

Keywords: familial polyposis; colorectal cancer; computed tomography; total proctocolectomy

INTRODUCTION

Familial adenomatous polyposis (FAP) is characterized by the presence of 100 or more adenomatous polyps in the mucosa of the colon, predisposing it to malignant transformation and contributing significantly to the cumulative risk of developing colorectal cancer (CRC) in patients diagnosed with this disease [1]. FAP can have different inheritance patterns. In most cases, it is an autosomal dominant disease, i.e., one copy of the mutated adenomatous polyposis coli (APC) tumor suppressor gene is sufficient to cause the disease [2]. This means that the affected individuals have a parent who is also affected by the disease. However, if FAP is caused by mutations in the MUTYH-associated polyposis (MAP) gene, it is inherited in an autosomal recessive manner [3]. Both copies of the gene must be mutated. In most cases, the parents of a person with an autosomal recessive disease each carry one copy of the mutated gene but do not show any symptoms of the disease themselves. Therefore, genetic testing is considered the gold standard for FAP diagnosis [4]. Once diagnosed, given the overall risk of CRC from a multifocal adenoma – carcinoma sequence, which often takes years, prophylactic colorectal surgery is the treatment of choice, followed by lifelong endoscopic screening [5]

CASE REPORT

A 42-year-old man presented to the emergency room with left flank pain, nausea, and diarrhea. Anamnestically, he reported blood in his stool and estimated that 12 hours had passed since symptom onset. Over the previous year, he had experienced similar episodes of pain and diarrhea, excluding the blood in stool. His medical history was otherwise unremarkable, whereas his family history included his mother's death from CRC at age 35. Upon physical examination, the patient had left-sided abdominal tenderness on palpation.

Abdominal ultrasonography (US) showed a discretely thickened bowel wall of the descending colon, no loop dilation, and hyperechoic left paracolic fat with scarce extraluminal fluid.

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Correspondence to:
Dragan VASIN
Pasterova 2
11000 Belgrade, Serbia
draganvasin@gmail.com

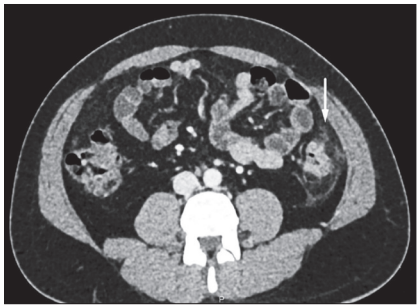

Figure 1. A contrast-enhanced axial CT scan showing bowel wall thickening in the descending colon (white arrow) with a "fat-stranding" pattern regionally

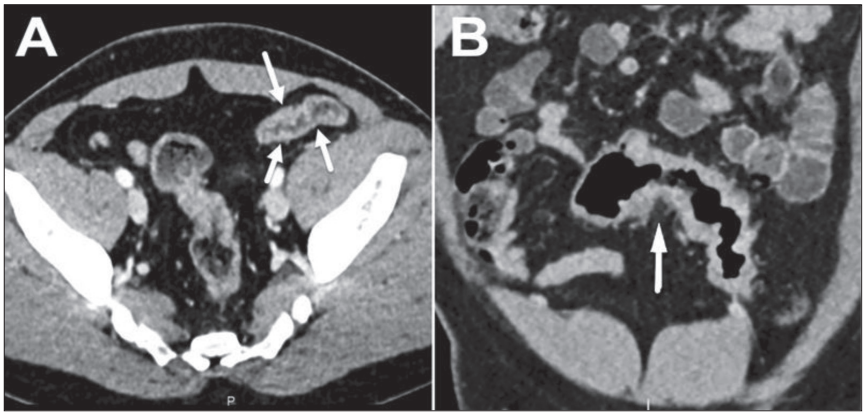

Figure 2. Contrast-enhanced axial (A) and coronal (B) CT scans showing the segmental (white arrows) thickening of the sigmoid colon wall highly suspicious of a neo-infiltrative process, but without clear visualization of multiple polyps on the mucosa

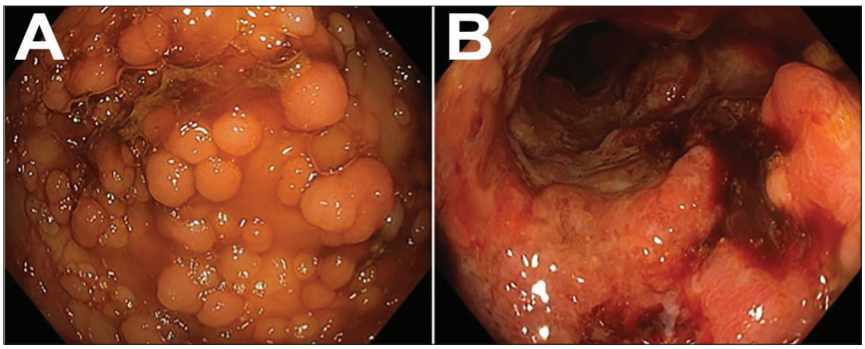

Figure 3. Colonoscopy findings in the transverse (A) and the sigmoid colon (B), showing a multitude of subcentimetric polyps with no healthy remaining mucosa and the previously described ulcero-infiltrative lesion, respectively

The US alone was inconclusive. However, that finding, coupled with the leading symptoms at the gastroenterological examination and the complete blood count and laboratory results that yielded leukocytosis measuring 12.8×10^9/L and hemoglobin 102 g/L warranted a computed tomography (CT) exam.

A subsequent CT scan revealed diffuse, irregular thickening of the colonic wall, with a lobulated luminal contour and discrete paracolic stranding of the fat plane (Figure 1). Along the wall of the colon, discrete intraluminal nodulations of the mucosa could be observed, which could not be adequately interpreted due to the inadequate preparation of the colon for examination (Figure 2A).

Distally, in the sigmoid colon, the CT also showed a focal, circular wall thickening, which is incompatible with an inflammatory etiology and most likely corresponds to a malignant change (Figure 2B). The patient was referred, and a colonoscopy was performed to gain further insight into the condition of the suspicious mucosa. It revealed hundreds of polyps throughout the colon with mild inflammation of the mucosa and no mucosal preservation, except for a small segment in the cecum. Most of the polypus lesions were 10 mm in size, whereas 10–20 of them were 20–25 mm in diameter (Figure 3A). In addition, on examination, an ulcero-infiltrative lesion was noted in the sigmoid colon, approximately 30 cm from the anocutaneous line, involving the entire circumference of the colon, extending proximally for approximately 10 cm, with relative luminal stenosis but allowing passage of the endoscope. The lesion bled during the biopsy (Figure 3B).

The histopathologic findings of the biopsied sigmoid mucosa confirmed a malignant transformation consistent with an advanced adenocarcinoma, which was a sufficient indication for surgical treatment. Consequently, a total proctocolectomy with ileo-anal anastomosis and bipolar ileostomy was performed. The macroscopic and microscopic specimens of surgical pathology are shown and described below (Figure 4).

The authors confirm that they have read the journal's position on issues involving ethical publication and affirm that this work is consistent with those guidelines. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written consent to publish all shown material was obtained from the patient.

DISCUSSION

FAP is characterized by the presence of 100 or more adenomatous polyps in the mucosa of the colon, predisposing it to malignant transformation and contributing significantly to the cumulative risk of developing CRC in patients diagnosed with this disease [1]. FAP can have different inheritance patterns. In most cases, it is an autosomal dominant disease, meaning that one copy of the mutated APC tumor suppressor gene in each cell is sufficient to cause the disease [2]. This means that affected individuals have a parent who also suffers from the disease. However, if FAP is caused by mutations in the MAP gene, it is inherited in an autosomal recessive manner [3]. Both copies of the gene must be mutated in each cell. In most cases, the parents

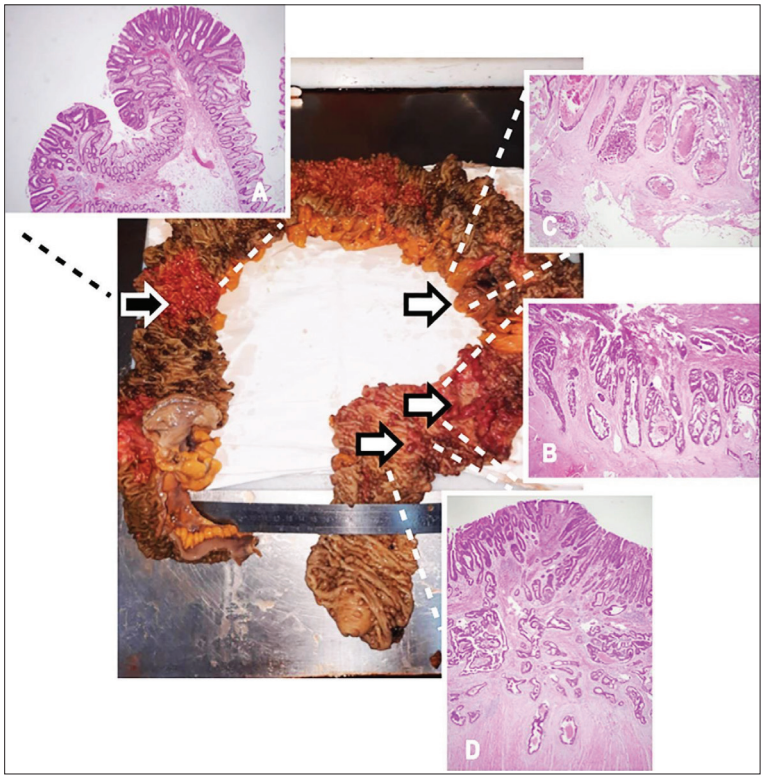

Figure 4. The intestinal resection specimen included a 112-cm-long colon, an 18-cm-long portion of the terminal ileum, and a 55-mm-long appendix; innumerable polyps were present throughout the entirety of the colorectal segment, from the Bauhin's valve all the way to 2–3 mm proximal to the lower resection line, with a dispersed distribution; most of them were sessile and semi-sessile, 2–10 mm in diameter, while a handful were pedunculated, polypoid, exceeding that size (black arrow); a photomicrograph of one such polyp is shown above (A); macroscopically, the resection specimen showed three well-demarcated tumors, two of which in the sigmoid and one in the descending colon (white arrows); the CT-suspected lesion was ulcero-vegetative, affecting the whole circumference of the sigmoid, with the infiltration depth of 10 mm and with a mixed tubular/tubulo-cribriform organization and expansive-infiltrative growth with signs of extramural invasion, microscopically (B); another tumor, smaller in size, was located proximally in the descending colon, also presenting as an ulcerative vegetative lesion, which demonstrated an infiltration depth of 10 mm (C); distal to the former the ulcerative-vegetative tumor of the sigmoid colon, a larger sessile polyp was observed within the same region and biopsied; the polyp showed suspicious infiltration into the submucosa and muscle layer on macroscopic cross-sections, a finding that was subsequently confirmed through histopathological examination as CRC (D); microscopically, both tumors (C and D) were mixed tubular/tubulo-cribriform adenocarcinomas, the first of which showed signs of extramural invasion (C), whereas the second one was intramural growth only (D)

of a person with an autosomal recessive disease each carry one copy of the mutated gene but do not show any symptoms of the disease themselves. Therefore, genetic testing is considered the gold standard for FAP diagnosis [4].

The family members of the proband are invited for screening before symptoms appear. The National Comprehensive Cancer Network guidelinec for screening for APC gene mutations recommends testing individuals at the age of 10 years and starting screening endoscopies at the age of 10–15 years [5]. They undergo prophylactic surgery as a preventive measure against cancer. The risk of developing cancer before the age of 20 years is very low, at only 1% of all FAP patients [6]. It is currently recommended that prophylactic surgery be performed at the age of 20 or 25 [7].

In an emergency situation where no genetic testing has been performed and where there is no information about possible FAP prior to the onset of symptoms, and even when the family history is positive for CRC at a young age, it is not surprising that the CT exam did not reveal diffuse mucosal polyposis, probably due to inadequate expansion of the bowel lumen and the presence of residual contents. However, as the underlying genetic disease successfully masked two of the three CRC lesions, we want to emphasize that a CT scan is inferior to MRI and CT colonography in terms of sensitivity when it comes to detecting CRC [8].

Conflict of interest: None declared.

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Породична аденоматозна полипоза и колоректални карцином – колико је компјутеризована томографија осетљива метода за откривање основне болести?

Софија Јовановић[1], Тијана Томић[1], Немања Бицић[2,3], Милош Распоповић[4], Драган Васин[1,5]

[1]Универзитетски клинички центар Србије, Центар за радиологију и магнетну резонанцу, Београд, Србија;
[2]Универзитетски клинички центар Србије, Клиника за дигестивну хирургију, Одељење за хепатобилиопанкреатичну хирургију, Београд, Србија;
[3]Универзитет у Београду, Медицински факултет, Катедра за хирургију, Београд, Србија;
[4]Универзитетски клинички центар Србије, Клиника за ургентну хирургију, Београд, Србија;
[5]Универзитет у Београду, Медицински факултет, Катедра за радиологију, Београд, Србија

САЖЕТАК

Увод Породична аденоматозна полипоза је аутозомно доминантно обољење које карактерише присуство 100 или више аденоматозних полипа на слузокожи дебелог црева, што представља предиспозицију за развој колоректалног карцинома.

Приказ болесника Овај рад представља приказ случаја претходно недијагностиковане породичне аденоматозне полипозе код болесника примљеног на одељење ургентне хирургије са сумњом на карцином сигмоидног колона. Налаз добијен компјутеризованом томографијом на мукози дебелог црева био је неконклузиван услед недовољне дистензије лумена црева и неадекватне припреме. Јасно је уочен едем зида, налаз на компјутеризованој томографији карактеристичан за формирани карцином у пределу сигмоидног колона, док су остала два фокуса малигне алтерације била маскирана дифузним, униформним задебљањем зида, које је каснијим колоноскопским прегледом јасно дијагностиковано као породична аденоматозна полипоза. Болеснику је урађена тотална проктоколектомија, након чега је наставио онколошко лечење.

Закључак Компјутеризована томографија је недовољно осетљива метода за дијагностику дифузне полипозе слузокоже колона, нарочито код ургентних стања када болесници нису припремљени за преглед, односно када није обезбеђена довољна дистензија лумена цревних вијуга. Како је основно обољење маскирало две од три лезије колоректалног карцинома на дебелом цреву код овог болесника, указујемо и на дијагностичку инфериорност компјутеризоване томографије у поређењу са колонографијом помоћу компјутеризоване томографије и магнетне резонанце, а нарочито са колоноскопијом као златним стандардом када је у питању откривање колоректалног карцинома.

Кључне речи: породична аденоматозна полипоза; колоректални карцином; компјутеризована томографија; тотална проктоколектомија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Isolated ipsilateral shoulder and elbow dislocation

Bojan Milenković[1], Miljan Bilanović[1], Slađan Timotijević[1], Miroslav Tatić[1], Đorđe Kolak[2,3]

[1]Bežanijska Kosa University Hospital Medical Center, Department of Orthopedics and Traumatology, Belgrade, Serbia;
[2]Kragujevac University Clinical Center, Clinic for Orthopedic Surgery and Traumatology, Kragujevac, Serbia;
[3]University of Kragujevac, Faculty of Medical Sciences, Department of Surgery, Kragujevac, Serbia

SUMMARY

Introduction Joint dislocation is the loss of congruence of the joint surfaces. This is a relatively common injury of the musculoskeletal injury. Among the large joints, shoulder joint dislocation has the highest incidence of 24/100,000/year, of which 94–97% are anterior, 1% are inferior, and the remaining injuries are posterior shoulder dislocations. The second most frequent injury is elbow dislocation, with an incidence of 5.2/100,000/year. Among these, over 80% are posterolateral dislocations.

Case outline A 57-year-old female patient was injured during an accidental ground-level fall sustaining combined injuries – anterior dislocation of the shoulder joint and a posterolateral dislocation of the elbow joint. Upon sustaining the described injuries, she was treated conservatively with good functional results.

Conclusion A review of the literature reveals that the combination of ipsilateral shoulder and elbow joint dislocations is a rare injury, with shoulder dislocation often being overlooked. Proper anamnesis, along with a thorough examination of the joints both proximally and distally to the injured joint, is crucial. If performed adequately, these injuries can often be successfully managed nonoperatively, with closed reduction, immobilization, and rehabilitation, leading to satisfactory functional recovery.

Keywords: shoulder dislocation; elbow dislocation; ipsilateral dislocation; isolated dislocation

INTRODUCTION

Reviewing the available literature, we identified two groups of combined shoulder and elbow dislocations. The first group includes patients with only shoulder and elbow joint dislocations, while the second group consists of patients with other associated injuries. Further differences among the presented cases refer to the type of dislocation (anterior, posterior, or inferior shoulder joint dislocation). Thus, Meena et al. [1] described a 30-year-old man, injured in a traffic accident as a motorcyclist, with ipsilateral dislocation of the shoulder joint and elbow joint, without associated injuries. Other studies describing isolated ipsilateral shoulder and elbow dislocations state that they occur as the result of low-energy trauma. Mandujano and Izaguirre [2] described an 86-year-old woman, injured during a ground-level fall, wherein she sustained an ipsilateral inferior shoulder joint dislocation and a posterior elbow joint dislocation, while some other authors described patients with ipsilateral anterior shoulder joint dislocation and posterior elbow joint dislocation [1, 3–7]. In the descriptions of the non-isolated shoulder and elbow dislocation injuries, associated injuries – open wounds, fractures, and neurovascular damage, are mentioned. Fractures of the greater tubercle of the humerus [8, 9], open wounds in the region of the medial epicondyle of the humerus [10, 11], and injuries of the axillary nerve [3, 9] are most commonly described.

CASE REPORT

A 57-year-old female patient was examined at our clinic complaining of pain in her left elbow and the inability to perform any movement of the elbow. She reported an injury sustained one hour before coming to our clinic, having tripped and fallen over a chair. She denied other injuries or any previous dislocations. The clinical findings showed swelling and deformity of the left elbow with the arm slightly abducted and externally rotated in the shoulder joint, with the elbow joint in flexion and neutral pronosupination. She supported the injured arm with her other hand. The neurovascular findings were normal. Also, the left shoulder joint was deformed, the lateral edge of the acromion was prominent, and the deltoid oval contour was distorted and flattened, the so-called epaulet sign. Encountering such a clinical finding, with the prior consent of the patient, we took photographs, which makes this case the only photo-documented case with such a combination of injuries in the currently available literature (Figure 1). After radiographic diagnostics, the diagnosis of anterior dislocation of the humeroscapular joint and posterolateral dislocation of the elbow joint was established, without any other osteoarticular lesions (Figures 2 and 3). With the excellent compliance of the patient, and with the use of analgosedation, we performed manual closed reduction of both joints. First, a closed reduction of the elbow joint was performed, with the patient positioned supine, by applying the

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Correspondence to:
Bojan MILENKOVIĆ
Bežanijska Kosa University Hospital Medical Center
Department of Orthopedics and Traumatology
11000 Belgrade, Serbia
bojanmilenkovic89@gmail.com

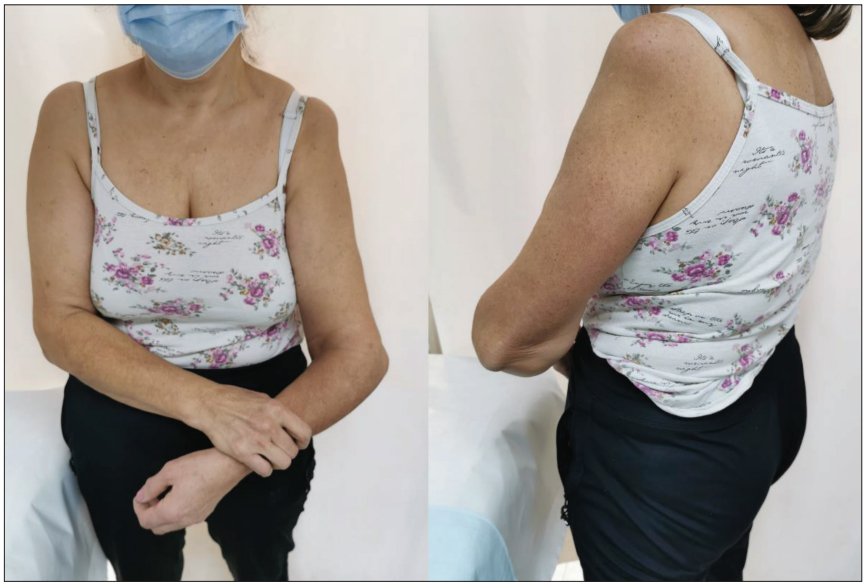

Figure 1. Clinical presentation of ipsilateral dislocation of the shoulder joint and the elbow joint [source: Photographic Archive, Bežanijska Kosa University Hospital Medical Center]

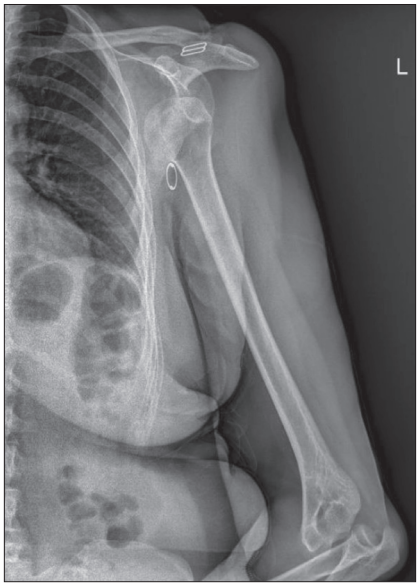

Figure 2. Anterior-posterior X-ray of the left humerus – isolated ipsilateral dislocation of the shoulder and elbow joint [source: Picture Archiving and Communication System (PACS), Bežanijska Kosa University Hospital Medical Center]

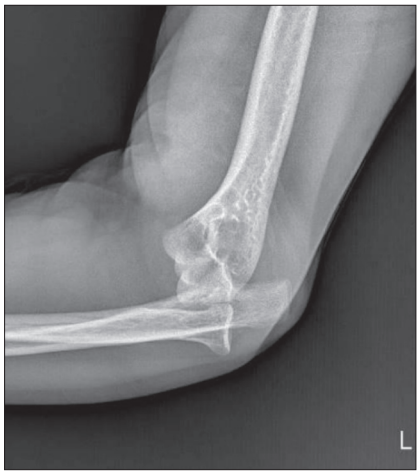

Figure 3. Lateral X-ray of the left elbow – posterolateral elbow joint dislocation [source: PACS, Bežanijska Kosa University Hospital Medical Center]

Figure 4. Clinical presentation after closed reduction of ipsilateral shoulder and elbow dislocations [source: Photographic Archive, Bežanijska Kosa University Hospital Medical Center]

technique of longitudinal traction to the forearm with the elbow in flexion and supination, while pushing the olecranon over the distal end of the humerus. Humeroscapular joint reduction was then performed using Cooper's technique [12]. After the reductions were performed, the radial pulse, motor function, and sensation were normal, and follow-up X-rays showed that both joints were congruent, without iatrogenic injuries in terms of fractures (Figures 4, 5, and 6). The arm was immobilized in a Desault orthosis for three weeks, whereupon the patient began physical therapy and rehabilitation. At the follow-up examination, eight weeks after the injury, the patient had palpable painful tenderness on the inner side of the elbow joint and a positive valgus stress test. Follow-up X-rays showed both joints to be congruent, with minor heterotopic calcifications in the region of the ulnar collateral ligament. The patient was advised to continue physical therapy. The functional outcome 12 weeks after injury was good. The patient was experiencing no pain, she had a full range of motion in the shoulder joint, a full range of pronation–supination motion, as well as flexion in the elbow joint. However, she had a loss of terminal extension of 20 degrees (Figures 7 and 8).

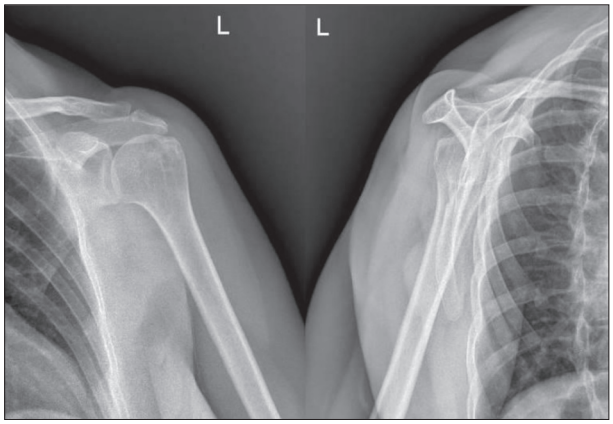

Figure 5. Anterior-posterior X-ray of the left shoulder and Y radiography of the scapula – status after closed reduction, the shoulder joint is congruent [source: PACS, Bežanijska Kosa University Hospital Medical Center]

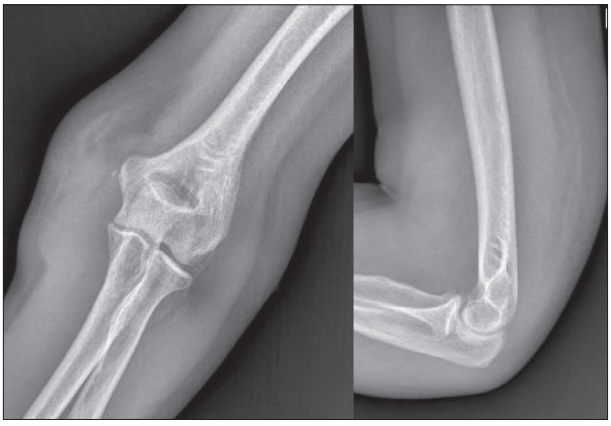

Figure 6. Anterior-posterior and lateral radiography of the left elbow – status after closed reduction, the elbow joint is congruent [source: PACS, Bežanijska Kosa University Hospital Medical Center]

Figure 7. Clinical presentation and range of motion, 12 weeks after injury – pronation and supination [source: Photographic archive, Bežanijska Kosa University Hospital Medical Center]

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written consent to publish all shown material was obtained from the patient.

DISCUSSION

Suman [13] was the first to describe this injury, in 1981. So far, seven cases have been reported with isolated injuries – ipsilateral dislocation of the shoulder joint and elbow joint, without associated soft tissue, osteoarticular and neurovascular injuries. Like the authors of similar studies, we concluded that in the clinical examination of the patient, it is most important to take a good history, to examine the injured joint as well as the neighboring joints, because a seemingly more serious injury can mask another one, and consequently lead to a potentially permanent decrease or loss of function in this joint.

While the clinical approach to diagnosing and managing these injuries has been outlined, several areas warrant further investigation. Future research should focus on refining diagnostic techniques, particularly in the early detection of associated injuries that may not be immediately obvious. Advanced imaging modalities such as MRI and CT scans could be explored to better visualize soft tissue and bone damage in patients with dual joint dislocations.

In terms of treatment, there is a need for standardized protocols addressing the management of both acute and chronic cases. The effectiveness of different reduction

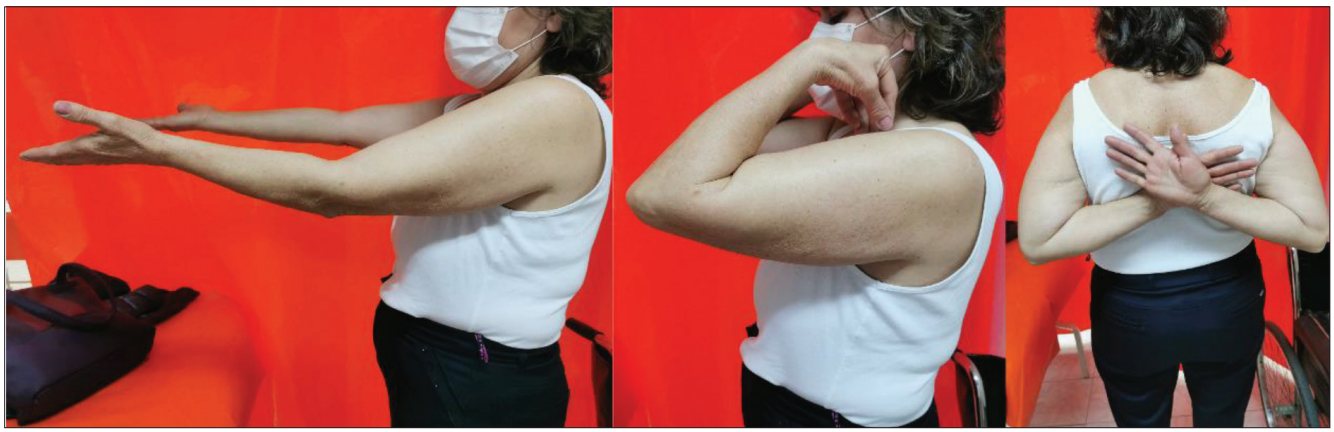

Figure 8. Clinical presentation and range of motion, 12 weeks after injury – extension and flexion of the elbow joint, internal rotation of the shoulder [source: Photographic Archive, Bežanijska Kosa University Hospital Medical Center]

techniques and post-reduction rehabilitation protocols could be evaluated through prospective clinical trials. Additionally, more attention should be given to the long-term outcomes of these patients, particularly in terms of joint stability, range of motion, and the potential for early onset arthritis or other degenerative conditions.

Follow-up care for patients with this type of injury is equally important. Research should aim to identify the most effective follow-up schedules and intervention strategies to monitor recovery and prevent complications. The development of evidence-based guidelines for follow-up care could improve patient outcomes and reduce the likelihood of permanent disability.

Conflict of interest: None declared.

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Изолована ипсилатерална луксација зглоба рамена и зглоба лакта

Бојан Миленковић[1], Миљан Билановић[1], Слађан Тимотијевић[1], Мирослав Татић[1], Ђорђе Колак[2,3]

[1]Клиничко-болнички центар „Бежанијска коса", Одељење ортопедије и ортопедске трауматологије, Београд, Србија;
[2]Универзитетски клинички центар „Крагујевац", Клиника за ортопедију и трауматологију, Крагујевац, Србија;
[3]Универзитет у Крагујевцу, Факултет медицинских наука, Катедра за хирургију, Крагујевац, Србија

САЖЕТАК

Увод Ишчашење зглоба представља губитак конгруентности зглобних површина, што је релативно честа повреда локомоторног апарата. Од великих зглобова, највећу инциденцу има ишчашење зглоба рамена, са учесталошћу од 24 на 100.000 становника годишње, при чему 94–97% чине предње луксације, 1% доње, а остало задње луксације. На другом месту је ишчашење зглоба лакта, са учесталошћу од 5,2 случаја на 100.000 становника годишње, при чему више од 80% чине постеролатералне луксације.

Приказ болесника Болесница стара 57 година повређена је при случајном паду на истом нивоу, при чему је задобила изоловане повреде – предње ишчашење зглоба рамена и постеролатерално ишчашење зглоба лакта. Лечена је конзервативно и постигнути су добри функционални резултати.

Закључак Прегледом литературе установили смо да је комбинација ипсилатералног ишчашења зглоба рамена и ишчашења зглоба лакта ретка повреда, код које се лако може превидети ишчашење зглоба рамена. Најважније је узети добру анамнезу и урадити детаљан преглед зглобова проксимално и дистално од повређеног. Ако се прегледу приступи адекватно, ове повреде се могу успешно лечити неоперативним путем, затвореном репозицијом, имобилизацијом и рехабилитацијом, уз добар функционални резултат.

Кључне речи: ишчашење зглоба рамена; ишчашење зглоба лакта; ипсилатерално ишчашење; изоловано ишчашење

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Biliary atresia associated with intestinal malrotation – unusual intraoperative presentation

Aleksandar Sretenović[1,2], Đorđe Topličić[1], Rastko Sava Jeremić[1], Dragana Vujović[1,2]

[1]University Children's Hospital, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia

SUMMARY

Introduction Biliary atresia (BA) is an idiopathic, progressive obliterative cholangiopathy of unknown etiology. The incidence of BA is 5–10 cases per 100,000 live births. Two clinical phenotypes of BA have been described: syndromic and non-syndromic form. From 1959, Kasai procedure is a standard therapeutic procedure. However, patients in whom there is an association of BA with other structural anomalies may have a worse outcome after the procedure. The goal was to present our patient with unusual form of BA associated with intestinal malrotation.

Case report We present a two-month-old female infant hospitalized because BA was suspected. On the echosonographic examination of the abdomen the gallbladder was not visible. Intraoperative diagnosis of BA was confirmed, and the Kasai procedure was performed. During the operation, intestinal malrotation with Ladd's bands was identified. In this case, after the complete Ladd procedure, we decided to trace the Roux coil through the mesoduodenum and then behind the duodenum towards the portal plate, where a portoenterostomy was then created in a standard way. During the "follow-up" the infant was free of complaints, the stools were normally discolored, and the values of liver function tests had a downward trend.

Conclusion Any doubt about the diagnosis of BA obliges us to determine the existence of all other possible anatomical abnormalities and associated anomalies, due to their potential importance in changing treatment plans and surgical approach, but also the impact on the outcome of treatment.

Keywords: biliary atresia; Kasai procedure; malrotation

INTRODUCTION

Biliary atresia (BA) is an idiopathic, progressive obliterative cholangiopathy of unknown etiology that affects the intra- and extra hepatic bile ducts, leading to cholestasis, progressive fibrosis and liver cirrhosis [1]. It is the most common cholestatic disorder of newborn age that occurs with a variable incidence of 5–10 per 100,000 live born, with female to male ratio of 1.4:1 [2].

BA is most often an isolated defect but can be associated with other congenital anomalies in up to 16% of cases. There are two clinical phenotypes of BA, the syndromic or embryonic form, often associated with other congenital anomalies such as polysplenia, intestinal malrotation, portal vein abnormalities, situs inversus, absence of the inferior vena cava and congenital heart disease. The incidence of syndromic forms of BA is around 10–20% [3, 4]. The acquired or perinatal form of BA is more common and represents 80–90% of cases [4].

The association of BA with intestinal malrotation is rare and has a very variable incidence ranging from 0.3% to 12%. The appearance of the Kasai procedure in 1959 enabled the long-term survival of patients with BA [5]. In patients with associated congenital anomalies, the results of this procedure may be worse due to altered anatomical relationships [6, 7].

The goal of our paper was to present an unusual case of BA associated with intestinal malrotation.

CASE REPORT

A female full-term infant, born by spontaneous vaginal delivery with a birth weighing 3300 g was hospitalized at the beginning of the third month of life (on the 67th day) due to jaundice and elevated values of liver function tests (LFT): direct bilirubin (DBil) 111.6 μmol/l, total bilirubin (TBil) 127 μmol/l, alanine aminotransferase (ALT) 173 U/L, aspartate aminotransferase (AST) 118 U/L. In the clinical findings at the beginning of hospitalization, apart from icterus, the liver was palpated 2 cm below the right lower rib arch, while the rest of the physical findings were normal. Echosonographic examination of the abdomen after fasting revealed enlarged left lobe of the liver (cranio-caudal diameter 45 mm, caudate lobe antero-posterior diameter 16 mm), and the gallbladder was not visible. Considering the symptoms and signs of the disease, laboratory and ultrasound findings, BA was suspected and surgical treatment indicated. At the age of 71 days, the patient was taken for intraoperative cholangiogram and to proceed to a Kasai procedure if indicated. Hypoplastic gallbladder and

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Correspondence to:
Aleksandar SRETENOVIĆ
University Children's Hospital
Tiršova 19
Belgrade
Serbia
alexmd64@gmail.com

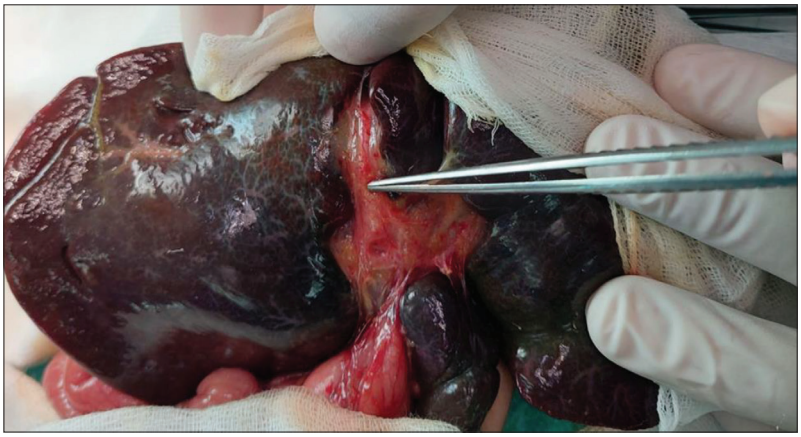

Figure 1. Macroscopic intraoperative finding of fibrotic liver parenchyma and absence of extrahepatic bile ducts

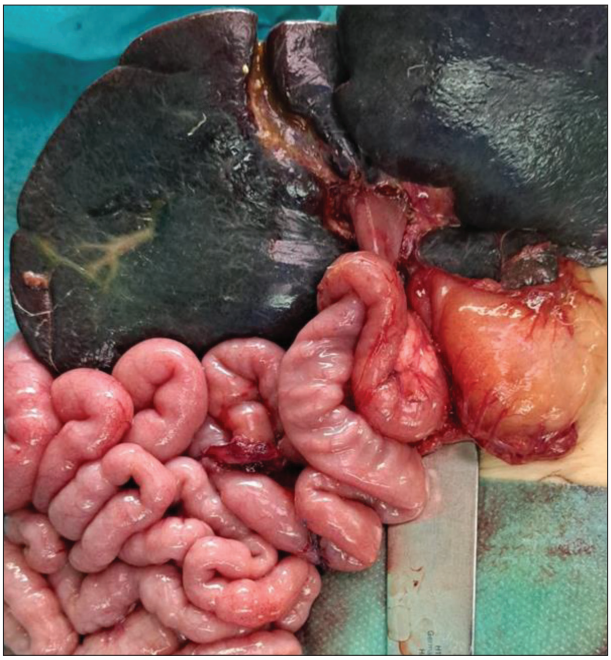

Figure 2. Porto-entero anastomosis – roux loop traced through the meso-duodenum

the absence of extra hepatic bile ducts were found intraoperative which confirmed the diagnosis of BA and the need to perform the Kasai procedure (Figure 1). During the operation, intestinal malrotation was identified with the duodenojejunal transition in the right half of the abdomen, the cecum and the appendix in the left upper quadrant of the abdomen. After the Ladd procedure was performed, the problem of the direction of the Roux-en-Y loop, which in the original technique is oriented transmesocolic, arose. After the Ladd procedure was completed, we decided to trace the Roux loop through the mesoduodenum, which enabled the best anatomical position of the intestinal loop to create a porto-entero anastomosis (Figure 2). After the appendectomy, the coils of the colon were placed in the left half of the abdomen and a liver biopsy was taken.

The infant started enteral feeding on the third postoperative day, and on the fifth postoperative day had normal discolored stools. Full enteral intake was established seven days after surgery. Bilirubin values showed a decreasing trend after the surgery. The infant was discharged from the hospital 17 days after surgery, on the 88th day of life. Bilirubin values at discharge were elevated (DBil 87.9 μmol/l, Tbil 104.2 μmol/l) as well as transaminase values (AST 221.0 U/L, ALT 389 U/L), stools were normally discolored, and body mass at discharge was 5020 grams. Pathological analysis showed congenital BA type 2.

At the follow-up examination three weeks after the operation, the bilirubin values remained unchanged, while the LFT values were decreasing, and the stools were usually discolored. Control abdominal ultrasound was performed one month after the operation showing no signs of intrahepatic bile ducts dilatation. Two months after the operation, the stools were usually discolored, with significant decrease of bilirubin and LFT values (DBil: 65.8 umol/L TBil: 83.7 umol/L, AST: 104 U/L ALT: 152 U/L).

The authors declare that the article was written according to ethical standards of the Serbian Archives of Medicine as well as ethical standards of institutions for each author involved. Informed consent was obtained from the parents/guardians of the patient involved in the report.

DISCUSSION

BA is a chronic progressive cholangiopathy, which occurs in a syndromic form with a frequency of 10–25% [1]. The association with intestinal malrotation is about 12% [2]. Although BA is known to coexist with different anatomical variations, studies have varied results on the prognosis of isolated BA compared with BA that is associated with congenital anomalies [3, 4, 5].

The outcome after surgery also depends on the conditions maintained in syndromic forms of BA [5]. However, due to limited number of cases the data on prognosis after the surgery is not yet adequately established [6].

Abdominal heterotaxy can have several surgical implications, primarily related to the orientation of the Roux-en-Y loop due to altered normal anatomical relationships [7, 8]. In the case of intestinal malrotation or non-rotation, the colon is located in the left side of the abdomen, while the duodenojejunal transition is located on the right side of the spinal colum, in the right side of the abdomen which can altered the normal orientation and positioning of the Roux-en-Y loop. Although it is unknown how these anatomical relationships would affect an infant's prognosis for BA, recognizing this abnormal anatomy is crucial both for the first exploration of hepato-portoenterostomy and for liver transplantation.

It can be expected that the presence of extra-hepatic anomalies and altered anatomy will negatively affect the outcome of surgical treatment. Differences in survival are primarily attributed to associated cardiac anomalies, however, studies showed that in experienced centers there is

no significant difference in the outcome of surgical treatment or in long-term biliary drainage [9]. The long-term follow-up of patients after portoenterostomy is necessary because the establishment of bile drainage alone does not mean a cure for such patients, because the Kasai procedure does not affect progressive fibrosis as a basic characteristic of the disease [7].

It is very important that these anatomical relations become recognized on time because even though it is unknown how they affect prognosis of BA, it interferes with surgical approach and selection of technique to be applied [10, 11].

The timely diagnosis of BA and associated conditions provides more effective management and good outcome [12].

If there are some associated conditions confirmed, they are not always diagnosed antenatal, but if they were, the diagnosis is always challenging and management usual involves surgical correction in the immediate postnatal period, or later on if possible [13].

For the next decade there are some expectations for the identification of new therapeutics for infants with cholestasis and potential use of precision medicine to optimize therapy for each infant [14, 15].

Even though that the liver transplantation is a gold standard for treatment of patients with BA, and the vast majority of patients reach adulthood, waitlist mortality negatively affects the overall prognosis and, in that case, it is not related to associated anomalies [16].

Although syndrome forms of BA are rare, the diagnosis of BA should always indicate further evaluation for another anatomical abnormalities because of their importance and potential influence on treatment plans and the surgical approach.

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Conflict of interest: None declared.

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Билијарна атрезија повезана са малротацијом црева – необична интраоперативна презентација

Александар Сретеновић[1,2], Ђорђе Топличић[1], Растко Сава Јеремић[1], Драгана Вујовић[1,2]

[1]Универзитетска дечја клиника, Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија

САЖЕТАК

Увод Билијарна атрезија је идиопатска, прогресивна облитеративна холангиопатија непознате етиологије. Њена инциденција је 5–10 случајева на 100.000 живорођених. Описана су два клиничка фенотипа билијарне атрезије: синдромски и несиндромски облик. Од 1959. Касаијева метода представља стандардни терапијски приступ. Међутим, пацијенти код којих постоји повезаност билијарне атрезије са другим структурним аномалијама могу имати лошији исход након операције.

Циљ овог рада је да прикажемо случај билијарне атрезије повезане са малротацијом црева.

Приказ случаја У овом раду представљамо двомесечно одојче хоспитализовано због сумње на билијарну атрезију. На ехосонографском прегледу абдомена жучна кеса није била видљива. Интраоперативно је потврђена дијагноза билијарне атрезије и примењена је Касаијева метода. Током операције идентификована је малротација црева са Ладовим бридама. У овом случају, након комплетне Ладове процедуре, одлучено је да се *Roux* вијуга усмери кроз мезодуоденум, а затим иза дуоденума ка портној плочи, где је потом на стандардни начин изведена портоентеростомија. Током редовног праћења одојче је било без тегоба, столица је нормално пребојена, а вредности тестова функције јетре су показивале опадајући тренд**.**

Закључак Свака сумња у дијагнозу билијарне атрезије обавезује нас да утврдимо постојање свих других могућих анатомских абнормалности и придружених аномалија, због њиховог потенцијалног значаја у измени планова лечења и хируршког приступа, али и утицаја на коначни исход лечења.

Кључне речи: билијарна атрезија; Касаијева метода; малротација

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

^{18}F-fluorodeoxyglucose positron emission tomography / computed tomography in the diagnosis of secondary malignancies in a patient with Hodgkin lymphoma

Nikola Pantić[1], Milica Stojiljković[1,2], Lenka Grujičić[1], Dragana Šobić Šaranović[1,2], Vera Artiko[1,2]

[1]University Clinical Center of Serbia, Center for Nuclear Medicine with PET, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia

SUMMARY

Introduction The incidence of a second cancer among patients who have been treated for Hodgkin lymphoma (HL) is higher than the incidence of cancer in the general population. ^{18}F-fluorodeoxyglucose (18F-FDG) positron emission tomography / computed tomography is used in the evaluation of a number of malignancies. The aim of the article is to emphasize the importance of including a second primary cancer as a differential diagnosis among patients at risk.

Case outline We present a case of a patient diagnosed with two separate malignancies almost two decades after the treatment of HL.

Conclusion In patients previously treated for HL, a biopsy of lesions that show high 18F-FDG uptake should be advised, particularly if the location of the lesion is unusual for the primary diagnosis.

Keywords: lymphoma, Hodgkin; positron emission tomography; second primary malignancy; chemotherapy; radiation therapy

INTRODUCTION

Hodgkin lymphoma (HL) is a chronic malignant lymphoproliferative disease with a bimodal distribution of occurrence in the third decade of life as well as in the sixth decade. It has a good prognosis and a high cure rate [1]. Lung cancer, on the other hand, causes an estimated 1.8 million annual deaths globally, which is 18% of all deaths attributed to cancer, and it is the leading cause of cancer death [2]. Meanwhile, non-Hodgkin lymphoma (NHL) is the most common hematological malignancy worldwide, with diffuse large B-cell lymphoma (DLBCL) being the most frequent subtype [3].

Among the late complications, second malignancies (SMN) are a great concern in cancer survivors [4]. Although second cancers can reflect the influence of lifestyle factors, environmental exposures, genetic contributions, and host factors, the late sequelae of treatment are paramount [5]. The risk for SMN is particularly high in HL survivors. In 2023, Núñez-García et al. [6] demonstrated the much higher than expected mortality in patients previously treated for HL, even when HL as the cause of death was eliminated, with patients younger than 30 years and women being especially vulnerable, most frequently secondary to SMN (37.8%). During the last three decades, the role of positron emission tomography / computed tomography (PET/CT) in diagnosis, staging, and follow-up of cancer patients has been revolutionary [7]. The majority of PET scans are performed using ^{18}F-fluorodeoxyglucose (18F-FDG), the gold standard of PET radiopharmaceuticals [8]. However, due to its non-specific nature, 18F-FDG PET/CT cannot distinguish between different types of cancer, and histopathological confirmation for lesions of an undetermined origin is a necessity.

In this case report, we present the development of two distinct malignancies two decades after the completion of Hodgkin lymphoma treatment.

CASE REPORT

A 62-year-old female patient was sent for an 18F-FDG PET/CT examination at the Center for Nuclear Medicine with Positron Emission Tomography of the University Clinical Center of Serbia for the initial staging of DLBCL, diagnosed 19 years after the patient had been treated for HL with chemotherapy (CTx) and radiation therapy (RTx). Previously, a sample of enlarged submandibular lymph nodes was taken. Histopathological specimens revealed pathological findings resembling DLBCL, the most common subtype of NHL. Therefore, the patient was scheduled for a PET/CT. Meanwhile, a standard-dose CT showed a mass in the right lung, without a definitive conclusion regarding the etiology. The patient complained of fatigue and coughing.

A three-dimensional PET scan and a low-dose non-enhanced CT scan were acquired

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Correspondence to:
Nikola PANTIĆ
University Clinical Center of Serbia
Center for Nuclear Medicine with PET
Višegradska 26
11000 Belgrade, Serbia
nikolapantic944@gmail.com

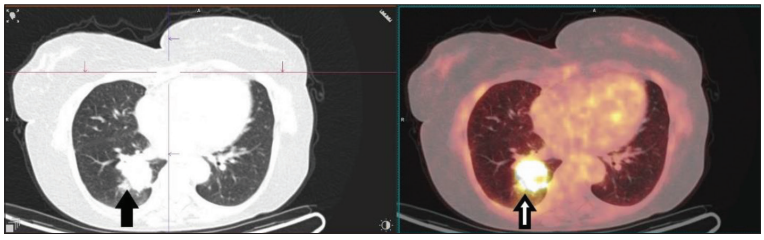

Figure 1. ^{18}F-fluorodeoxyglucose positron emission tomography / computed tomography: the axial image depicts a lesion in the posterobasal segment of the right lower lobe of the lungs on the low-dose CT (black arrow) and an increased accumulation of a radiopharmaceutical on the fused image (white arrow); histopathology revealed squamous cell carcinoma

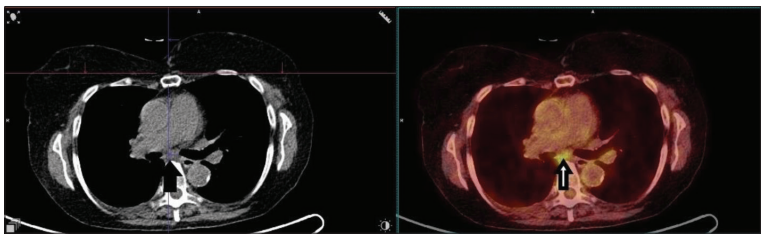

Figure 2. ^{18}F-fluorodeoxyglucose positron emission tomography / computed tomography: the axial image depicts an enlarged subcarinal lymph node on the low-dose CT (black arrow) and an increased accumulation of a radiopharmaceutical on the fused image (white arrow)

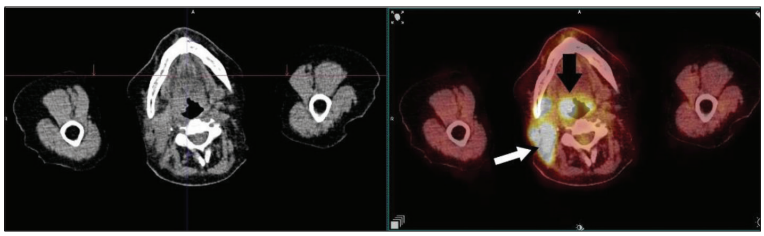

Figure 3. ^{18}F-fluorodeoxyglucose positron emission tomography / computed tomography: the axial image depicts an increased accumulation of a radiopharmaceutical in the right tonsil (black arrow) and in enlarged right cervical lymph nodes (white arrow); histopathology revealed diffuse large B-cell lymphoma

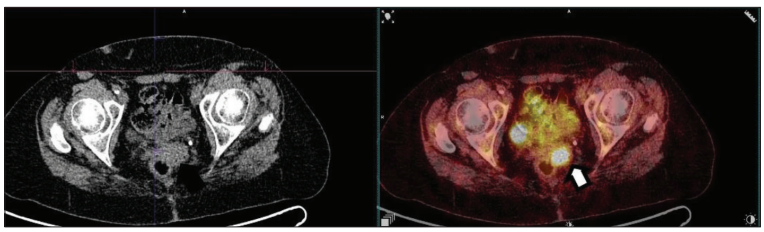

Figure 4. ^{18}F-fluorodeoxyglucose positron emission tomography / computed tomography: the axial image depicts a soft tissue mass in the cervix of the uterus on the low-dose CT (black arrow) and an increased accumulation of a radiopharmaceutical on the fused image (white arrow); histopathology revealed diffuse large B-cell lymphoma

from the base of the skull to the mid-thigh. On PET/CT exam, we found an increased metabolism of glucose in a lesion in the posterobasal segment of the right lower lobe of the lungs (Figure 1), previously seen on a standard-dose CT, and in the subcarinal lymph node (Figure 2). Dimensions of the pulmonary lesion were 42 × 38 × 41 mm with the maximum standardized uptake value (SUVmax) of 22.2, while the subcarinal lymph node measured 16 × 9 mm in diameter with the SUVmax of 4.7. Primary carcinoma of the lungs was suspected, without the exclusion of a lymphoma as a possible cause. PET/CT examination also disclosed an increased accumulation of 18F-FDG in the right tonsil and right cervical lymph nodes (Figure 3), up to 36 × 20 mm in size with SUVmax of 36.4, which showed an increased metabolism due to DLBCL, and a focal zone of an increased metabolism of glucose in a soft tissue mass in the cervix of the uterus (Figure 4), 22 × 17 mm in diameter with the SUVmax of 14.7. Later on, bronchoscopy was performed, and a biopsy of the pulmonary lesion revealed squamous cell carcinoma. Specimens from both the submandibular lymph nodes and a pulmonary lesion were sent for revision, and the findings confirmed two separate malignancies of different locations and histological types. Afterwards, the gynecology specialist did a comprehensive evaluation. The patient stated that she noticed blood spots on her underwear in the last seven days. Ultrasound confirmed a soft tissue mass in the projection of the cervix. The sample of the lesion was taken. Based on the morphological and immunohistochemical analysis of the tumor cells, the pathologist concluded that infiltration by the previously diagnosed DLBCL was most likely. Two weeks later, the patient arrived at the emergency department with family members who reported that the patient was disoriented and could not walk. CT of the head was performed. A scan revealed an oval, hypodense lesion in the frontobasal region of the right cerebral hemisphere, suspected to be a metastasis. Further treatment will be planned based on the decision of the lung cancer council.

A decision (668/6) dated April 19, 2018, by the University Clinical Center of Serbia's Ethics Committee allows employees of the Center for Nuclear Medicine with PET of the University Clinical Center of Serbia to use anonymous data and obtained patient results for scientific research purposes.

DISCUSSION

HL, a type of cancer that arises from B-lymphocytic cells, is one of the most curable malignancies [9]. In recent years, the use of lower doses of alkylating CTx and RTx, limited to smaller volumes and at lower doses, has improved survival of patients with HL, such that the cure rate for this disease currently exceeds 80% [6]. However, as more patients survive longer, the impact of late complications of treatment is increasingly clear, with second primary malignancy (SPM) and cardiovascular, pulmonary, and infectious diseases being the leading cause of mortality in survivors of HL [10]. NHL and lung cancer are among the most common SPMs in patients previously treated for HL [11], both of which occurred in the case we presented. Moreover, the patient received CTx and RTx, both of which increase the risk for SPM in HL survivors. 18F-FDG PET/CT at baseline has proven to be highly sensitive in determining sites of disease

for DLBCL [12], an aggressive lymphoma subtype. It shows higher sensitivity, specificity, and accuracy compared to contrast-enhanced CT in the initial assessment and staging of lymphoma [13]. However, since a large percentage of malignant lesions show 18F-FDG avidity, the type of cancer cannot be determined based on 18F-FDG-PET/CT findings alone, and further evaluation is needed if SMN is suspected. Furthermore, most non-cancerous cells use glucose as the main source of energy. Consequently, the increased glucose metabolism is not only specific for malignant neoplastic lesions but is also observed in many other sites, such as pathologically benign lesions, especially inflammatory lesions, and normal organs [14].

A nuclear medicine specialist should be aware of a possibility of two or more different types of malignant lesions in a patient, especially in patients who have an increased risk thereof due to earlier treatment. In cases where the etiology of the lesion is uncertain, a biopsy should be advised, in order to determine a proper therapeutic strategy.

Conflict of interest: None declared.

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Позитронска емисиона и компјутеризована томографија са ^{18}F-флуородеоксиглукозом у дијагностици секундарних малигнитета код болесника са Хоџкиновим лимфомом

Никола Пантић[1], Милица Стојиљковић[1,2], Ленка Грујичић[1], Драгана Шобић Шарановић[1,2], Вера Артико[1,2]

[1]Универзитетски клинички центар Србије, Центар за нуклеарну медицину са ПЕТ, Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија

САЖЕТАК

Увод Инциденца другог карцинома код болесника који су лечени од Хоџкиновог лимфома већа је него у општој популацији. Позитронска емисиона томографија и компјутеризована томографија са ^{18}F-флуородеоксиглукозом користе се у евалуацији бројних малигнитета. Циљ рада је да се истакне важност укључивања другог примарног карцинома као диференцијалне дијагнозе код болесника са повећаним ризиком.

Приказ болесника Представљен је случај болесника са два одвојена малигна обољења дијагностикована скоро две деценије након лечења Хоџкиновог лимфома.

Закључак Код болесника који су претходно лечени од Хоџкиновог лимфома препоручује се биопсија лезија које показују повишено преузимање ^{18}F-флуородеоксиглукозе, посебно ако је локација лезије неуобичајена за примарну дијагнозу.

Кључне речи: Хоџкинов лимфом; позитронска емисиона томографија; други примарни малигнитет; хемотерапија; зрачна терапија

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HISTORY OF MEDICINE / ИСТОРИЈА МЕДИЦИНЕ

Guidelines for the preparation and dosage of medicines, instructions for their use, and methods of application according to the *Hilandar Medical Codex No. 517*

Zoran Vacić

Serbian Medical Society, The Academy of Medical Sciences, Belgrade, Serbia

SUMMARY

The Hilandar Medical Codex is a medieval manuscript produced in the translation and copying workshop of the Hilandar Monastery (*scriptorium*). It is regarded as the most significant document for studying the history of Serbian medicine. The manuscript dates back to the mid-16th century.

The aim of this paper is to identify and explain the measures for quantity outlined in the Hilandar Medical Codex for medicine preparation, dosage, physician instructions, contraindications, and methods of application.

The analyzed material includes the deciphered text of the Hilandar Medical Codex and its translation into contemporary Serbian. To identify the scientific names of individual remedies, references were made to Dioscorides' De Materia Medica, Antidotarium of Nicholas of Salerno, as well as general and specialized dictionaries.

The prescribed weight and volume measures for the quantities of ingredients used in medicine preparation include the *aksag* (about 4.55 grams or 1/6 ounces), ounce, liter, cup, drop, and "handful."

In the medication dosage instructions, the measurements, with the exception of ounces, are not precise but largely descriptive.

The warnings and instructions provided to the doctor regarding the use of certain drugs included the recommended duration of use and contraindications.

The means of drug application included a quill (for instillation into the eye or nose), a balloon made from a pig's bladder (*vesica urinaria*) with a goose quill *calamus* (for flushing the ureter and administering enemas), a wick (*funiculus incendiarius*) for applying medicine into body cavities (such as the vagina, rectum, or nose), and a sponge (*litus*) for applying liquid medicines to the skin's surface.

Keywords: Hilandar Medical Codex; measurement for quantity; instructions; contraindications; means of drug application

INTRODUCTION

The Hilandar Medical Codex (HMC) is a medieval manuscript produced in the translation and copying workshop of the Hilandar Monastery (*scriptorium*). It is considered the most significant document for studying the history of Serbian medicine. In 1952, while studying the collection at the Hilandar library, Đorđe Sp. Radojičić observed that "there are significant texts for the history of our medicine (...)" Among them was a larger manuscript, consisting of 204 pages, titled *Manuscript on the Recognition of Diseases by Palpation of Veins* [бєсѣда wт(ъ) позианиа болѣсти по пипанию ж нлъъ] [1]. Paleographic analysis has dated the manuscript to the mid-16th century [2, 3].

His discovery demonstrated: (1) that the scientific medical advancements of Latin Europe were accessible to Serbian doctors during the Middle Ages, (2) that these achievements could be learned in their own language – an exception in a time when Latin and Greek were the dominant languages of all sciences, including medicine, and (3) the lexical richness of the Serbian language in terms of terminology related to anatomy, physiology, pathophysiology, and pharmacotherapy.

The pharmacological records, which serve as the most important source for our research, were created by translating documents such as *Practica JO. Serapionis dicta breviarium*, *Liber Serapionis de Simplici Medicina*, *Liber de Simplici Medicina dictus Circa Instans Practica Platearis* by Matthaeus Platearius (†1161) ("The Book of Simple Medicines"), [4, 5] *Antidotarium Parvum* by Nicolaus Salernitanus (1140–?), ("The Book of Compound Medicines") [6] and Avicenna's *Canon of Medicine* ("The Book of Poisons") [7].

The translation of these documents into our vernacular, "with certain admixtures of Old Slavic" [8], provides a detailed insight into the development of medical terminology in the Serbian language during the Middle Ages.

The aim of this paper is to identify and clarify the measures for quantity outlined in HMC for the preparation of compound medications, the dosage of both simple and compound medications, instructions for physicians, contraindications for specific medications, and the methods for administering these medications.

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Correspondence to:
Zoran VACIĆ
Serbian Medical Society
Džordža Vašingtona 19
11000 Belgrade
Serbia
jeremija1955@gmail.com

METHODS

The deciphered and transcribed text of the Hilandar Medical Codex No. 517, along with its translation into contemporary Serbian, has been analyzed [9]. During the text analysis, significant inconsistencies between the deciphered text and the contemporary Serbian translation were addressed by consulting the photographed pages of the Codex manuscript preserved in the Serbian Academy of Sciences and Arts' archive [10]. In addition to pharmacological manuscripts ("The Book of Simple Medicines," "The Book of Compound Medicines," and "The Book of Poisons"), other manuscripts from the Hilandar Medical Codex were analyzed. These documents detail specific simple drugs, their effects, the methods for preparing compound drugs, and other instructions relevant to the topic of this paper.

To identify the scientific names of individual remedies and their equivalents in contemporary Serbian, classical manuscripts such as Dioscorides' *De Materia Medica* (Greek: Πεδάνιος Διοσκουρίδης; Latin: *Pedanius Dioscorides*) [11] and the *Antidotarium* of Nicolaus Salernitanus [12] were consulted, along with general and specialized dictionaries [13–17].

RESULTS AND DISCUSSION

The measures for the preparation of medications

Specific measures are prescribed for the quantities of ingredients and the preparation method of medicines. For example: "Take one *aksag* (about 4.55 grams or 1/6 ounces) of Stinking gum (*Ferula assa-foetida L.*) and boil it in one liter of water with an equal amount of honey" [18], "Take one ounce of *Agaricus L.*, two ounces of honey, one ounce of barley flour yeast, two ounces of olive oil, one ounce of salt, and two cups of water. Boil everything together and strain" [19], "Take and mix one *aksag* of powdered Snake Root (*Polygonum bistorta L.*), ginger (*Zingiber officinale Roscoe*), clove (*Eugenia caryophyllata Thunb.*), cinnamon (*Cinnamomum ceylanicum Breyn*), mastic (*Pistacia lentiscus L.*), and nutmeg (*Myristica fragrans Houtt*)" [20], etc. In some prescriptions, the quantities of ingredients are not precisely specified: "Take a small amount of saltpeter powder (KNO3), a large quantity of salt water, honey, and olive oil. Mix everything together, cook, and use it for an enema" [21], etc. In some prescriptions, however, the ingredients are specified in equal parts: "Take coral powder (Coelenterate system) and mix it with an equal amount of juice from Bermuda grass (*Cynodon dactylon L.*)" [22], or in relative proportions to the main ingredient: "Take one part of *Senna alexandrina* powder (*Cassia acutifolia Del.*), three parts of wine, and rose water" [23], etc.

The measures (weight and volume) mentioned in the prescriptions of HMC include *aksag*, ounce, liter, cup, drop, and "handful."

Aksag (Greek: εξαγιον, Latin: *exagium*) is a unit of weight used for valuables. It was widely employed during the Middle Ages. The earliest mention of *aksags* in our region comes from Dubrovnik, where, in a 1305 treatment contract, four gold rings, each weighing four "aksadjes," were pledged to a wound-healer as collateral for the payment of treatment costs. In the fourth century, when the gold coin known as the solidus was introduced, its weight was standardized to the Roman sextula, or the Greek εκαγιον, as 72 parts of a liter. As a result, one *aksag* of *a fine scale* weighed 4.55 grams. Valuables such as gold, silver, and pearls were measured using *a fine scale*, as were medicinal substances that were considered precious due to their limited quantities in nature or the great distance from their country of origin [24].

The ounce (Latin: *uncia*), a unit of weight used since Roman times, was widely used in the Serbian lands during the Middle Ages. From the mid-16th century, the ounce became a standard measure for medicines in the region. The Roman ounce weighed 27.288 grams, the Byzantine ounce 27.30 grams, and the Dubrovnik fine ounce 27.328 grams in the 14th century, increasing to 27.427 grams in the 16th century. A fine ounce was equivalent to six *aksags* [25].

The liter (Greek: λίτρα, Latin: *libra*) is one of the oldest publicly recognized units of weight. In the *Karyes Typikon* of St. Sava (1199), it was stipulated that the Hilandar Monastery was obliged to provide "60 liters of oil" for Sava's cell [26]. The Roman and Byzantine liters both weighed 327.45 grams, while the fine Venetian liter (*libra*) weighed 302 grams, and the Serbian medieval liter weighed approximately 316 grams [27]. In the HMC drug prescriptions, the liter, as a unit of weight, is equivalent to 72 *aksags* and corresponds to the Roman or Byzantine liter.

The liter was also used as a unit of volume. The volume of one liter is determined by a container calibrated to hold one liter by weight, typically referring to water, although it can also be applied to other liquids.

The cup (Latin: *cuppa*) was primarily used as a measure for wine, but also for other substances. It is difficult to determine the exact volume of the cup, as various descriptions in the literature refer to cups of differing capacities.

The drop was also used as a unit to measure the quantity of a medicinal ingredient in a preparation. For example: "Mix one drop of balm [єдиꙋ капю балшамда] with a small amount of warm water" [28].

The handful is mentioned as a unit of measure in the prescription for bitter electuary [лѣтꙋарнѡ горкѡ]. "Take Dutchman's pipe [*Aristolochia (L.) Tourn.*] and the great yellow gentian (*Gentiana lutea L.*), each in one *handful*" [ѡтъ сбакога .а. рꙋчицꙋ]. The "handful" refers to a bundle that is "just the right size to be easily held by hand" [29]. A common Serbian term for this measure is *rukovet*.

In some prescriptions, instructions specify using ingredients in quantities sufficient for preparing the medicine, such as "add as much honey as needed" [и медa колико потрѣбꙋє] [30].

Medication dosing

In "The Book of Simple Medicines," the dosage measures for medicines are provided. These measures, with the exception of ounces, are not precise, as is often the case with

prescriptions, but are mostly descriptive. For example, the medicine was given in amounts corresponding to the size of a hen's egg [ꙗнцє] or a goose egg [гꙋш'чїє ꙗнцє]. Other measures used for the medicine included a spoon, a teaspoon, a cup, or a small cup. Solid forms of medicines are defined by the size of various fruits, such as a hazelnut, a walnut, a kidney bean, or a pinch of pepper. The size of a suppository was intended to correspond to the length of a finger [дꙋго и дебел окако пръстъ], a middle finger [дꙋго и дебел окако срѣднїи пръстъ], or the shape and size of a date [како фїнїкꙋ].

Doses of medications were adjusted based on age, with different amounts prescribed for children and adults. For example, when using aloe (*Aloë sp.*) as an anthelmintic, the adult dose is half an *aksag* of aloe powder [ако бꙋдє великъ чловѣкъ даи мꙋ полъ аѕакы], while the children's dose is an amount that fits in half a hazelnut shell [ако бꙋдє мало дѣтѣ даи мꙋ алоє колико полъ лѣшнъика] [31].

Medicines were administered according to specific instructions: "take the medicine for three days, every morning," "take it every morning and evening," "drink warm every morning," "take the pills five times a day," etc. In some cases, the duration of treatment was based on the patient's improvement: "give him this until you see that he is better," or "repeat this several times until his condition improves." Some medicines intended for the treatment of chronic conditions or bodily dysfunctions (such as stomach weakness, meteorism, flatulence, etc.) could be taken by the patient "whenever he/she wished" and "as much as he/she desired." Pills and powders for oral administration were taken with water, wine, or the juice of a specific plant, with the wine and juice serving a therapeutic role.

Warnings and instructions

"The Book of Simple Medicines" also includes warnings for the physician regarding the use of certain medicines, such as the recommended duration of use, contraindications, and the appropriate time of year for administering the medicine.

The application time is specified when using ointments for the treatment of ringworm, old wounds, and for hair removal in cases of hirsutism and hypertrichosis. The ointment for ringworm and old wounds is made from cashew (*Anacardium occidentale L.*) and orpiment (As_2S_3), while the hair removal ointment is composed of orpiment and quicklime [вара несквашена] [32]. In the contemporary Serbian translation of the HMC, it is stated that the ingredients of the ringworm ointment are cashew and asphaltite. However, the correct component of this ointment is not asphaltite, but orpiment, as indicated in the deciphered text of the Codex [и стꙋци толикожде аꙋрипимен'та] [33]. Both prescriptions include instructions to apply the ointment for no longer than one hour. After this time, the treated area should be promptly washed with warm water. If the ointment is left on longer, "his skin will peel off" [да дръжи оиꙋи мастъ лихо .а. часъ єр ако внєкѥ ꙋѕъдръжи хокѥ мꙋ сє кожа огꙋлити] [32, 33]. Orpiment is the active ingredient in these ointments and can cause the aforementioned complication if left on the skin for too long.

In the section of the text referring to cashew, there is a warning that "anyone who eats cashew or drinks it without other medicines may develop morphea" [кто ꙗст анакарди или пиє безъ дрꙋгаго билїа може мꙋ ꙋчинит мор'фиа] [33].

The author of "The Book of Simple Medicines" cautions doctors never to use white coral as a medicine unless explicitly instructed to "take white coral" [нъ никъда немои поставити бѣлѣ коралїи ꙋ билїа докле не рєчє възми бѣлѣ коралїи]. In recommendations regarding the use of corals in medicine, they almost always refer specifically to red corals [къдъ говори ꙋѕми коралїи тои ѕна да говори ꙋѕми цръвенѣ коралиїє] [22].

Rockcap fern (*Polypodium vulgare L.*) is not used in the preparation of medicines that do not contain anise (*Pimpinella anisum L.*) or fennel (*Foeniculum vulgare Mill.*) [34]. Additionally, kerosene should not be administered orally in summer, but only in winter [35].

A medicine made from saffron crocus powder (*Crocus sativus L.*) and wine, used for a weak stomach or palpitations, should not be given to individuals with an excess of black bile, as it may irritate the stomach and induce vomiting [36]. Coral pills with barley water should not be used by individuals with bloody diarrhea [ако бꙋдє ималъ ѕдола течєнїа ѡтъ кръвїи]. Instead, the pills should be prepared with buckhorn (*Plantago lanceolata L.*) juice [22]. An enema containing saffron crocus (*Crocus sativus L.*) should not be given to individuals with general weakness or dizziness, particularly those suffering from rheumatism or gout. In such cases, instead of saffron crocus, a bigroot geranium (*Geranium macrorrhizum L.*) should be used in the enema [37].

According to the HMC, Dioscorides writes that medicines containing honey are contraindicated for those with acute fever [кои има острꙋ ѡгницꙋ], as honey is considered hot and unsuitable for treating this condition [38]. For the same reasons, honey should not be given to individuals who are excessively thirsty [39].

Pregnant women should not be allowed to eat celery [коꙗ жена нѣс(тъ) праѕна не трѣбꙋє дати и селин(ъ) да гриѕє] [40].

The medicine applied to the ear should be warm, while the medicine applied to the eye should be cold [и да ѕнаш(ъ) єръ свакꙋ сътвар(ъ) требꙋє топло поставити ꙋ ꙋхо а хладно ꙋ око] [41]. The enema fluid should be warmed to lukewarm temperature before use [и съгрѣи да бꙋдєтъ млачиѡ] [42].

Means of drug application

For the application of non-oral drugs, the following methods were used:

(1) To apply drops to the eye or nose, a quill was dipped into the prepared medicine (solution) [и ѡмакꙋи перо] and then the liquid was dripped from the quill into the eye [стави ꙋ очїи] or nose [стави ꙋ носъ] [43, 44].

(2) A pig's bladder [мѣехыръ свиньски, *vesica urinaria*] was used for flushing the ureter [чини мꙋ глистиръ водопꙋстомъ],

and likely also for enemas. A liquid for rinsing or an enema was poured into the bladder, and then a hollow goose quill was inserted into it [кран ѡтъ гушчїега пера, *calamus*]. The quill was securely tied to prevent any leakage at the connection with the bladder. Under pressure, the rinsing solution (or enema) was then introduced into the ureter or rectum [35].

(3) A wick [стѣнилѡ] was used to apply medication into body cavities (such as the vagina, rectum, or nose) or wounds. It appears that "wick" was a general term for the tool used to administer medicine. Relja Katić referred to it as *funiculus incendiarius* [10, 45]. A wick was used for the application of ointments, salves, and powders "Take one *aksag* of musk (*Muscus*) and three *aksags* of Frisco clover (*Trifolium fibrinum*), mix them together, and soak a paper towel with the mixture. Place the towel in the woman's vagina" [ꙋзми москосъ .а. аѯаж и триферамана .г. аѯак(а) і смѣшан ꙁаєдно и смочи стѣнилѡ бꙋбакюрнѡ и постави женѣ въ плодвѣ] [46]. Paper and cloth were used to make a wick. "Create a dressing from cloth, coat it with honey, sprinkle birthwort powder on top, and apply it to the raw wound" [шчинъı стѣнило ѡт(ъ) кръпѣ, и помажи га медѡм(ъ) и поспли ꙁгора прахѡм(ъ) ѡт(ъ) арщологїе и постави ꙋ живинꙋ] [47]. Unfortunately, the other dictionaries we consulted do not provide a definition for this term. However, based on how the use of a wick is described in the deciphered text of the HMC, we believe that, in addition to Katić's interpretation, it should be noted that a wick could also take the form of a funnel (such as paper fiche) or another shape.

(4) A sponge (*litus*) was used to apply liquid medicines to the skin's surface [ѡт(ъ) блюванїа] скваси гꙋбꙋ морскꙋ въ оцтъ и постави на стомах(ъ) [48].

CONCLUSION

The Hilandar Medical Codex is a manuscript created by translating and compiling key texts from European and Arabic medieval scientific medicine. It was written as a practical manual for physicians.

The analysis of the HMC text identified the measurements of weight and volume mentioned in the prescriptions for preparing compound medicines (*aksag*, ounce, liter, cup, drop, and handful), as well as the measurements for dosing both simple and compound medicines. It also outlined the methods of administering the medicines, provided instructions for the doctor regarding contraindications, and described the means of applying drugs that are not taken orally.

We found that the measures for quantity and instructions for preparing medicines were clearly defined, with careful attention given to the dosage, duration of therapy, and age-appropriate dosing. Additionally, the prescriptions included explicit guidelines on contraindications for certain drugs. This reflects the advanced understanding that physicians of the time had regarding the benefits and potential risks of specific remedies.

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20. „O potprstnici (Potentilla bistorta) [ѡт(ъ) бишор'тъ]", Hilandarski medicinski kodeks № 517, 115, 312.
21. „O šalitri (Nitrium) [ѡт(ъ) нитриꙋм(ъ) сърѣч(ъ) саланитръ]", Hilandarski medicinski kodeks № 517, 143, 341.

22. „O koralima (Coralli) [ѡт(ъ) коралн]“, Hilandarski medicinski kodeks № 517, 129, 326.
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32. „O auripigmentu ili saru zrnaku (Auripigmentum) [ѡт(ъ) ауріпнмента съръч(ъ) сари зрънакъ]“, Hilandarski medicinski kodeks № 517, 110, 307.
33. „O pipku (Anacardium occidentale) [ѡт(ъ) анакардн]“, Hilandarski medicinski kodeks № 517, 108, 307.
34. „O slatkoj paprati (Polypodium vulgare) [ѡт(ъ) полнподнум(ъ) съръч(ъ) ѡт(ъ) папратно цвлѣ кореніе нже растѣ по каменнн]“, Hilandarski medicinski kodeks № 517, 147, 346.
35. „O petroleju ili nafti (Petroleum) [ѡт(ъ) петролеум(ъ) съръч(ъ) нафда]“, Hilandarski medicinski kodeks № 517, 147, 346.
36. „O kaćunku ili šafranu (Crocus sativus) [ѡт(ъ) крокуш(ъ) съръч(ъ) шафранъ]“, Hilandarski medicinski kodeks № 517, 128, 325.
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38. „Ovde počinje o tome kako se priprema đulap ili sirup [ѡвдѥ почнѣ како сѥ чин(н) гюлап(ъ) і ширѡп(ъ)]“, Hilandarski medicinski kodeks № 517, 168, 369.
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48. „O povraćanju [ѡт(ъ) блювання]“, Hilandarski medicinski kodeks № 517, 60, 245.

Смернице за израду и дозирање лекова, упутства за употребу и средства за апликацију лека у *Хиландарском медицинском кодексу* бр. 517

Зоран Вацић

Српско лекарско друштво, Академија медицинских наука, Београд, Србија

САЖЕТАК

Хиландарски медицински кодекс је средњовековни рукопис настао у преводилачкој и преписивачкој радионици (скрипторијуму) манастира Хиландар и најзначајнији документ за проучавање историје српске медицине. Рукопис је датиран у средину XVI века.

Циљ рада је да се попишу и разјасне мере за количину које су коришћене приликом израде лекова, дозирање лекова, упутства лекару, контраиндикације и средства за апликацију лекова описаних у *Хиландарском медицинском кодексу*.

Анализирани су рашчитани текст Хиландарског медицинског кодекса и његов превод на савремени српски језик. За утврђивање научног имена појединих симплиција (једноставних лекова) консултовани су Диоскоридова *De Materia Medica*, *Antidotarium* Николе из Салерна, општи и специјализовани речници.

Прописане мере за количине састојака који се користе у припреми лека укључују *аксаї* (износио је око 4,55 грама или 1/6 унце), унцу, литар, шољу, кап и „шаку”. У упутствима за дозирање лекова, мере, са изузетком унци, нису прецизне, већ углавном описне. Упозорења и упутства лекару у вези са употребом одређених лекова укључивала су препоручено трајање употребе и контраиндикације.

Средства за апликацију лека су перо (за укапавање у око или нос), балон од свињског мехура (*vesica urinaria*) са каламусом гушчијег пера (за испирање уретера и за клистирање), стенило (фитиљ, *funiculus incendiarius*) за апликацију лекова у телесне шупљине (вагина, ректум, нос), сунђер за апликацију течних лекова (*litus*) на површину коже.

Кључне речи: *Хиландарски медицински кодекс*; мере за количину; упутства; контраиндикације; средства за апликацију лека

IN MEMORIAM

Radmilo Rončević (January 20, 1938 – January 18, 2024)

Milan T. Stojičić[1,2], Maja Nikolić Živanović[2]

[1]University of Belgrade, Faculty of Medicine, Serbia;
[2]University Clinical Center of Serbia, Clinic for Burns, Plastic, and Reconstructive Surgery, Belgrade, Serbia

Radmilo Rončević was born on January 20, 1938, in Žanjevica, a village in the Gacko municipality, in the Kingdom of Yugoslavia. He completed his high school education in Novi Sad and attended the Military Academy at the School Center in Ljubljana. In 1967 he graduated from the Faculty of Dentistry, and in 1969 from the Faculty of Medicine at the University of Belgrade. He specialized in maxillofacial surgery at the Military Medical Academy in Belgrade in 1972, in oral surgery at the Faculty of Dentistry in Zagreb in 1979, and in plastic and reconstructive surgery at the Faculty of Medicine at the University of Zagreb in 1984. In 1978, he defended his doctoral dissertation titled "Physical, biochemical, and immunological values of *parotid saliva* in the pathogenesis, prognosis, and diagnosis of chronic recurrent parotitis" at the Military Medical Academy in Belgrade. He completed a subspecialization in craniofacial surgery at the Mayo Clinic in Rochester, MN, USA, in 1987 and further training in oculoplastic surgery in New York in 1989.

Figure 1. Dr Radmilo Rončević, MD, DDS, PhD (January 20, 1938 – January 18, 2024)

Dr. Radmilo Rončević was appointed as an assistant professor at the Faculty of Medicine in Novi Sad in 1980, teaching maxillofacial surgery. He was a visiting professor at the Faculty of Medicine at the University of Niš in 2000, where he taught surgery with war surgery. He also lectured at the State University of Pennsylvania in the USA, the Medical Faculty in Hanover, Germany, and the State University in St. Petersburg, Russia.

In 1994, he obtained the title of scientific advisor at the Faculty of Medicine at the University of Belgrade and served as a scientific advisor at the Ministry of Science of the Republic of Serbia. As a surgeon, he worked at the Military Medical Academy in Belgrade, the Military Hospital in Zagreb, and the Clinical Center of Serbia. Dr. Radmilo Rončević performed a wide range of surgeries in the fields of plastic, reconstructive, and craniofacial surgery. He was dedicated to performing the most complex surgeries on advanced tumors of the craniofacial region, congenital, and vascular anomalies, and mutilating injuries and their consequences. At the turn of the millennium, he became known both in Serbia and globally as one of the pioneers in orbital surgery. He particularly excelled in the surgical treatment of Graves' ophthalmopathy using a three-wall decompression method, performing over 250 successful orbital surgeries. In 1989, he co-authored the paper "Surgical treatment of thyrotoxic exophthalmos" with renowned craniofacial surgeon Ian Jackson, published in *Plastic and Reconstructive Surgery*, the leading international journal in the field.

Dr. Rončević was a member of the Executive Board of the Belgrade City Assembly, responsible for healthcare from 1997 to 2000. He was a member of numerous professional associations, including the Serbian Medical Society, the Serbian Association for Plastic, Reconstructive, and Aesthetic Surgery, the Balkan Association for Plastic, Reconstructive, and Aesthetic Surgery, the European Association for Plastic, Reconstructive, and Aesthetic Surgery, the World Association for Plastic, Reconstructive, and Aesthetic Surgery, the Serbian Association

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Correspondence to:
Milan T. STOJIČIĆ
Solunska 25/18
11158 Beograd
Serbia
doktorat123@gmail.com
milan.stojicic@med.bg.ac.rs

for Maxillofacial Surgery, the European Association for Maxillofacial Surgery, the International Association for Maxillofacial Surgery, the International Association for Craniofacial Surgery, and the Balkan Association for Otorhinolaryngology.

Dr. Rončević published approximately 300 scientific and professional papers, including 40 in leading international journals. According to the Scopus database, he was cited 260 times. He presented over 100 papers at international congresses, many of which were published as abstracts. He was a member of the editorial boards of four journals and a reviewer for numerous global journals. He wrote the book *Orbital Surgery*, which was recognized as a scientific monograph of national significance. In 2016, he published the book *Surgery of the Orbital Cavity: No-Man's-Land* in New York and co-authored several Serbian and American textbooks.

He delivered approximately 40 invited lectures at major global centers, including the Mayo Clinic, the New York Medical University Center, University Hospital in London, the Medical Institute in Sofia, Cairo, Rome, Glasgow, Tokyo, San Francisco, Miami, St. Petersburg, Moscow, Ufa University, and the Pennsylvania State University, and performed demonstration surgeries at three major global centers.

He led four domestic scientific projects and was a mentor or co-mentor for numerous master's and doctoral theses. He was fluent in English, Russian, and Slovak.

Dr. Rončević was also the author and petitioner for a scientific revision of the Theory of Evolution. In his public and political engagements, he advocated for the respect of Serbian national interests.

As a surgeon, Dr. Rončević was known for his vast knowledge, expertise, and dedication to his patients. He selflessly shared his experience, offering advice and support to younger colleagues, inspiring them to continually improve and become better doctors and people.

Dr. Radmilo Rončević passed away on January 18, 2024, after a short illness. He was buried at the New Cemetery in Belgrade.

His work and legacy will live on through his sons Dušan, Nemanja, and Vuk.

Milan T. Stojičić
University of Belgrade, Faculty of Medicine,
University Clinical Center of Serbia,
Clinic for Burns Plastic and Reconstructive Surgery
Maja J. Nikolić Živanović
University Clinical Center of Serbia,
Clinic for Burns Plastic and Reconstructive Surgery

Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонорарашу. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике, регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови**,** наручени коментари, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози. Оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови и актуелне теме, публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, $Б_{12}$, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

ЕТИЧКА САГЛАСНОСТ. Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу

оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant* – Cross Check (iThenticate). Радови код којих се докаже плагијаризам/аутоплагијаризам биће одбијени, а аутори санкционисани.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (http://www.nlm.nih.gov/mesh)*.

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом. Уколико је рад у целости на српском језику, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад и претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе и актуелну тему чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°*C*), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада који чине – насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, рад из историје медицине и преглед литературе до 5000 речи, а за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3000 речи; радови за остале рубрике могу имати највише 1500 речи.
Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике“ у CA се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).
Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видеоприказа у *e*-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публи-

кације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примере навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *http://www.nlm.nih.gov/bsd/uniform_requirements.html*. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био разматран за објављивање у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 9. Статута Друштва) и измирити накнаду за обраду чланака (*Article Processing Charge*) у износу од 3000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*Article Processing Charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату / накнаду за обраду чланка, као доказ о уплатама, уколико издавач нема евиденцију о томе. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за обраду чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

НАПОМЕНА. Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доле наведене адресе и број телефона.

АДРЕСА:
Српско лекарско друштво
Уредништво часописа „Српски архив за целокупно лекарство“
Ул. краљице Наталије 1
11000 Београд
Србија

Телефони: (+381 11) 409-2776, 409-4479
E-mail: *office@srpskiarhiv.rs*
Интернет адреса: *http://www.srpskiarhiv.rs*

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The papers are always submitted with Summary in both English and Serbian, included in the manuscript file. The text of the manuscript should be typed in *MS Word* using the *Times New Roman* typeface, and font size 12 pt. The text should be prepared with margins set to 25 mm and onto A4 paper size, with double line spacing, aligned left and the initial lines of all paragraphs indented 10 mm, without hyphenation. Tabs and successive blank spaces are not to be used for text alignment; instead, ruler alignment control tool and *Toolbars* are suggested. In order to start a new page within the document, *Page Break* option should be used instead of consecutive enters. Only one space follows after any punctuation mark. If special signs (symbols) are used in the text, use the *Symbol* font. References cited in the text are numbered with Arabic numerals within parenthesis (for example: [1, 2]), in order of appearance in the text. Pages are numbered consecutively in the right bottom corner, beginning from the title page.

When writing text in English, linguistic standard American English should be observed. Write short and clear sentences. Generic names should be exclusively used for the names of drugs. Devices (apparatuses, instruments) are termed by trade names, while their name and place of production should be indicated in the brackets. If a letter-number combination is used, the number should be precisely designated in superscript or subscript (i.e., 99Tc, IL-6, O2, B12, CD8). If something is commonly written in italics, such as genes (e.g. BRCA1), it should be written in this manner in the paper as well.

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The authors should enclose the description of contribution to the article of every co-author individually (within the Submission Letter). Funding, collection of data or general supervision of the research group alone cannot justify authorship. All other individuals having contributed to the preparation of the article should be mentioned in the *Acknowledgment* section, with description of their contribution to the paper, with their written consent.

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control. The manuscripts with approved plagiarism/auto-plagiarism will be rejected and authors will not be welcome to publish in Serbian Achieves of Medicine.

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SUMMARY. Along with the original article, preliminary and short communication, review article, case report, article on history of medicine, current topic article, article for language of medicine and article for practitioners, the summary not exceeding 100–250 words should be typed on the second page of the manuscript. In original articles, the summary should have the following structure: Introduction/Objective, Methods, Results, Conclusion. Each segment should be typed in a separate paragraph using boldface. The most significant results (numerical values), statistical analysis and level of significance are to be included. The conclusion must not be generalized, it needs to point directly to the results of the study. In case reports, the summary should consist of the following: Introduction (final sentence is to state the objective), Case Outline (Outline of Cases), Conclusion. Each segment should be typed in a separate paragraph using boldface. In other types of papers, the summary has no special outline.

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If an article is entirely in Serbian (e.g. article on history of medicine, article for "Language of medicine," etc.), captions and legends of all enclosures (tables, graphs, photographs, schemes) – if any – should be translated into English as well.

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If the manuscript is entirely in the Serbian language, tables and corresponding legend should be both in Serbian and English. Also, the table cells should contain text in both languages (do not create two separate tables with a single language!).

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ACKNOWLEDGMENT. List all those individuals having contributed to preparation of the article but having not met the criteria of authorship, such as individuals providing technical assistance, assistance in writing the paper or running the department securing general support. Financial aid and all other support in the form of sponsorship, grants, donations of equipment and medications, etc., should be mentioned too.

REFERENCES. The reference list is the responsibility of the authors. Cited articles should be readily accessible to the journals readership. Therefore, following each reference, its DOI number and PMID number (if the article is indexed for MEDLINE/PubMed) should be typed. References should be numbered in Arabic numerals in order of citation in the text. The overall number of references should not exceed 30, except in review articles, where

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ADDRESS:
Serbian Archives of Medicine
Editorial Office
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11000 Belgrade
Serbia
Phones: (+381 11) 409-2776, 409-4479
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CONTENTS

ORIGINAL ARTICLES

Milica Jovanović-Medojević, Dragana Jovanović, Mina Medojević, Strahinja Nedić, Đorđe Stratimirović, Sanja Vujašković, Mirjana Vujašković

THE PREVALENCE AND SEVERITY OF MOLAR INCISOR HYPOMINERALIZATION IN GROUP OF STUDENTS – A CROSS-SECTIONAL STUDY
128–139

Igor Đukić, Zoran Jezdić, Mirsad Kadić, Anesa Pirović Kadić, Ognjenka Janković, Aleksandra Radulović, Ivan Arsić, Aleksandar Jakovljević, Nenad Nedeljković

EXPLOITING MAXILLARY SINUS DIMENSIONS FOR SEXUAL DETERMINATION IN THE BOSNIAN AND HERZEGOVINIAN POPULATION
140–145

Uroš Anđelić, Marko Uzelac, Filip Filipović, Mihailo Ille, Marija Đurić, Jelena Jadžić

TRABECULAR MICROCALLI IN LUMBAR VERTEBRAE OF ADULT MEN WITH ALCOHOL-ASSOCIATED LIVER DISEASE: POSTMORTEM MICRO-COMPUTED TOMOGRAPHY ASSESSMENT
146–152

Liuxiu Yu, Mingfeng Xiong, Xiaoyu Li, Chunhong Wu, Liqin Wang, Yao Gao, Minxia Long, Qin Gan

EFFICACY OF XIAOZHOUTIAN FIRE DRAGON MOXIBUSTION COMBINED WITH TRADITIONAL CHINESE MEDICINE ON POLYCYSTIC OVARY SYNDROME WITH KIDNEY YANG DEFICIENCY – A RANDOMIZED CONTROLLED TRIAL
153–162

Saša S. Živić, Ivan Nikolić, Marko Jović, Vladimir Petrović, Dragan C. Bogdanović, Vladimir Stojiljković, Aleksandar Kamenov

MORPHOMETRIC CHARACTERISTICS OF THE GREAT SAPHENOUS VEIN AS GRAFT FOR SURGICAL MYOCARDIAL REVASCULARIZATION IN RELATION TO SEX
163–169

Željka Rogač, Aleksandar Dimitrijević, Ivana Andrić, Vesna Miranović, Dejan Stevanović

THE IMPACT OF CERTAIN ANTI-SEIZURE MEDICATIONS ON COGNITIVE STATUS, BEHAVIOR, ANXIETY, AND DEPRESSION IN SCHOOL-AGED CHILDREN WITH NEWLY DIAGNOSED EPILEPSY – A SIX-MONTH FOLLOW-UP STUDY
170–175

Jera Grabnar, Rina R. Rus

RENAL INJURY IN CHILDREN WITH A CONGENITAL SOLITARY KIDNEY – A SINGLE CENTER EXPERIENCE
176–180

Marina Nikitović, Aleksandar Stepanović, Tatjana Arsenijević, Nina Petrović

RELATION OF ONCOGENIC MICRORNA-10B AND MICRORNA-21 TO GLIOBLASTOMA SIZE AND LOCALIZATION
181–186

Xiaomei Huang, Hanxue Cai, Wen Zeng, Xueqin Zeng, Xinhong Xiao, Yi Liu, Yanhong Xu, Xiaomei Liu, Qinghua Zhong

EFFECT OF AURICULAR POINT ACUPRESSURE COMBINED WITH THREE-STEP ANALGESIC THERAPY ON CANCER PAIN
187–192

CASE REPORTS

Sofija Jovanović, Tijana Tomić, Nemanja Bidžić, Miloš Raspopović, Dragan Vasin

FAMILIAL ADENOMATOUS POLYPOSIS AND COLORECTAL CANCER – HOW SENSITIVE IS COMPUTED TOMOGRAPHY IN DETECTING THE UNDERLYING DISEASE?
193–196

Bojan Milenković, Miljan Bilanović, Slađan Timotijević, Miroslav Tatić, Đorđe Kolak

ISOLATED IPSILATERAL SHOULDER AND ELBOW DISLOCATION
197–200

Aleksandar Sretenović, Đorđe Topličić, Rastko Sava Jeremić, Dragana Vujović

BILIARY ATRESIA ASSOCIATED WITH INTESTINAL MALROTATION – UNUSUAL INTRAOPERATIVE PRESENTATION
201–204

Nikola Pantić, Milica Stojiljković, Lenka Grujičić, Dragana Šobić Šaranović, Vera Artiko

^{18}F-FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY / COMPUTED TOMOGRAPHY IN THE DIAGNOSIS OF SECONDARY MALIGNANCIES IN A PATIENT WITH HODGKIN LYMPHOMA
205–207

HISTORY OF MEDICINE

Zoran Vacić

GUIDELINES FOR THE PREPARATION AND DOSAGE OF MEDICINES, INSTRUCTIONS FOR THEIR USE, AND METHODS OF APPLICATION ACCORDING TO THE *HILANDAR MEDICAL CODEX NO. 517*
208–212

IN MEMORIAM

Milan T. Stojičić, Maja Nikolić Živanović

RADMILO RONČEVIĆ (JANUARY 20, 1938 – JANUARY 18, 2024)
213–214