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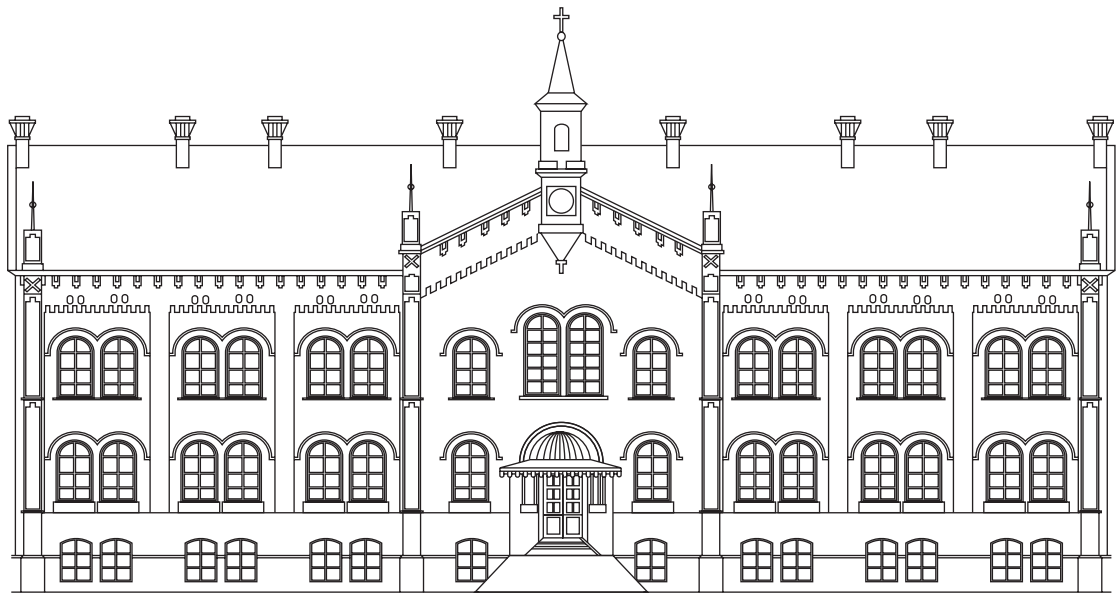

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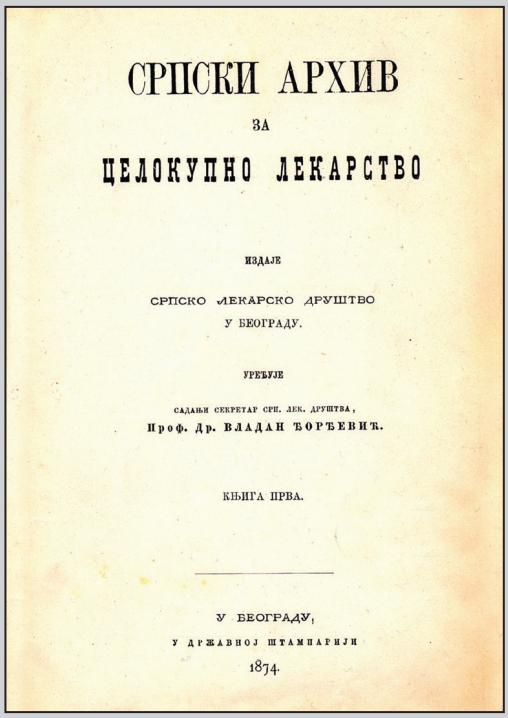


СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

ИЗДАЈЕ

СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

УРЕЂУЈЕ

САДАШЊИ СЕКРЕТАР СРП. ЛЕК. ДРУШТВА,

Проф. Др. ВЛАДАН ЂОРЂЕВИЋ.

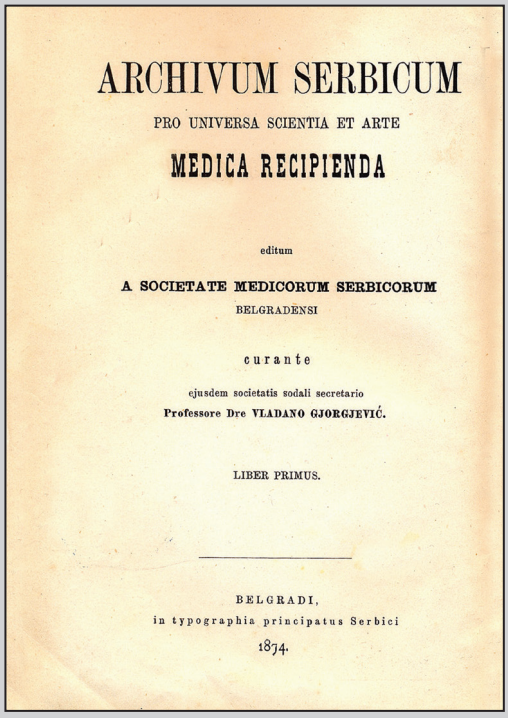
КЊИГА ПРВА.

У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

Прва страна првог броја часописа на српском језику



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A SOCIETATE MEDICORUM SERBICORUM

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curante

ejusdem societatis sodali secretario

Professore Dre VLADANO GJORGJEVIĆ.

LIBER PRIMUS.

BELGRADI,

in typographia principatus Serbici

1874.

The title page of the first journal volume in Latin

Српски архив за целокупно лекарство је часопис Српског лекарског друштва основаног 1872. године, први пут штампан 1874. године, у којем се објављују радови чланова Српског лекарског друштва, претплатника часописа и чланова других друштава медицинских и сродних струка. Објављују се: 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 и 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.

Сви рукописи који се разматрају за штампање у „Српском архиву за целокупно лекарство“ не могу да се поднесу или да буду разматрани за публиковање на другим местима. Радови не смеју да буду претходно штампани на другим местима (делимично или у потпуности).

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Радови се штампају на енглеском језику са кратким садржајем на енглеском и српском језику (ћирилица), односно на српском језику, са кратким садржајем на српском и енглеском језику.

Аутори прихватају потпуну одговорност за тачност целокупног садржаја рукописа. Материјал публикације представља мишљење аутора и није нужно одраз мишљења Српског лекарског друштва. С обзиром на брз напредак медицинске научне области, корисници треба да независно процењују информацију пре него што је користе или се на њу ослањају. Српско лекарско друштво, уредник или Уређивачки одбор „Српског архива за целокупно лекарство“ не прихватају било какву одговорност за наводе у радовима. Рекламни материјал треба да буде у складу с етичким (медицинским) и правним стандардима. Рекламни материјал укључен у овај часопис не гарантује квалитет или вредност оглашеног производа, односно тврдње произвођача.

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Srpski Arhiv Za Celokupno Lekarstvo (Serbian Archives of Medicine) is the Journal of the Serbian Medical Society founded in 1872, and with first issue published in 1874. Serbian Archives of Medicine publishes articles of the Serbian Medical Society members, subscribers, as well as members of other associations of medical and related fields. The journal publishes the following article types: editorials, original papers, preliminary and short communications, case reports, video-articles, images in clinical medicine, review articles, current topics, articles for practitioners, history of medicine articles, language of medicine articles, medical ethics (clinical ethics, publication ethics) and regulatory standards in medicine, congress and scientific meeting reports, personal view articles, invited commentaries, letters to the editor, book reviews, professional news, In memoriam and other articles.

All manuscripts under consideration in the Serbian Archives of Medicine may not be offered or be under consideration for publication elsewhere. Articles must not have been published elsewhere (in part or in full).

The submitted manuscripts are forwarded by the Editorial Board to reviewers for editing and evaluation. If the reviewers find that the manuscript needs to be modified or amended, the copy of the report is sent to the author(s), requiring of them to make necessary modifications or amendments of the text or to provide argumentative explanation of their disagreement with the suggested reviewer's remarks. The final decision on acceptance of the article for publication is made by the Editor-in-Chief.

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Српски архив за целокупно лекарство

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EDITORIAL / УВОДНИК

Serbian Archives of Medicine – the 2024 highlights

At the beginning of each new year, people glance at the memories left behind and then hopefully look forward to creating new, even more inspiring ones in the year ahead. And it is the same for us at the Serbian Archives of Medicine, the official journal of the Serbian Medical Society, the oldest society of its kind in modern medical Serbian history, founded in Belgrade (Serbia), on April 22, 1872 (according to the Julian calendar), i.e., May 4, 1872 (according to the Gregorian calendar) [1, 2].

In its most recent history, more precisely over the past six years that this Editorial Board was in charge, and amidst endeavors to render our journal more *en vogue* and keep the pace with global trends, including electronic submissions and editorial office handling of manuscripts, while at the same time encourage authors to embrace these novelties, the COVID-19 pandemic hit and did not spare a bit the medical and scientific community. Irrelevant of the challenging three years of pandemic and the following three in modest conditions linked to limited financial resources of the Serbian Medical Society that burdened the technical section of the Editorial office with additional chores, we are glad that we managed to conclude the year 2024 according to existing plans. We preserved its publishing continuity established back in December 2018, while the growing trend of submissions noted in 2023 was maintained throughout 2024, but now with a significant increase of foreign authors and acceptance of foreign reviewers to review papers *pro bono*.

Even in 2020, in spite of COVID-19 pandemic, we have called the attention of the growing global medical issue i.e., burnout, work-related syndrome, which at that time reached an epidemic level, and therefore has been recognized by the World Health Organization [3, 4]. Sadly, this issue while taking its toll on all employed in the healthcare sector, has proportionately grown in popularity research-wise [5], but we have only recently started to see submissions addressing it on a national scale.

Among other pressing issues, we have also tried to emphasize the necessity of relevant stakeholders' and legislators' engagement linked to most vulnerable in our society such as women, elderly and children as patients and women as healthcare providers who face additional daily challenges in a society that has been increasingly normalizing all forms of violence, starting from neglect in providing timely healthcare [6, 7]. We are grateful that in this effort to better our society as a whole, we could count on the support of the professors from Faculty of Law, University of Belgrade and private Universities as well who have participated both as authors and reviewers.

Over the past year, due to the exponentially growing number of submissions where ones from abroad stopped being a surprise, the Editorial Board has played an important part in supporting the Editor-in-Chief who due to aforementioned misfortunate events (the pandemic and ensuing financial scarcity, or maybe even *vice versa*) has taken upon herself numerous time-consuming tasks bellow the pay grade of the position. So, for the second year in a row, we have faced all options of compensating the technical support failures to be exhausted, despite our ongoing efforts. Authors' training to use the Serbian Archives of Medicine submission system in all stages of a submission have continued during 2024 which remains a time-consuming effort for although CEON offers a guide available to download online, a very small percentage of Serbian authors use it or shows willingness to. With a growing number of submissions, the number of registered users has increased as well, and since the process is not simple, it demands

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patience and tolerance in clarifying causes of encountered issues and further advisements on resolving it. The situation was additionally aggravated by the fact that internet providers encountered difficulties in their own area caused by additional increase in the number of internet users – a fact our authors are unaware of, which can cause additional frustration of the painstaking process that is independent of the Journal's requirements. Besides, academic networks have been targeted by hackers and malware, which furthermore aggravated occasional exchanges with the authors and reviewers', as much as the mere power shortages that can cause similar problems. Fortunately, our reviewers have fully embraced the electronic submission and review process.

The importance of use of iThenticate aiming to diminish plagiarism has helped discourage authors to use copy/paste during editing, although the program itself has its inherent logical drawbacks. Communicating the results of this analysis prior addressing a submission to further review, has been a delicate and complex process whether in contact with the authors or the reviewers.

I would like to take this opportunity to extend our heartfelt thanks to our partners – Službeni Glasnik and their Department in charge of our Journal's publishing process, in particular to Ms. Jasmina Živković, and Bytesoft company that has provided our website maintenance even when frugality made us end our formal contract, they provided us with an invaluable collaborator, Mr. Nemanja Anđelov who worked tirelessly all year round. Thanks to these two amazing people who never cared whether it was a working day or a weekend, our Journal hit both the stands and was available online in expected deadlines. I am convinced that the Serbian Medical Society and the Republic of Serbia shall find the needed resources to improve our Journal's logistic support and make one more step forward – or shall I say backward – and rejoin the PubMed search engine where it was last listed back in 2017.

Finally, I would like to take this opportunity to thank all members of the Editorial Board, the Publisher's Advisory Board and our reviewers (Table 1) for their selfless engagement during the past year.

At the end of my six-year term, I owe a special debt of gratitude to our two Editors gone too soon – Academicians Milorad Mitković (1950–2021) and Zoran Krivokapić (1955–2022). Not only did they personally review papers in record times, but they offered continuous support in advising in reviewers process beyond orthopedic and abdominal surgery which were their respective subspecialty fields.

Our Editorial Board lived some festive moments as well during 2024, and those were its members promotions: a corresponding member of the Serbian Academy of Sciences and Arts, professor Tatjana Simić, was promoted to the rank of academician only a month after stepping in her new role of the dean of the Faculty of Medicine of the University of Belgrade in October 2024, as the third woman in our faculty's 104-year-long history and professor Gordana Kocić joined the Serbian Academy of Sciences and Arts as corresponding member. Both of our amazing and inspiring colleagues to all women in medicine, always found time to both review and guidance on adequate reviewers whenever turned to for advice.

At the end of my term, allow me to express special thanks to academician professor Radoje Čolović, a stellar President of the Serbian Medical Society who not only entrusted me with this challenging and inspiring role, but also gifted me this honor each doctor and scientist in Serbia can be bestowed with and that is to become the Editor-in-Chief of the Serbian Archives of Medicine.

The Serbian Medical Society since December 2023 is led by its new President, professor Milan A. Nedeljković, upon whose proposal, on February 6, 2025, the Assembly of the Serbian Medical Society, has decided to endorse the Editorial Board with one more Editor-in-Chief, professor Siniša Stojković, full and active professor of the Faculty of Medicine of the University of Belgrade whom I wish a warm welcome aboard on this occasion while I hope this is just a first step announcing more involvement of colleagues from other medical schools nation-wide.

Conflict of interest: None declared.

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Honorary President, International Committee of Symposia on Morphological Sciences,
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Table 1. The list of the Serbian Archives of Medicine reviewers in 2024

1. Antić Andrija
2. Arsenijević Miloš
3. Avram Nada
4. Babović Ivana
5. Baljošević Ivan
6. Baraba Anja
7. Barać Aleksandra
8. Basarić Dragan
9. Baščarević Zoran
10. Bašić Dragoslav
11. Belojević Goran
12. Bila Jelena
13. Bila Jovan
14. Bilanović Dragoljub
15. Biljana Medjo
16. Binić Ivana
17. Bokun Jelena
18. Božić Marija
19. Bulat Petar
20. Cerovac Nataša
21. Chen Guang
22. Chen Guo-An
23. Chen Ziang
24. Clichici Simona Valeria
25. Crnogorac Snežana
26. Čolić Snježana
27. Čolović Milica
28. Čolović Radoje
29. Ćeranić Miljan
30. Dimitrijević Lidija
31. Dimitrijević Milovan
32. Ding Lingling
33. Dinić Ljubomir
34. Divac Nevena
35. Dizdarević Sabina
36. Đikanović Bosiljka
37. Đorđević Igor
38. Đorđević Vladimir
39. Đorđević Jocić Jasmina
40. Đukanović Ljubica
41. Đukić Vojko
42. Đurić Stefanović Aleksandra
43. Franić Damir
44. Gajić Ina
45. Galun Danijel
46. Galunić Bilić Lea
47. Gjorgijevska Elizabeta
48. Glišić Branislav
49. Gojnić Miroslava
50. Golubović Emilija
51. Gotić Mirjana
52. He Mu
53. He Wei
54. Ilić Đorđe
55. Ilić Miroslav
56. Ille Tatjana
57. Ivanov Olivera
58. Jakšić Vesna
59. Janković Aleksandra
60. Janković Radmila
61. Janković Svetlana
62. Janjić Vladimir
63. Ječmenica Lukić Milica
64. Jeremić Jelena
65. Ješić Miloš
66. Jiang Xiangao
67. Jotić Ana
68. Jovanović Milan
69. Jovanović Tanja
70. Jovanović Medojević Milica
71. Jovanović Simić Jelena
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77. Kocić Gordana
78. Kojić Zvezdana
79. Konstantinović Ljubica
80. Kontić Milica
81. Kontić Olivera
82. Kostić Mirjana
83. Kostić Svetislav
84. Kovač Jelena
85. Kovačević Igor
86. Kravljanac Ružica
87. Krstić Ivana
88. Krstić Miodrag
89. Kuzmanović Miloš
90. Lačković Maja
91. Lađević Nebojša
92. Lalić Nensi
93. Lalošević Dušan
94. Latas Milan
95. Lazarević Dragana
96. Lazić Zoran
97. Lečić Toševski Dušica
98. Lešić Aleksandar
99. Maksimović Nataša
100. Mandinić Zoran
101. Marić Gorica
102. Marić Nebojša
103. Marinković Darko
104. Marinković Vesna
105. Marković Dubravka
106. Marković Roberta Teofilo
107. Marković Nikolić Nataša
108. Martinović Tamara
109. Matić Aleksandar
110. Matić Slađana
111. Micev Marjan
112. Micić Ivan
113. Mihajlović Goran
114. Mihalj Marija
115. Mijač Dragana
116. Mijajlović Milija
117. Milenković Pavle
118. Miličković Maja
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128. Mitrović Nikola
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140. Özer Zülfünaz
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147. Peco Antić Amira
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155. Poskurica Mileta
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159. Radosavljević Aleksandra
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163. Rakić Snežana
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171. Samardžić Janko

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191. Svetel Marina
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196. Tasić Velibor
197. Teofilovski Parapid Gordana
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201. Tomić Dragan
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203. Trenkić Marija
204. Tudzarova Gjorgova Smilja
205. Vacić Zoran
206. Valla Frederic
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211. Veljković Andrej
212. Videnović Nebojša
213. Vojinov Saša
214. Vojvodić Nikola
215. Vujotić Ljiljana
216. Vuletić Biljana
217. Yang Zhan-Min
218. Zdraveska Marija
219. Zlatković Švenda Mirjana
220. Žagar Ivana

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Significance of T-Scan™ in recording occlusion parameters in orthodontic patients

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SUMMARY

Introduction/Objective In orthodontics accurate records about occlusal aspects: contacts, forces, loads, the total load force and bilateral force distribution are essential. The aim of this prospective clinical study was to evaluate occlusal parameters in different malocclusions and normal occlusions using the T-Scan III Novus (Tekscan Inc., Boston, MA, USA).

Methods Group of 43 patients, was divided in three types of malocclusions (class I, II, III), normal occlusion. A multi-bite scan was registered, using T-Scan III Novus (Tekscan Inc.). Data was analyzed with T-Scan software v 10 (Tekscan Inc.). The total force on the first molars was analyzed, and average force percentage compared. For bilateral load distribution, we analyzed total forces in the first; fourth versus the second; third quadrant, for each malocclusion, average force was assessed and compared.

Results The first molar's occlusal load showed that tooth #26 was favored to bear the highest load of all first molars in class II, III, and normal occlusion. In class I malocclusion all molars had similar forces. The highest occlusion force mean on the right side was in class III, and at the left side in class II. The highest discrepancy was in class II, then class III, class I, and the lowest in the normal occlusion.

Conclusion Normal occlusion was the most equilibrated, with the best load distribution, lowest discrepancy and highest force values, while in other classes there was a need for load equilibration and similar force distribution throughout dental arches to minimize discrepancy between left and right side of the jaws.

Keywords: T-Scan; malocclusion; occlusal load

INTRODUCTION

The orthodontic therapeutic goal is to achieve an ideal alignment between the teeth in the dental arch and to allow even distribution of the generated forces during the act of mastication [1]. For instance, any premature occlusal contact can generate occlusal stress which leads to alterations in the tooth-supporting tissues, the masticatory muscles, and temporo-mandibular joint [1]. Occlusal articulation relations can be recorded using several occlusal analyzers. Articulating paper being the most used occlusal analyzer for determining contact points between the maxillary and mandibular arch. However, the paper can only record contact points and is unable to accurately quantify their intensity and/or determine the magnitude of the generated occlusal forces [1].

Clinicians use occlusal contact detection to identify the height of restorations, equilibrate occlusion [2], and to perform post-orthodontic adjustments [3, 4, 5]. However, these static indicators only mark the surface area of dental contact, and do not have the ability to assess the degree of occlusal force within the contact or quantify it is time variance. These methods are based on clinician's "subjective interpretation" combined with the patient's feeling and verbal feedback [6]. The correlation between the size of occlusal marking and the actual relative occlusal force contained within the marking is only 21%, if the largest paper mark on a tooth represents the most forceful contact, may result in wrong contact adjustment [7]. There is not enough scientific evidence that shows articulating paper can reproduce occlusal force, to justify its continued use as a diagnostic aid [8, 9].

Maness invented the T-Scan system for computer occlusal analysis in 1987 which allows real-time measurements of occlusal forces to be captured with intraoral sensor. The tool was upgraded over the years, with software and hardware modifications until current version of the system, known as T-Scan III Novus (Tekscan Inc., Boston, MA, USA). Graphical interface is supported by the T-Scan software v 10 (Tekscan Inc.) [8]. The program utilizes the data and displays it in full color 3D or 2D images. The resultant occlusal contacts are visualized as contours or cellular pictures on dental arch in 2D graphics. Moreover, the left and right sides can be displayed in distinct color codes (green on the left, red on the right), with the respective occlusal forces given underneath [9–12]. The dentition can also be divided into

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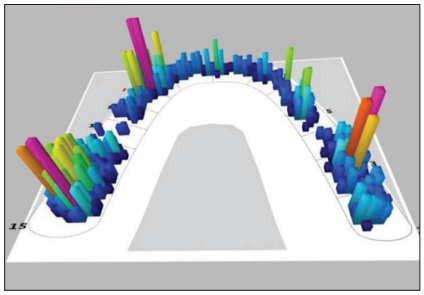

Figure 1. 3D occlusal load interpretation

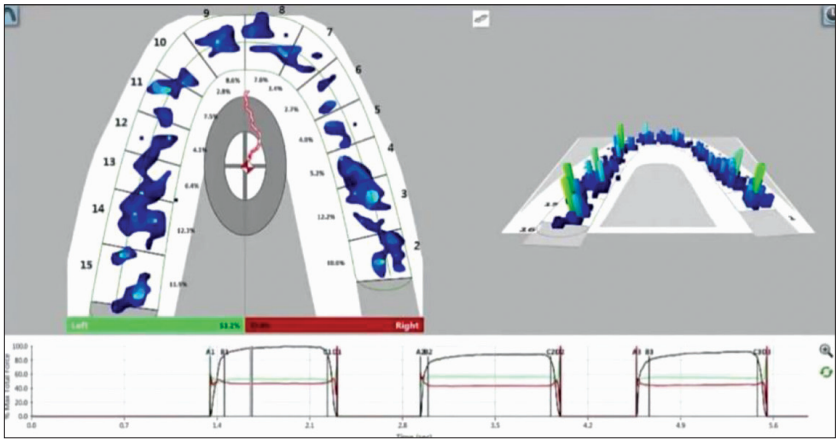

Figure 2. 2D occlusal load interpretation

two halves: anterior and posterior, dividing it in four study segments [13, 14] (Figures 1 and 2).

The aim of this prospective clinical study was to evaluate occlusal parameters in different malocclusions and normal occlusions using the T-Scan III Novus (Tekscan Inc.).

METHODS

This prospective clinical study was performed at Ras Al Khaimah college of dental sciences, Dubai, United Arab Emirates. The study was approved by the ethics committee of the School of Dental Medicine, University of Belgrade (No. 36/24)598 and it meets the criteria for medical research involving human subjects according to the ethical principles described in the Declaration of Helsinki. Study included 43 patients, with different types of malocclusions and normal occlusion, divided into four groups. Age range was 18–60 years old. All the subjects were given written consent.

Inclusion criteria: class I malocclusion (normal molar relationship, with crowding, misalignment of the teeth, rotations, cross-bites, and other alignment irregularities), class II malocclusion, class III malocclusion, normal occlusion. Exclusion criteria: patients with TMJ disorders, patients with severe malocclusion who require surgical treatment. Participants were assessed, a multi-bite scan was registered, using the T-Scan III Novus (Tekscan Inc.) for each patient to record the occlusal parameters.

Two variables were assessed:

1. NET discrepancies of forces generated at maximum intercuspation position between the left and the right side of the mouth.

2. the total average occlusal force of the first molars withstanding at maximum intercuspation position.

The patients were seated on the dental chair with the lower and upper half of the body positioned at an angle of 90º. Data acquisition using the T-Scan III Novus device (Tekscan Inc.) consisted of registering occlusal contacts with a sensor film, data transfer though a module called the 'handpiece' which is linked to a computer, with data processing software, to visualize the parameters on the computer screen (Figure 3).

Figure 3. T-Scan III Novus (Tekscan Inc.) handle and sensor film for load registration

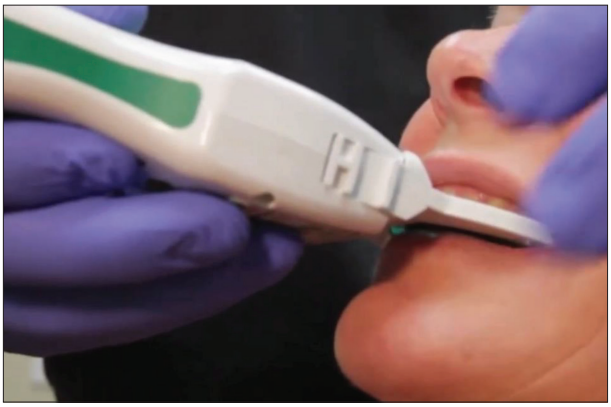

Figure 4. T-Scan intraoral load registration

The recording sensor was inserted intraorally between the dental arches so that the central mark is positioned between the central incisors of a patient. Recording started with pressing the button on the handlebar; the patient was instructed to occlude firmly to complete intercuspation. A multi-bite scan was recorded for each subject consisting of three bites consequently, to minimize the possibility of an error. Values of the three readings were assessed for each patient. Nevertheless, the maximum intercuspation position – the B point interval, was also taken into consideration in this study (Figure 4).

Scan records were analyzed using the T-Scan III Novus software v 10 (Tekscan Inc.). The total force on the first molars was analyzed on each scan. For these selected teeth, an average force percentage was calculated and compared. For bilateral load distribution assessment, we analyzed the

total forces in the first and fourth quadrant versus the second and third quadrant, for every patient and each malocclusion. Data was analyzed and average force for the right side (first : fourth quadrants) *vs.* left side (second : third quadrants) was assessed.

Data was processed using the IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA) software. Both descriptive and inferential statistics were used to describe the sample, identify differences in mean values between each tooth. The exact (and approximate) 95% confidence intervals, statistics test values, and p-values were reported. The p-value ($p < 0.05$) was defined as statistically significant.

Descriptive statistics was used to summarize the mean and standard deviation of each molar variables. One-way analysis of variance (ANOVA) was used to determine whether there are any significant differences in occlusion force means between groups. Post hoc was used to figure out which groups in the sample differ and to compare every mean with another.

RESULTS

Patient assessment:

At clinical assessment, the subjects were sorted out according to Angle's classification of malocclusion. In total, 14 subjects were diagnosed with class I malocclusion, eight subjects were diagnosed with class II malocclusion, nine subjects were diagnosed with class III malocclusion, and finally 12 subjects had no malocclusion (normal occlusion).

T-Scan III Novus data assessment:

The results in Table 1 display the mean values of occlusal force for each molar independently. The results showed that the highest occlusal force in the normal occlusion was noted in tooth (T) 26 of (B1, B2, B3), (B – point interval of maximal intercuspation), the mean of B1 was (14.8), B2 (14), and B3 (14.6). On the other hand, the readings of class I malocclusion were approximately close to one another, which ranged 9.4–12.5. Similarly in class II malocclusion, the values of T26 were the highest. B1, B2, and B3 had readings of mean values (11.6, 10, and 9.7) respectively. Finally, in regards of class III malocclusion, the readings of occlusion force of T26 and T36 were approximately the same, but they were higher compared to T16 and T46 (Table 1).

As the first molars are Angle's keys of the occlusion, they were of particular interest for this assessment. For purpose of this study, the analysis was narrowed to specific teeth: 16 – upper right first molar, 46 – lower right first molar, as opposed to each other they form an occlusal unit. As well as 26 – upper left first molar, 36 – lower left first molar on the opposite side of dental arch. Table 2. shows that the lowest occlusion force was noted in class II malocclusion between teeth 16 (5.7444 ± 5.98567) and 46 (3.0519 ± 4.18051). On the other hand, the highest occlusion force was noted in the normal occlusion between teeth 16 (13.5917 ± 10.50322) and 46 (14.4296 ± 5.79900). In regards of class I malocclusion, the occlusion force between teeth 16 and 46 was slightly higher compared to teeth 26 and 36. In contrast, the occlusion force in class III malocclusion of teeth 26 and 36 was higher than in 16 and 46. In total the highest values were noted at normal occlusion.

Table 1. Descriptive statistics for each molar by classification

Normal occlusion				
	T-16	T-26	T-36	T-46
B1	12.06	14.8	10.8	10.3
B2	10.68	14	11.7	8.9
B3	14.13	14.6	8.79	11.2
Class I malocclusion				
	T-16	T-26	T-36	T-46
B1	9.869	12.523	11	11
B2	10.692	11.783	10	10
B3	11.220	10.090	10	9.4
Class II malocclusion				
	T-16	T-26	T-36	T-46
B1	4.81	11.6	8.5	3.37
B2	5.06	10	6.8	4.82
B3	8.53	9.7	8.5	2.23
Class III malocclusion				
	T-16	T-26	T-36	T- 46
B1	9.712	12.51	12.17	7.21
B2	10.1	11.50	11.54	7.53
B3	7.875	10.87	11.55	6.28

T – tooth number; B – point interval maximal intercuspation

One-way analysis of variance (ANOVA) is used to determine differences in occlusion force mean values between groups. Table 3 shows the descriptive statistics of occlusion force by ANOVA. The highest occlusion force mean at the right side was reported in class III malocclusion (53.3019 ± 13.32165). While on the left side highest values were noted in class II (57.3854 ± 12.29782). The NET discrepancy indicates, that the highest value was noted in class II malocclusion, followed by class III

Table 2. Descriptive statistics of malocclusion as a group

Parameters	Class I malocclusion	Class II malocclusion	Class III malocclusion	Normal
16 (B1 + B2 + B3)	10.1267 ± 7.01293	5.7444 ± 5.98567	8.0815 ± 7.78995	13.5917 ± 10.50322
26 (B1 + B2 + B3)	9.8367 ± 7.48175	9.0074 ± 7.91359	10.4852 ± 14.45189	13.2944 ± 10.56608
36 (B1 + B2 + B3)	10.0333 ± 8.10563	6.7556 ± 6.40297	10.5519 ± 15.47594	9.5111 ± 7.91757
46 (B1 + B2 + B3)	9.9400 ± 6.07756	3.0519 ± 4.18051	7.5905 ± 8.31459	14.4296 ± 5.79900

B – point interval maximal intercuspation

Table 3. Descriptive statistics result by Analysis of Variance (ANOVA)

Parameters		N	Mean	Standard deviation	Standard error	95% confidence interval for mean		Minimum	Maximum	Significance #
						Lower bound	Upper bound			
Right	Normal	12	46.5423	9.74603	2.70306	40.6528	52.4318	27.60	61.63	p = 0.238
	Class I	14	44.7231	11.29099	3.01765	38.2039	51.2423	29.17	71.17	
	Class II	8	42.6146	12.29782	4.34794	32.3333	52.8958	26.67	60.23	
	Class III	9	53.3019	13.32165	4.44055	43.0619	63.5418	31.60	74.53	
Left	Normal	12	53.4577	9.74603	2.70306	47.5682	59.3472	38.37	72.40	p = 0 .238
	Class I	14	55.2615	11.27031	3.01212	48.7543	61.7688	28.83	70.83	
	Class II	8	57.3854	12.29782	4.34794	47.1042	67.6667	39.77	73.33	
	Class III	9	46.6981	13.32165	4.44055	36.4582	56.9381	25.47	68.40	
NET Discrepancy	Normal	12	15.1000	13.59180	3.76969	6.8866	23.3134	0.47	44.80	p = 0.544
	Class I	14	19.3766	14.97600	4.00250	10.7297	28.0234	2.47	42.33	
	Class II	8	24.7292	12.46588	4.40736	14.3074	35.1509	3.90	46.67	
	Class III	9	20.0556	17.50490	5.83497	6.6001	33.5110	1.00	49.07	

N – number of patients; #one way ANOVA

Table 4. Multiple comparison (post-hoc Tukey) of dependent variable

Dependent variable			Mean difference (I–J)	Standard error	Significance	95% confidence interval	
						Lower bound	Upper bound
Right	Normal	Class I	1.81923	4.42333	1.000	-10.4593	14.0978
		Class II	3.92772	5.16055	1.000	-10.3973	18.2527
		Class III	-6.75954	4.97991	1.000	-20.5831	7.0640
	Class I	Normal	-1.81923	4.42333	1.000	-14.0978	10.4593
		Class II	2.10849	5.08986	1.000	-12.0203	16.2373
		Class III	-8.57877	4.90661	0.528	-22.1989	5.0413
	Class II	Normal	-3.92772	5.16055	1.000	-18.2527	10.3973
		Class I	-2.10849	5.08986	1.000	-16.2373	12.0203
		Class III	-10.68727	5.58035	0.376	-26.1776	4.8030
	Class III	Normal	6.75954	4.97991	1.000	-7.0640	20.5831
		Class I	8.57877	4.90661	0.528	-5.0413	22.1989
		Class II	10.68727	5.58035	0.376	-4.8030	26.1776
Left	Normal	Class I	-1.80385	4.42079	1.000	-14.0754	10.4677
		Class II	-3.92772	5.15758	1.000	-18.2445	10.3890
		Class III	6.75954	4.97705	1.000	-7.0561	20.5752
	Class I	Normal	1.80385	4.42079	1.000	-10.4677	14.0754
		Class II	-2.12388	5.08693	1.000	-16.2445	11.9968
		Class III	8.56339	4.90379	0.531	-5.0489	22.1757
	Class II	Normal	3.92772	5.15758	1.000	-10.3890	18.2445
		Class I	2.12388	5.08693	1.000	-11.9968	16.2445
		Class III	10.68727	5.57714	0.375	-4.7941	26.1687
	Class III	Normal	-6.75954	4.97705	1.000	-20.5752	7.0561
		Class I	-8.56339	4.90379	0.531	-22.1757	5.0489
		Class II	-10.68727	5.57714	0.375	-26.1687	4.7941
NET Discrepancy	Normal	Class I	-4.27656	5.67110	1.000	-20.0188	11.4657
		Class II	-9.62917	6.61628	0.920	-27.9951	8.7367
		Class III	-4.95556	6.38468	1.000	-22.6786	12.7675
	Class I	Normal	4.27656	5.67110	1.000	-11.4657	20.0188
		Class II	-5.35261	6.52564	1.000	-23.4669	12.7617
		Class III	-0.67900	6.29071	1.000	-18.1412	16.7832
	Class II	Normal	9.62917	6.61628	0.920	-8.7367	27.9951
		Class I	5.35261	6.52564	1.000	-12.7617	23.4669
		Class III	4.67361	7.15450	1.000	-15.1863	24.5335
	Class III	Normal	4.95556	6.38468	1.000	-12.7675	22.6786
		Class I	0.67900	6.29071	1.000	-16.7832	18.1412
		Class II	-4.67361	7.15450	1.000	-24.5335	15.1863

malocclusion, then class I malocclusion, and the lowest discrepancy was found in the normal occlusion; (24.7292 ± 12.46588, 20.0556 ± 17.50490, 19.3766 ± 14.97600, and 15.1000 ± 13.59180) respectively (Table 3). The results showed that there was no statistically significant difference between the mean values of occlusion force between the selected teeth (16, 26, 36, and 46) within groups. There was no statistically significant difference within groups as determined by one-way ANOVA in regards of right and left side ($p = 0.238$), similarly, to the NET discrepancy there was "no" statistically significant difference between groups ($p = 0.544$) (Table 3).

Multiple comparisons show which groups differed from each other. In Table 4 the results showed that there are no significant differences between the groups as whole. The Tukey post-hoc test was used for conducting post-hoc tests on a one-way ANOVA. A Tukey post-hoc test showed that there were no significant differences between the groups as whole as the p-value ranged from 0.375 to 1.000.

The crosstabulation table showed that seven (25.9%) of the participants had discrepancy compared to five (35.3%) without discrepancy, with normal occlusion. While eight (29.6%) participants had a discrepancy compared to six (35.3%) in class I malocclusion. Moreover, seven (25.9%) participants vs one (5.9%) participant in class II malocclusion had discrepancy in occlusion force. In class III malocclusion, five (18.5%) had a discrepancy compared to four (23.5%) without discrepancy. In total, 27 participants had a discrepancy compared to 16 participants without discrepancy (Table 5) (Figure 5).

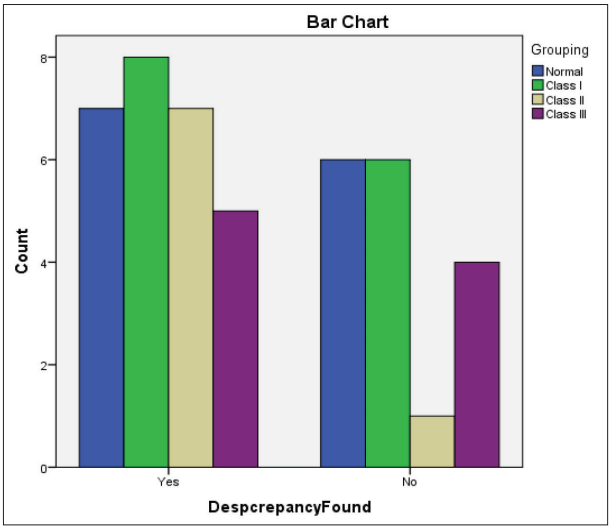

Figure 5. Discrepancies of occlusion force of all situations as outcome of χ^2

Table 5. Grouping cross-tabulation

Parameters		Grouping				Significance
		Normal	Class I	Class II	Class III	
Discrepancy found	Yes	7 (53.8%)	8 (57.1%)	7 (87.5%)	5 (55.6%)	p = 0.416
	No	5 (46.2%)	6 (42.9%)	1 (12.5%)	4 (44.4%)	
Total		12 (100%)	14 (100%)	8 (100%)	9 (100%)	

DISCUSSION

An important part of dental assessment in orthodontics, prosthetics, implantology, and other branches of dentistry is information about occlusal contacts. Over the years this information was obtained in many ways of which the most used occlusal analyzer for determining contact points between the maxillary and mandibular arch was articulating paper. Chowdhary and Sonnahalli [1]. stated that this manner of intermaxillary contact assessment resulted as less accurate, since the only information are the dots and shapes that cannot be quantified. Nevertheless, the novel generation of intraoral digital occlusal contact identifier T-Scan III Novus (Tekscan Inc.) is the most reliable system for dental contact assessment. This system provides 2D and 3D visualization of dental contacts and measures the force between the teeth. In this study statistical analysis was done with information obtained with T-Scan III Novus (Tekscan Inc.) and measurement of occlusal force.

Other authors emphasized the role of the first molars in balanced occlusion and Angle was the first who stated that the key of the occlusion were the first molars, that is why the first molar load distribution was of particular interest for this study [2–5]. First of all, the individual load of the first molars was assessed, tooth T26 in normal occlusion showed the highest values of load barring (B1 14.8, B2 14.0, B3 14.6). The T16 in the normal occlusion had similar but somewhat lower values of load barring (B1 12.06, B2 10.68, B3 14.13). Which indicates that the highest load was measured in region of upper first molars. In class I malocclusion tooth T26 was also barring the highest load (B1 12.523, B2 11.783, B3 10.090), but the differences between the measured teeth (T16, T26, T36, T46) were not as high, ranging from 9.4 to 12.523. This indicates similar load distribution in each one of the first molars. Class II malocclusion also showed the highest load on the tooth T26 – B1 11.6, B2 10, B3 9.7. While in class III malocclusion T26 and T36 had higher readings compared to the T16 and T46, which indicates higher load force in the first molar region on the left side of upper and lower dental arch (Table 1).

When the first molar occlusal units (16:46;26:36) were assessed the data showed that the lowest force was in class II malocclusion between teeth 16 and 46 (5.7444 ± 5.98567 and 3.0519 ± 4.18051) respectfully. Nevertheless, the highest force was noted in normal occlusion between teeth 16 and 46 (13.5917 ± 10.50322 and 14.4296 ± 5.79900) respectfully. In class I malocclusion the occlusion force between teeth 16 and 46 was slightly higher than between 26 and 36. In contrast, the occlusion force in class III malocclusion between teeth 26 and 36 was higher than between 16 and 46. In total the highest values were noted at normal occlusion (Table 2). This illustrates the load distribution through contact surfaces in different classes, the load is changing depending on number and size of contacts.

As Rubió-Ferrer et al. [3] stated slight lateral asymmetries in occlusal contact area and masticatory muscle force are relatively frequent, because maximum bite force and occlusal contact area are key to masticatory performance,

mastication is more frequently dominant on one side which usually offers the most efficiency. This was also suggested in our study where one-way analysis of variance ANOVA was used to determine whether there are any differences in occlusion force mean values between groups, which showed that the highest occlusion load mean was noted at the right side in class III malocclusion, while on the left side the highest mean value was noted in class II. NET discrepancy showed that the highest mean value was in class II followed by class III than class I and normal occlusion (24.7292 ± 12.46588, 20.0556 ± 17.50490, 19.3766 ± 14.97600, and 15.1000 ± 13.59180) respectfully (Table 3). This data demonstrates that the normal occlusion with lowest NET discrepancy mean, showed the most balanced relationship between left and right side. Analysis of variance ANOVA showed that there were no statistical differences within groups between the mean values of occlusion force of the teeth 16, 26, 36, and 46. This illustrates that in every group there was similar load distribution in each one of the first molars as shown in Table 3.

The results of comparison of groups to establish how groups differed from each other showed that there were no statistically significant differences between groups as whole, which indicates that groups did not differ in a significant manner. Post-hoc Tukey test on one way ANOVA showed that there were no statistically significant differences between groups as whole since the p value ranged 0.375–1.000 (Table 4).

CONCLUSION

Normal occlusion was the most equilibrated, with the best load distribution, lowest discrepancy and highest force values, while in other classes there was a need for load equilibration and similar force distribution throughout dental arches to minimize discrepancy between left and right side of the jaws.

ACKNOWLEDGMENT

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Conflict of interest: None declared.

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Значај Т-скена у регистровању оклузалних параметара код ортодонтских пацијената

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САЖЕТАК

Увод/Циљ У ортодонцији је од великог значаја тачна евиденција о оклузалним аспектима: контактима, силама, оптерећењима, укупној сили оптерећења и билатералној расподели силе.

Циљ ове проспективне клиничке студије била је процена оклузалних параметара код различитих малоклузија и нормалне оклузије коришћењем *T-Scan III Novus*-а (Tekscan Inc., Бостон, MA, САД).

Методе Група од 43 пацијента подељена је у три типа малоклузије (класа I, II, III) и нормалну оклузију. Регистровано је скенирање више загрижаја коришћењем *T-Scan III Novus*-а (*Tekscan Inc.*). Подаци су анализирани софтвером *T-Scan v* 10. Анализирана је укупна сила на првим моларима и упоређен је просечни проценат силе. За билатералну расподелу оптерећења анализирали смо укупне силе у првом и четвртом наспрам другог и трећег квадранта, а за сваку малоклузију процењена је и упоређена просечна сила.

Резултати Оклузално оптерећење првих молара показало је да је зуб #26 поднео највеће оптерећење од свих првих молара у класама II, III и нормалној оклузији. У класи I малоклузије сви молари су имали сличне силе. Највећа средња сила оклузије на десној страни била је у класи III, а на левој страни у класи II. Највеће одступање било је у класи II, па у класи III и класи I, а најмање у нормалној оклузији.

Закључак Нормална оклузија је била најуравнотеженија, са најбољом расподелом оптерећења, најнижим одступањем и највећим вредностима силе, док је у другим класама постојала потреба за уравнотежењем оптерећења и сразмерном расподелом силе кроз зубне лукове како би се минимизирало неслагање између леве и десне стране вилица.

Кључне речи: Т-скен; малоклузија; оклузално оптерећење

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Maxillary sinus augmentation utilizing Xenograft, Bichat`s fat pad tissue and low-level light therapy – cone beam computed tomography and resonance frequency analysis results of a prospective randomized clinical study

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SUMMARY

Introduction/Objective Implant placement in the posterior maxilla may be often hard to achieve because of insufficient bone volume and the presence of a highly pneumatized maxillary sinus. In these situations, sinus floor augmentation frequently has been proposed as the treatment possibility, conventionally performed utilizing xenograft materials. This research aims to study whether fragmented fat tissue from the Bichat's fat pad mixed with bovine-derived bone yields better results than the use of bovine-derived bone alone in maxillary sinus augmentation. The secondary aim was to evaluate the influence of low-level light therapy on bone regeneration in patients treated with fragmented fat tissue mixed with bovine-derived bone.

Methods Six patients were included in the study; 12 maxillary sinus augmentation procedures were performed, and patients were randomly assigned into three groups. Six months after surgery a cone-beam computed tomography bone density analysis was performed, and resonance frequency analysis (RFA) was performed on 12 placed implants.

Results Bone density results yielded notable differences in Hounsfield units, with experimental groups (499.94 ± 88.43) resembling natural bone more when compared with the control group (674.57 ± 217.12). RFA data shows that the results exhibit a degree of comparability or moderately better stability in the experimental groups (56.88 ± 6.03) compared to the control group (53 ± 20.12).

Conclusions The given Hounsfield units and RFA analysis serve as clear indicators of the substantial potential of fragmented fat tissue and xenograft mixture in maxillary sinus augmentation, by its complete integration and provision of significant stability to the inserted implants. Xenograft mixed with Bichat's fat pad tissue may represent an important novel entity in the field of bone regeneration.

Keywords: novel graft; bone regeneration; fat tissue; low-level light therapy

INTRODUCTION

Implant placement in the posterior maxilla may often be hard to achieve because of insufficient bone volume and the presence of a highly pneumatized maxillary sinus. In these situations, sinus floor augmentation frequently has been proposed as a treatment possibility. Grafting the floor of the maxillary sinus has emerged as the most common surgical modality for correcting this inadequacy. This technique, first published in 1980 by Boyne [1], and subsequently modified by other clinicians, can result in an increase in bone height that allows the placement of implants of conventional length in the grafted sites. Crestal sinus lift involves accessing the maxillary sinus through the alveolar crest, typically via the implant osteotomy site, to elevate the Schneiderian membrane and place a bone graft material. This technique is suitable for cases with minor to moderate bone deficiency. Lateral sinus lift is a surgical procedure designed to increase bone height in the posterior maxilla when there is significant bone loss. This technique involves creating a lateral access window in the maxillary sinus wall to elevate the Schneiderian membrane and place a bone graft material. As surgical treatment was modified, with time and concomitant improved insight into technology and regenerative medicine, grafting materials were also modified. Various surgical techniques and biomaterials have been developed to make possible the successful placement of dental implants in resorbed alveolar bone, and multiple bone grafting techniques including natural and synthetic graft materials have been tested for this purpose. The process of osteogenesis has

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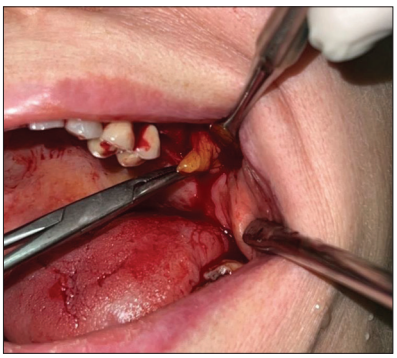

Figure 1. A small fragment of Bichat's fat pad is harvested through the existing flap with no additional morbidity

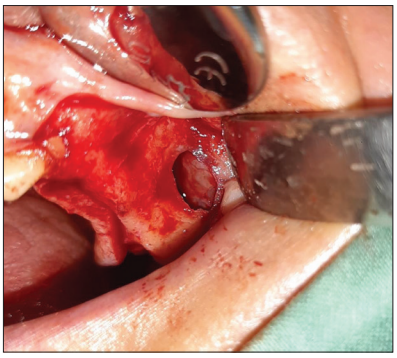

Figure 2. Lateral maxillary sinus augmentation approach

been described as the direct transfer of vital cells to the area that will regenerate new bone. Osteoconduction embraces the principle of providing space and a substratum for the cellular and biochemical events progressing to bone formation. The space maintenance requirement for many of the intraoral bone augmentation procedures allows the correct cells to populate the zone of focus. Osteoinduction embodies the principle of converting pluripotential, mesenchymal-derived cells along an osteoblast pathway with the subsequent formation of bone. With this in mind, it is imperative to design and employ a graft with a significant and optimal regenerative potential.

Fat tissue characterization and subsequent utilization in tissue reconstruction have been found in contemporary literature. Adipose tissue contains a multipotent cell population with similar properties, although not identical, to those of marrow-derived mesenchymal stem cells [2]. Adipose-derived stem cells are shown to be pluripotent *in-vitro* as well as *in-vivo* [3], and utilization of whole fat tissue is also shown to produce bone in critical size bone defects [4].

This research aims to study whether fragmented fat tissue from the Bichat's fat pad mixed with bovine-derived bone yields better results than the use of bovine-derived bone alone in maxillary sinus augmentation. Additionally, a secondary objective was to examine the effect of low-level light therapy (LLLT) to investigate its potential enhancement of bone regeneration.

METHODS

Before commencement, this study received approval from the Ethical Committee of the School of Dental Medicine, University of Belgrade (Approval No. 36/11). The research adhered to the principles outlined in the Declaration of Helsinki. The investigation was conducted at the Department of Maxillofacial Surgery and the Department of Periodontology and Oral Medicine, School of Dental Medicine, University of Belgrade. Patient inclusion, surgical procedures, data collection, and analysis were carried out during a two-year period (June 28, 2022 – June 28, 2024).

All patients were informed about the study/surgical protocol and provided their informed consent for participation in the study. The study sample comprised patients who were presented at the School of Dental Medicine, University of Belgrade, for implant rehabilitation and were diagnosed with partial edentulism with atrophy of the posterior maxilla. Unilateral and bilateral atrophy cases were included. The inclusion criteria were the presence of a periodontally healthy frontal maxillary segment due to utilization of a computer-guided system, and a residual bone height of 1–4 mm in the posterior maxilla. Six patients (one female and five males) were included in this study. A total of 12 maxillary sinus augmentations were performed and 12 implants were inserted.

Exclusion criteria were the following: acute or chronic sinusitis, active sinus or nasal infections, sinus membrane perforation during surgery, history of surgery in the sinonasal region, history of radiation therapy in the head or neck region, systemic diseases such as uncontrolled diabetes or autoimmune diseases, pregnancy or lactation and history of significant bone metabolic disorders.

Cone beam computed tomography (CBCT) scans were performed before surgery, and patients were randomly assigned into three groups:

Group 1 (control): maxillary sinus augmentation (n = 4) using bovine-derived bone (Bio-Oss, Geistlich Pharma AG, Wolhusen, Switzerland);

Group 2 (Xenograft + fat tissue): maxillary sinus augmentation (n = 4) using fragmented fat tissue mixed with bovine-derived bone (Bio-Oss, Geistlich Pharma AG) in a 50:50 ratio;

Group 3 (Xenograft + fat tissue + LLLT): maxillary sinus augmentation (n = 4) using fragmented fat tissue mixed with bovine-derived bone (Bio-Oss, Geistlich Pharma AG) in a 50:50 ratio, treated with 635 nm pulsing LLLT (Repuls 7, Repuls Lichtmedizintechnik GmbH, Wien, Austria).

In all three groups, after maxillary sinus augmentation, the lateral bone window is covered with a collagen membrane (Bio-Gide, Geistlich Pharma AG).

Preparation of graft:

Xenograft-Bichat's fat pad mixture (Xenofat graft) was prepared utilizing fat tissue harvested from the Bichat's fat pad (Figure 1). This adipose tissue was obtained concomitantly with the flap used for the lateral sinus lift procedure (Figure 2), thereby minimizing additional morbidity. Fragments of fat tissue were meticulously excised from Bichat's fat pad and subsequently washed with a physiological solution. Following this, the fat tissue was fragmented into smaller particles (Figure 3) and mixed in a 50:50 ratio with bovine-derived bone graft material. The prepared mixture (Figure 4) was carefully placed within the maxillary sinus, positioned between the Schneiderian membrane and the floor of the sinus cavity, as part of the sinus augmentation procedure.

Figure 3. Fragmented tissue of the Bichat's fat pad

Figure 4. Mixture of Bichat's fat pad tissue and xenogenic bone in a 50:50 ratio

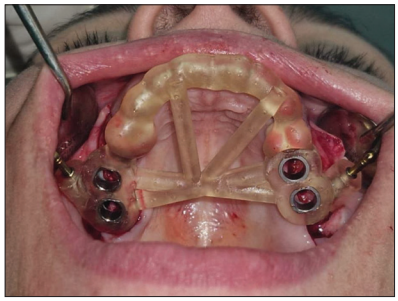

Figure 5. Fully computer-guided implant protocol

Surgical procedure

First stage

Antibiotic prophylaxis (1 g of amoxicillin with clavulanic acid or 600 mg clindamycin in cases of penicillin hypersensitivity) was prescribed to each patient, starting one hour before the surgery. Dexamethasone (0.004 g) was administered subcutaneously before surgery. The surgical procedures are performed in the conditions of local anesthesia (Septanest, Septodont, Saint-Maur-des-Fossés, France). All three groups underwent the sinus augmentation procedure using a lateral bone window approach (Figure 2). Surgical procedures in the first group include a conventional maxillary sinus augmentation procedure with the use of bovine-derived bone, while in the second and third groups, Xenograft + Bichat's fat pad tissue was utilized for sinus augmentation. The wounds were sutured with interrupted resorbable sutures. Patients from the third group are additionally treated with 635 nm LLLT (Repuls Lichtmedizintechnik GmbH) in five sessions starting from the third postoperative day.

Second stage

Six months after maxillary sinus augmentation, CBCT scans were obtained and patients were scheduled for implant placement (Bone Level Tapered®, Institute Straumann AG, Basel, Switzerland). The CBCT scans were utilized to meticulously assess the available bone volume and quality in the posterior maxillary segment, as well as to plan the optimal implant position related to future prosthetic work (Figure 6 and 7). A computer-guided system was employed to navigate the implants into the ideal positions. Customized surgical guides were fabricated and guided implant placement was performed in the posterior maxillary segment (Figure 5). Resonance frequency analysis (RFA) of the placed implant was performed with the use of Penguin RFA device (Integration Diagnostics Ltd., Gothenburg, Sweden).

CBCT bone density analysis:

Six months after maxillary sinus augmentation, CBCT scans were performed for dual objectives. Firstly, they were conducted to facilitate the precise planning of computer-guided implants (Figure 6). Secondly, the scans were utilized to analyze the bone density within the surgical site. The evaluation of bone density was conducted utilizing Planmeca Romexis analysis software (Planmeca Oy, Helsinki, Finland). Within this software, a cubical region of interest measuring 7 × 7 × 7 mm was delineated, resulting in a volume of 343 mm^3. Cubical markings were

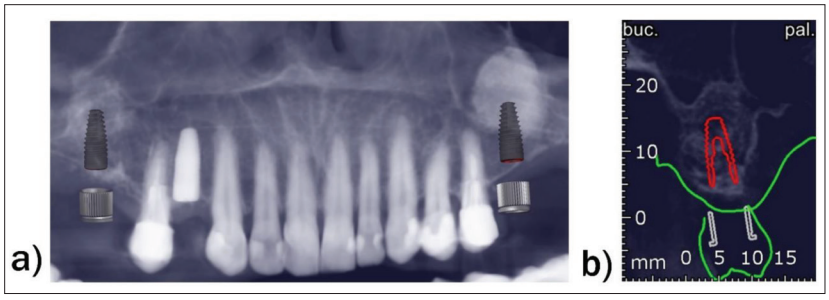

Figure 6. a) Panoramic radiograph image with planned implants in previously augmented maxillary sinuses; b) bucco-palatal ideal position of the implant in the previously augmented maxillary sinus

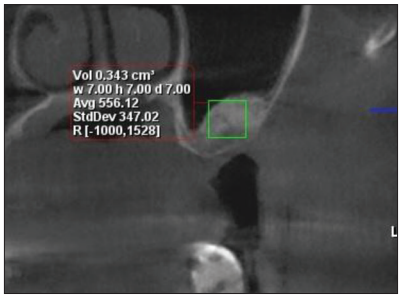

Figure 7. Hounsfield units analysis

Table 1. Differences in Hounsfield units across groups

Parameters	HU	p-value (*vs.* xenograft) [b]	RFA	p-value (*vs.* xenograft) [b]
Xenograft	674.57 ± 217.12		53.0 ± 20.12	
Xenograft + Fat	459.68 ± 86.54	0.141	60.75 ± 6.45	0.491
Xenograft + Fat + LLLT	540.19 ± 80.22	0.313	53.0 ± 1.83	1.000
p-value [a]	0.239		0.207	
Xenograft	674.57 ± 217.12		53.0 ± 20.12	
Xenograft+ Fat/ Xenograft + Fat + LLLT	499.94 ± 88.43		56.88 ± 6.03	
p-value [b]	0.207		0.729	

HU – Hounsfields units; RFA – resonance frequency analysis;
LLLT – low-level light therapy;
[a]ANOVA;
[b]t test

positioned in bone regions where guided implant placement was subsequently planned. The bone density within this region of interest was automatically quantified and expressed in Hounsfield units (HU), a standardized measure of radiodensity commonly used in radiographic imaging analysis (Figure 7).

Statistical analysis

The sample size was calculated based on the formula for determining the size of independent samples. Results are presented as mean ± standard deviation. Groups were compared using t-test (two samples) or ANOVA (three samples). Correction for unequal variances was applied where appropriate. All p-values less than 0.05 were considered significant. All data were analyzed using IBM SPSS Statistics for Windows, Version 29.0.2.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Notable differences in HU across groups are present (Table 1). Specifically, the control group exhibited significantly higher HU (674.57 ± 217.12) compared to the experimental groups (499.94 ± 88.43), which demonstrated Hounsfield unit values that were closer to those observed in natural native bone. Examination of RFA data shows that the results exhibit a degree of comparability or moderately better stability in the experimental groups (56.88 ± 6.03) compared to the control group (53 ± 20.12).

DISCUSSION

Bone regeneration of the posterior maxilla remains a significant entity in implant-prosthetic rehabilitation due to the high prevalence of bone atrophy in this area. Placement of endosseous implants in patients with highly pneumatized maxillary sinus often requires a two-stage approach, the first stage being maxillary sinus augmentation, conventionally performed utilizing xenograft materials. Implementing effective bone regeneration strategies is crucial for ensuring the success and longevity of implant treatment, which is why continuous and persistent efforts are invested in investigating different graft materials, driven by the imperative to achieve biomimetic bone composition. Body-derived additives to graft materials, such as various forms of growth factors, including platelet-rich plasma, platelet-rich fibrin, and plasma rich in growth factors; or mineralized tissues such as autologous bone and tooth-derived bone graft are frequently implemented in regenerative procedures [5, 6, 7].

Stem cell research is also, among other fields, focused on the need for bone regeneration in cranial, maxillofacial and oral surgery, especially because of the enormous social and psychological impact of bone defects in these regions. Stem cells are shown to be capable of differentiation under appropriate *in vitro* conditions to mesoderm-type cells, e.g. osteoblasts, adipocytes and chondrocytes [8]. This was also shown in clinical settings [9]. In clinical conditions, the accessibility of suitable cell sources is a critical consideration. An abundant tissue in most individuals and amenable to minimally invasive harvesting procedures is adipose tissue, which also emerged in the literature as a promising reservoir of stem cells [10]. Adipose tissue is readily accessible for clinical use via minimally invasive procedures, which is especially apparent in this study design since there was no additional morbidity in obtaining the Bichat's fat pad tissue, whose stem cells were shown to have similarities in cell yield, morphology, and multilineage differentiation with other adipose-derived stem cells while demonstrating faster proliferation and greater tendency of producing colonies [11]. Buccal fat-pad-derived stem cells were used successfully in the treatment of large alveolar bone defects [12], as well as in the augmentation of the atrophic posterior mandible as an additive to xenogenic bone [13]. Adipose tissue was also utilized as unprocessed with success in experimental [14], as well as clinical [4], highlighting its efficiency and clinical applications. Based on HU analysis, fragmented Bichat's fat pad tissue in this study showed contribution to achieving graft anatomy that closely aligns with natural bone anatomic structure, while at the same time providing high stability for implants.

With the advancement of technology and science, various innovative approaches have emerged to stimulate tissue regeneration. Among these approaches, the utilization of light therapy has gained prominence [15]. LLLT, also known as photobiomodulation, encompasses a spectrum of techniques that harness the therapeutic properties of light to modulate cellular activities and promote tissue repair. This non-invasive modality involves the application of specific wavelengths of light to targeted tissues, where it interacts with chromophores within cells, initiating a cascade of biological responses. Through mechanisms such as photobiomodulation, light therapy has been shown to enhance cellular metabolism, accelerate wound healing [16, 17], reduce inflammation, and promote angiogenesis

[18, 19] and collagen synthesis [20, 21]. Moreover, light therapy offers versatility in its application, with various modalities that include also possible intraoral and extraoral applications. An extraoral approach with pulsing LED 635 nm light was shown to successfully penetrate soft and hard tissues in the maxillofacial region, proving the possibility of reaching deeper tissues and achieving therapeutic goals [22]. Several studies showed the beneficial effect of LLLT on bone regeneration. Bai et al. [23] demonstrated the promotion of blood vessel, collagen fiber and bone tissue formation, while a systematic review by Kheiri et al. [24] showed evidence of stimulation of osteogenesis in critical-size bone defects as well as enhancement of fibroblast and osteoblast proliferation with the use of LLLT. The addition of LLLT to the first experimental group in this study yielded similar results, however, given that the expected influence of LLLT primarily pertains to angiogenesis, further studies and biopsy analysis will be crucial in elucidating its specific impact.

Zizelmann et al. [25] evaluated the use of autologous cancellous bone graft, which is the gold standard, in maxillary sinus augmentation and obtained bone density of 266–551 HU, which resembles natural bone density and is also comparable with experimental groups of this study. Al-Obaidi et al. [26] performed graftless maxillary sinus augmentation, utilizing only gelatine sponges in order to organize the blood clot under the elevated Schneiderian membrane, so the obtained bone was native bone whose bone density (595.5 ± 159.4 HU) is also comparable with results of this study. Maxillary sinus augmentation utilizing calcium phosphate bioceramics granules demonstrated a higher mean bone density (766.9–1018.7 HU) when compared with the lower density of the native bone control group (482.6–891 HU) [27]. The ideal bone graft material should emulate the structural, mechanical, and biological properties of native bone tissue. This study utilized current knowledge and advancements in biomaterial science, tissue engineering, and regenerative medicine which hold promise in obtaining a step closer to this goal. In this study, analysis of CBCT findings in the control group compared to the experimental groups individually and collectively reveals that both experimental groups exhibit bone morphology more closely resembling natural native bone. All groups yielded high implant stability, with slightly better stability in experimental groups, however, a larger sample size is needed to get more insight. Further histological characterization and additional analyses will contribute to a deeper understanding of the achieved results and may potentially serve as guidelines for further scientific inquiry.

The given HU and RFA analysis serve as clear indicators of the substantial potential of this graft mixture, by its complete integration and provision of significant stability to the inserted implants. Xenograft mixed with Bichat's fat pad tissue may represent a novel entity in the field of bone regeneration.

CONCLUSION

The findings of this study support that the utilization of fragmented Bichat's fat pad tissue xenograft mixture may enhance the regenerative process in terms of obtaining bone more resembling native bone compared with the utilization of xenograft alone. Additionally, the augmented graft demonstrates high implant stability, indicating its potential for successful integration and long-term support. The addition of LLLT also resulted in bone resembling native bone while maintaining high implant stability. Further analyses based on tissue biopsies are ongoing and will provide additional insights, enhancing our understanding of the observed results.

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Аугментација максиларног синуса коришћењем ксенографта, ткива Бихатовог масног јастучета и терапије светлошћу ниског интензитета – резултати проспективне рандомизоване клиничке студије компјутеризоване томографије конусног снопа и радиофреквентне аблације

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САЖЕТАК

Увод/Циљ Уградња денталних имплантата у регији постериорне максиле може представљати изазов услед честе појаве недовољног волумена кости и присуства високопнеуматизованог максиларног синуса. У оваквим ситуацијама као терапијски модалитет препоручује се аугментација максиларног синуса и конвенционално се изводи помоћу ксеногених материјала.

Циљ овог истраживања је анализа резултата употребе фрагментисаног масног ткива Бихатовог масног јастучета у комбинацији са коштаним замениоцем говеђег порекла, у односу са употребу само коштаног замениоца говеђег порекла код аугментације максиларних синуса. Секундарни циљ студије је евалуација утицаја терапије светлошћу ниског интензитета на регенерацију кости код пацијената третираних фрагментисаним масним ткивом у комбинацији са коштаним замениоцем говеђег порекла.

Методе У студију је укључено шест пацијената, учињено је 12 аугментација максиларних синуса, а пацијенти су насумично распоређени у три групе. Шест месеци после хирургије урађена је компјутеризована томографија конусног снопа густине кости и анализа резонантне фреквенције 12 уграђених имплантата.

Резултати Резултати анализе густине кости показали су значајне разлике у Хоунсфилдовим јединицама, са вредностима експерименталне групе (499,94 ± 88,43) које су сличније вредностима природне нативне кости у поређењу са вредностима контролне групе, које су више (674,57 ± 217,12). Анализа резонантне фреквенције имплантата показала је сличне вредности контролне групе (53 ± 20,12) и експерименталних група, са благо вишим нивоом стабилности имплантата код експерименталних група (56,88 ± 6,03).

Закључак Дате Хоунсфилдове јединице и анализе резонантне фреквенције јасни су индикатори значајног потенцијала употребе комбинације фрагментисаног масног ткива и ксеногеног коштаног замениоца код аугментације максиларних синуса, демонстрирајући комплетну интеграцију и пружање значајне стабилности уграђеним имплантатима. Фрагментисано масно ткиво у комбинацији са ксенографтом може представљати значајан нови ентитет у области коштане регенерације.

Кључне речи: нови графт; регенерација кости; масно ткиво; терапија светлошћу ниског интензитета

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Can creatine kinase levels be an indicator of the need for hemodialysis?

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SUMMARY

Introduction/Objective This study aimed to evaluate the relationship between changes in creatine kinase (CK) levels and the need for dialysis in patients with crush syndrome (CS).

Methods We conducted a retrospective analysis of patients with CS who were admitted to our hospital after the earthquake from February to May of 2023. We recorded demographic and laboratory data of the patients and divided them into two groups based on the change in CK levels within 48 hours. The groups were compared based on the need for dialysis and mortality rates.

Results A total of 84 patients with crush injuries participated in the study (41 males and 43 females). The average age was 33.65 ± 13.1 years. Nineteen patients received hemodialysis, and 18 patients underwent fasciotomy due to compartment syndrome. The patients were divided into two groups, Group 1 consisted of patients with more than a 50% decrease in CK levels within 48 hours, while Group 2 included patients with a decrease of less than 50% in CK levels during the same period. We compared the two groups regarding the frequency of dialysis and mortality. No statistically significant differences were found between the groups (p = 0.328 for dialysis and p = 0.89 for mortality).

Conclusion Although CK is an important enzyme for diagnosing CS and indicates ongoing muscle damage, changes in CK levels during follow-up do not reliably predict the need for dialysis or mortality risk.

Keywords: crush syndrome; creatine kinase; dialysis; mortality

INTRODUCTION

On February 6, 2023, one of the biggest earthquake catastrophes in Turkey and Syria's history hit the southeast part of Turkey and Syria, which are inhabited by more than 20 million people. More than fifty thousand people lost their lives according to official numbers revealed by the Health Ministry of Turkey [1, 2, 3]. In Syria, which is a war zone, a less-studied and less-secure region compared to Turkey, more than 4000 people lost their lives as declared by the World Health Organization [3]. Over 160,000 buildings were demolished, and 16 hospitals collapsed following the earthquake, blocking access to health care in the first hours after the earthquake. Most patients were transferred to the hospitals adjacent to earthquake-hit cities [1, 2, 3].

Crush syndrome (CS) could be defined as trauma – or compression-associated muscle injury caused by disasters, accidents, or explosions. Generally, it involves the skin, subcutaneous tissue, muscle, tendon, and bones and frequently involves the lower extremities [4]. Following muscle injury, major intracellular electrolytes such as potassium and phosphate and enzymes such as creatine kinase (CK) are released into the systemic circulation. This can cause electrolyte imbalance and might require medical treatment, sometimes resulting in renal replacement therapy [5, 6, 7].

Acute kidney injury (AKI) is one of the most serious complications of CS. According to different studies, the rate of AKI might differ 15–50% among patients with CS [8, 9, 10]. As a treatment, it is vital to initiate fluid resuscitation therapy as soon as access to treatment is available and renal replacement therapy when necessary [11, 12]. CK is an enzyme primarily present in muscles, the brain, and visceral tissues (in small amounts). It is an intracellular enzyme and very sensitive to hypoxia and injury. Following trauma, hypoxia, or myocardial ischemia, serum CK levels can be detected as elevated. Despite its sensitivity, it is not specific to any disease due to its presence in different tissues [13, 14]. Similarly, in CS patients, CK levels can be measured higher than 10,000 U/L [9]. Following muscle injury, CK levels rise within 2–12 hours, peak between 24 and 72 hours, and decline by the third to fifth day. Persistently elevated CK levels or a failure to decrease as expected may indicate ongoing muscle damage, a pre-existing muscle disorder, or compartment syndrome [15].

Change in CK levels, as a sign of injury, could theoretically be a sign of ongoing nephrotoxic effects on the kidney. Therefore, in this study, we aimed to determine whether the change in CK levels could be a sign of the need for renal replacement therapy.

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METHODS

Setting and participants

We retrospectively included adult CS patients in our study. Patients admitted to our hospital following the catastrophic earthquake that occurred on February 6, 2023, in our region with a history of trauma, and whose CK levels were 10,000 U/L or above, were diagnosed with CS. In addition, according to the KDIGO Guidelines, among these patients, those who developed acute kidney injury were recorded [16].

Patients under 18 years of age, with end-stage renal disease, and with a history of recent surgical intervention were excluded from the study.

Patient characteristics and procedures

Patient data were obtained from the hospital's patient database system. Demographic characteristics such as age and gender were noted. Laboratory findings at the time of admission, including white blood cell count, neutrophil, hemoglobin, hematocrit, platelets, urea, creatinine, CK, sodium, potassium, total calcium, phosphate, and C-reactive protein, were recorded. The time spent under the rubble until the first medical intervention, length of hospital stay, and the rate of fasciotomy, dialysis, and mortality were retrieved from the electronic health records of the participants. Although not optimal, since continuous renal replacement therapy (CRRT) was not an option in our clinic, conventional intermittent hemodialysis was applied to every patient requiring dialysis.

In addition to CK levels at the time of admission, CK levels after the 48th hour of admission were recorded. The patients were divided into two groups: the "fast responders" (decreased more than 50% within 48 hours) and the "slow responders" (decreased less than 50% within 48 hours) according to the decrease in the level of CK within 48 hours. These two groups were compared in terms of frequency of dialysis, mortality, length of hospital stay, and need for fasciotomy.

Statistical analysis

The program used for statistical analysis was IBM SPSS Statistics, Version 26.0 (IBM Corp., Armonk, NY, USA). The distribution of the data was assessed using the Kolmogorov–Smirnov test. The data with normal distribution were presented as mean ± standard deviation (SD), and skewed data were expressed as median and inter-quartile range (IQR). Categorical variables were reported as a percentage of the total. The difference between continuous and skewed variables was compared with the Student's t-test and the Mann–Whitney U test, respectively. The χ^2 test was used to determine the relationship between categorical variables. Point biserial correlation was calculated to evaluate the potential correlation of CK levels with hemodialysis, fasciotomy, mortality, and length of hospital stay. P values less than 0.05 were accepted as statistically significant.

Ethics

The institutional Clinical Research Ethics Committee approved the study protocol. All procedures followed the Turkish Medicine and Medical Devices Agency Good Clinical Practices Guidelines and the Declaration of Helsinki.

RESULTS

This study included 84 patients with a median age of 29 years (min: 18, max: 72, IQR: 15), and 51.2% (n = 43) of them were female. The demographic characteristics, including age and gender, and baseline laboratory findings of participants were given in Table 1. The median CK levels of the enrollees at the time of admission were 7868 U/L (IQR: 23,351), and 32.1% (n = 27) of them were diagnosed with AKI. While the CK levels of patients in the "fast responders" group at the time of admission were significantly higher, the CK levels at the 48th hour were found to be similar in both fast and slow responders.

While 22.6% (n = 19) of patients received hemodialysis, 21.4% (n = 18) of patients underwent fasciotomy due to compartment syndrome. The in-hospital mortality rate was 6% (n = 5). The comparison of fast and slow responder

Table 1. General characteristics, and baseline laboratory findings of patients

Characteristic	Total (n = 84)	Fast responders (n = 36)	Slow responders (n = 48)	p*
Age (Years), median (IQR)	29 (15)	28.5 (14)	29.5 (16)	0.238
Sex, n (%), female	43 (51.2)	20 (23.8)	23 (27.4)	0.488
WBC (109/L), mean (SD)	15 (6.6)	14.4 (7.2)	15.4 (6.1)	0.968
Neutrophil (109/L), mean (SD)	12.3 (5.9)	11.6 (6.5)	12.8 (5.5)	0.717
Hemoglobin (mmol/L), mean (SD)	7.97 (2.02)	8.24 (2.15)	7.76 (1.92)	0.119
Hematocrit, mean (SD)	0.39 (0.1)	0.4 (0.1)	0.38 (0.9)	0.148
Platelets (109/L), mean (SD)	232 (93.8)	237.4 (79.9)	229.3 (104.7)	0.787
Urea (mmol/L), median (IQR)	7.58 (9.32)	9.07 (8.99)	7.41 (11.49)	0.603
Creatinine (µmol/L), median (IQR)	76.91 (130.83)	79.56 (228.96)	65.42 (128.18)	0.235
CK (admission) (U/L), median (IQR)	7868 (23351)	15,823 (46808)	5326 (16,102)	0.005
CK (48th hour) (U/L), median (IQR)	5294 (13462)	5294 (13292)	5515 (13526)	0.910
Sodium (mmol/L), median (IQR)	137 (5)	137 (7)	137 (4)	0.647
Potassium (mmol/L), median (IQR)	4.26 (0.88)	4.4 (1.12)	4.09 (0.88)	0.097
Calcium (mmol/L), median (IQR)	2.15 (0.21)	2.15 (0.24)	2.16 (0.19)	0.645
Phosphorus (mmol/L), median (IQR)	1.33 (6.46)	1.33 (7.10)	1.33 (5.49)	0.927
CRP (mg/L), median (IQR)	122 (143.3)	119 (132.5)	123 (147)	0.964

IQR – interquartile range; WBC – white blood count; CK – creatine kinase CRP – C-reactive protein;
*p-values < 0.05 were considered statistically significant

groups in terms of the need for dialysis treatment and in-hospital mortality revealed that neither the need for dialysis (11.9% *vs.* 10.7%, p = 0.328) nor in-hospital mortality rates (2.4% *vs.* 3.6%, p = 0.890) were significantly different between groups (Table 2). However, the mortality rate was significantly higher among patients who received hemodialysis (4.8% *vs.* 1.2%, p = 0.002). In addition, fasciotomy rates (8.3% *vs.* 13.1%, p = 0.701) and the length of hospital stay (12 days *vs.* 11.5 days, p = 0.553) were similar between both groups (Table 2).

While the CK levels at the time of admission and the amount of drop in the first 48 hours were significantly correlated with the need for hemodialysis (r = 0.371, p < 0.001, r = 0.302, p = 0.005, respectively), we did not observe such correlation for mortality rates, fasciotomy rates, or the length of hospital stay (p > 0.05 for all) (Table 3).

Table 2. The comparison of outcomes between slow and fast responders

Characteristic	Total (n = 84)	Fast responders (n = 36)	Slow responders (n = 48)	p*
Hemodialysis, n (%)	19 (22.6)	10 (11.9)	9 (10.7)	0.328
Fasciotomy, n (%)	18 (21.4)	7 (8.3)	11 (13.1)	0.701
Mortality, n (%)	5 (6)	2 (2.4)	3 (3.6)	0.894
Length of hospital stay (days), median (IQR)	12 (15)	12 (14)	11.5 (19)	0.553

IQR – interquartile range;
*p-values < 0.05 were considered statistically significant

Table 3. The Point Biserial Correlation of patients' CK levels, Δ CK levels, and outcomes

Characteristic	CK (admission)		Δ CK (first 48 hours)	
	r	p*	r	p*
Hemodialysis	0.371	0.001	0.302	0.005
Fasciotomy	0.188	0.088	0.154	0.161
Mortality	0.164	0.136	0.103	0.351
Length of hospital stay (days)	0.126	0.252	0.147	0.182

CK – creatine kinase; Δ CK – the amount of fall in CK in the first 48 hours;
r – Point Biserial Correlation;
*p-values <0.05 were considered statistically significant

In our study, we examined electrolyte imbalances in all patients. The rate of patients with hyperkalemia and hypokalemia was 8.3% (n: 7) and 9.5% (n: 8), respectively. In addition, hypocalcemia and hyponatremia, which are common electrolyte disorders in patients with CS, were 41.6% (n: 35) and 30.9% (n: 26), respectively.

DISCUSSION

Crush syndrome (CS), defined by Bywaters 60 years ago and also known as Bywaters syndrome, is a medical condition consisting of elevated CK levels and renal failure resulting from the destruction of muscle cells following an injury or hypoxia. In its etiology, different events or diseases can play significant roles, such as trauma and injury caused by natural and unnatural disasters (earthquake, typhoon, war, mine accident), toxins, viral infection, and strenuous exercise [17, 18]. Elevated CK levels can be observed in CS and remain elevated if CS continues. Change in CK levels, as a sign of injury, could theoretically be a sign of ongoing nephrotoxic effects on the kidney. Therefore, in this study, we aimed to determine whether the change in CK levels could be a sign of the need for renal replacement therapy.

In our study, comparable to previous studies, the median age was 29 (18–72). The fact that the rate of patients over the age of 65 in our study was 2.4% (n: 2), and this situation was lower than the distribution of the population over the age of 65, may be related to the high mortality rate of elders at the scene [19, 20, 21]. Although the unknown age distribution of deaths at the scene makes this situation difficult, our study is comparable to the previous study of Erek et al. [22]. Tanida et al. [19] reported that in the Hanschin earthquake that occurred in Japan in 1995, the mortality rate was found to be six times higher in patients older than 80 years compared to patients younger than 50 years. This low rate may be related to previous observations that the geriatric population is more prone to earthquake-related injury but has a much higher on-the-spot mortality rate [19, 20]. In terms of mortality rate between different sexes, there was no difference between the patients (male: 41, female: 43) (p = 0.684).

AKI caused by CS is one of the severe complications observed at different levels in various studies. In a study conducted in China after the Wenchuan Earthquake, the rate of AKI was detected at 41.6% among hospitalized patients [23]. However, in one of the leading studies conducted in Turkey by Sever et al., the rate of AKI was 12% in the patients [12]. Therefore, it might be challenging to estimate the exact rate of AKI, which might be caused by different factors. In our study, the rate of acute kidney injury was 32.1%, which was compatible with other studies.

In terms of electrolyte imbalances, which can be observed in CS, hyperkalemia, hyperphosphatemia, and hypocalcemia are the most common disturbances that can be life-threatening if not treated on time. Aggressive fluid management with the control of urine output is the first-line treatment of CS. Urine alkalization and dialysis intervention to control electrolyte imbalances can be applied. Hyperkalemia is the most fatal complication and one of the leading factors of mortality observed in patients with CS [24, 25]. In the Marmara Earthquake, Erek et al. [22] reported that hyperkalemia is the most frequent electrolyte imbalance observed in cases (42% of them) that died from CS. And hyperkalemia is more commonly observed than hypokalemia. In our study, despite no statistical difference observed (p > 0.05), the rate of hypokalemia was higher than hyperkalemia, 9.5% (n: 8) and 8.3% (n: 7), respectively. In this regard, our data are more like the study conducted in the Wenchuan Earthquake in China, which showed that hypokalemia (18.2%) was more common than hyperkalemia (15.9%) [23]. Apart from these, hypocalcemia and hyponatremia were detected at the rate of 41.6% (n: 35) and 30.9% (n: 26), respectively.

In rhabdomyolysis-associated AKI, elevated CK is an indicator of ongoing muscle damage, which may be an

indicator of increased risk of kidney damage [26]. For this reason, the absence of an increase in CK level and a decrease in CK level theoretically creates less need for dialysis. In this study, we compared the patients whose CK levels decreased by more than 50% within the first 48 hours from the moment of admission with the patients whose CK levels did not decrease, in terms of dialysis. We could not detect any statistical difference in dialysis frequency between these two groups ($p = 0.328$). In a study conducted by Xiao et al. [27] on 86 patients, no significant difference was detected between the CK level of the patients and the need for CRRT, and no statistically significant relationship between CK and renal function was observed there. In our study, patients received conventional intermittent dialysis, as our clinic did not offer the option of CRRT. This approach allowed us to monitor and assess the effectiveness of intermittent treatment. Notably, the outcomes we observed closely aligned with the findings reported by Xiao et al. [27], reinforcing the validity of our study results.

Mortality in CS can be caused by many factors, such as the duration of exposure under the rubble, the time of transfer of the patient, the moment of starting the first treatment, the presence of electrolyte imbalances, the development of acute kidney injury, the availability of dialysis, fasciotomy, or trauma-related surgery. Although the overall mortality rate reaches 20% in the population with kidney damage, this rate may vary [28, 29]. This rate may be even higher in patients with multiorgan damage. In a study conducted by Erek et al. [22] in 639 patients, they found the mortality rate in patients with CS who were on dialysis and who were not on dialysis to be 17.2% and 9.3%, respectively. In our study, death occurred in five patients, and this rate was determined as 5.9%. Reasons for the lower mortality rate compared to other studies may be related to many factors, such as our low number of patients, the partial distance of our center from the epicenter of the earthquake, and the increase in treatment opportunities for CS.

Our study has several limitations. This study was performed retrospectively with patients who received medical treatment under the extreme and devastating conditions of a massive earthquake that caused power and internet outages, staff shortages, and usage of field hospitals. So, we could not retrieve the data of initial treatments applied to the patients (type, dose, duration of treatments, etc.) and information on diuresis adequately and accurately from the hospital's patient database system. Therefore, we couldn't compare the initial treatments of responders and non-responders. Apart from this, the number of patients in our study compared to previously published studies was relatively low, which makes it hard to draw broad conclusions. The urgency of the situation was challenging and obstructed our study to be designed prospectively. Therefore, our study was designed retrospectively. These were the limitations encountered during the study.

CONCLUSION

Although CK is a significant enzyme in the diagnosis of CS and can be a sign of ongoing muscle damage, the present study revealed that the changes in CK levels during the first 48 hours have no predictive value for the need for dialysis and in-hospital mortality. However, due to the retrospective design of this study and a relatively small number of participants, future studies are warranted to confirm the current findings. Besides this, electrolyte imbalances are frequent and can be fatal in CS. Therefore, awareness of clinicians and on-time treatment is crucial.

Conflict of interest: None declared.

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Могу ли нивои креатин киназе бити показатељ потребе за хемодијализом?

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САЖЕТАК

Увод/Циљ Ова студија је имала за циљ да испита однос између промена у нивоима креатин киназе (КК) и потребе за дијализом код болесника са трауматском рабдомиолизом.

Методе Урађена је ретроспективна анализа болесника са трауматском рабдомиолизом који су примљени у нашу болницу након земљотреса у периоду од фебруара до маја 2023. године. Забележени су демографски и лабораторијски подаци болесника, који су потом подељени у две групе на основу промене у нивоима КК унутар 48 сати. Групе су упоређене у односу на потребу за дијализом и стопу морталитета.

Резултати У студији је учествовало укупно 84 болесника са повредама услед дробљења (41 мушкарац и 43 жене). Просечна старост била је 33,65 ± 13,1 година. Деветнаест пацијената је подвргнуто хемодијализи, док је 18 пацијената подвргнуто фасциотомији због синдрома компартмана. Болесници су подељени у две групе: Група 1 обухватала је болеснике код којих је дошло до смањења КК за више од 50% у року од 48 сати, док су у Групи 2 били болесници са смањењем КК мањим од 50% у истом периоду. Упоређивање две групе у погледу учесталости дијализе и стопе морталитета није показало статистички значајне разлике ($p = 0,328$ за дијализу и $p = 0,89$ за морталитет).

Закључак Иако је КК важан ензим за дијагнозу трауматске рабдомиолизе и указује на текуће оштећење мишића, промене у нивоима КК током праћења нису поуздан предиктор потребе за дијализом или ризика од морталитета.

Кључне речи: трауматска рабдомиолиза; креатин киназа; дијализа; морталитет

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

A cross-sectional study on the factors influencing drug resistance in clinical *Mycobacterium tuberculosis* in Hulunbuir, Inner Mongolia

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Introduction/Objective This study aimed to improve the understanding of drug-resistant tuberculosis (TB) by conducting a retrospective analysis of clinical data from TB patients in Hulunbuir, Inner Mongolia, collected between 2015 and 2017.
Methods A retrospective analysis was performed on clinical data from patients with TB. The data were used to determine monodrug resistance rankings for ethambutol, isoniazid, rifampicin (RIF), and streptomycin. Additionally, the study examined drug resistance rates for multidrug resistance (MDR) combinations, specifically isoniazid + RIF + streptomycin and isoniazid + RIF + ethambutol + streptomycin. A multivariate logistic regression analysis was conducted to assess risk factors for drug resistance, including sex, hospitalization status, age, and treatment history.
Results The findings revealed that both MDR combinations had resistance rates of 4.51%, the highest among the combinations analyzed. Ethambutol, isoniazid, and RIF showed the three highest monodrug resistance rates. Patients undergoing retreatment had higher rates of monodrug resistance, MDR, and polydrug resistance compared with those receiving initial treatment. The multivariate logistic regression analysis indicated that women had a lower risk of developing drug resistance than men, and hospitalized patients were found to be at lower risk than outpatients. Being 20–40 years old and undergoing retreatment were identified as significant risk factors for developing drug-resistant TB.
Conclusion In the Hulunbuir region of Inner Mongolia, there was a notable presence of drug resistance among patients with TB, with specific demographic and treatment history factors contributing to this resistance. These findings underscore the importance of considering these factors in developing targeted treatment strategies and public health policies to combat drug-resistant TB.
Keywords: *Mycobacterium tuberculosis*; drug resistance; influence factor

INTRODUCTION

Tuberculosis (TB) is a disease primarily caused by *Mycobacterium tuberculosis* (Mtb) infection and poses a significant threat to public health [1]. Between 2016 and 2020, the World Health Organisation designated China as a high-burden country for TB, TB-HIV co-infection and multidrug-resistant TB (MDR-TB). The high burden of MDR-TB is a key factor hindering TB control [2]. Multidrug-resistant TB is difficult to treat, severely impacting patients' physical and mental health and livelihoods and endangering their lives.

In recent years, with the implementation of the National Tuberculosis Prevention and Control Programme, some progress has been made in the prevention and control of drug-resistant TB in China. However, the epidemic of drug-resistant TB remains inadequately controlled in economically disadvantaged, remote areas [3]. Recent reports indicate a significant upward trend in drug resistance rates, particularly for rifampicin (RIF), multidrug-resistant strains and fluoroquinolones among TB patients in Inner Mongolia [4]. These remote regions face unique challenges due to economic disparities. This study aims to investigate the drug resistance status of TB and its associated influencing factors in the Hulunbuir area to guide individualized treatment plans. It also addresses a gap in the understanding of specific factors affecting drug resistance, which is essential for developing effective control measures.

METHODS

Study design and population

A cross-sectional study was conducted using convenience sampling of 688 patients with TB who were newly diagnosed or previously treated and who sought medical care, were referred to it, or were tracked in the Hulunbuir Second People's Hospital in the Hulunbuir region between January 1, 2015 and December 31, 2017. A questionnaire survey was then undertaken to gather basic information, medical history and treatment history from the patients. The diagnostic criteria used in this study followed the Diagnostic Criteria for Pulmonary Tuberculosis formulated by the Tuberculosis Branch of the Chinese Medical Association [5]. The inclusion criteria were as follows: (1) patients with

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positive sputum culture identification for Mtb, (covering pulmonary TB and extrapulmonary TB) and (2) patients who underwent drug susceptibility testing (DST). The exclusion criteria were as follows: (1) patients who were on treatment during the investigation period, (2) patients declining DST, and (3) patients unwilling to participate in the survey.

Research methods

Sputum samples from all active patients with TB included in the study were cultured on a solid medium using Roche culture medium. Positive cultures were subsequently subjected to strain isolation, identification and DST. The DST for TB was divided into two parts. First, sputum samples were collected to culture Mtb; subsequently, the drug susceptibility of the strains was tested using kits. Strain identification was accomplished by qualified researchers using a gene chip method, which employed a DNA microarray chip to qualitatively detect samples from clinically suspected patients for Mtb and non-tuberculous mycobacteria. The detailed procedures followed the Laboratory Testing Guidelines for Tuberculosis [6]. To ensure the accuracy and reliability of the gene chip method in strain identification, a series of quality assurance measures were implemented, including the establishment of standardized operating procedures, the adoption of double-blind testing processes, the introduction of positive and negative controls, inter-laboratory comparisons, the development of quality control metrics, ongoing quality improvement plans, detailed record-keeping and adherence to ethical standards. Through these stringent quality control measures, the consistency and traceability of the experimental results were ensured and enhanced the scientific validity of the research. The acidic Roche culture medium was manufactured by Hangzhou Innovation Biotechnology Co., Ltd (Hangzhou, Zhejiang, China). The DST kit and gene chip were purchased from Beijing Boao Biological Group Co., Ltd. (Beijing, Beijing, China), both compliant with national standards.

Quality control

Patient questionnaires were completed by professional physicians to ensure accuracy in the patients' medical history and treatment records. Smear examination, bacterial culture and DST were conducted in accordance with the Diagnostic Criteria for Pulmonary Tuberculosis [5] and the Laboratory Testing Guidelines for Tuberculosis [6].

Relevant definitions

Newly diagnosed patients were defined as individuals who had never used anti-TB drugs or had used them for less than one month and had discontinued treatment for less than two months. Retreatment patients, by contrast, included those who underwent irregular anti-TB treatment for at least one month, as well as those who experienced treatment failure or relapse. The drug resistance classification was as follows:

1. Any drug resistance: Mtb is resistant to a particular anti-TB drug, including but not limited to that specific drug;
2. Monodrug resistance: Mtb is resistant to a single first-line anti-TB drug;
3. MDR: Mtb is resistant to both isoniazid and RIF concurrently;
4. Polydrug resistance: Mtb is resistant to two or more anti-TB drugs, excluding simultaneous resistance to both isoniazid and RIF.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics, Version 23.0 (IBM Corp., Armonk, NY, USA). Descriptive and analytic statistics were employed to depict and examine drug resistance profiles and rates. Counts were described using proportions or percentages, and group differences were assessed using the chi-squared (χ^2) test. A logistic regression model was utilized to analyze the factors influencing drug resistance in patients with TB. The outcomes were visualized using GraphPad Prism 9.3 (GraphPad Software, San Diego, CA, USA). Two-tailed tests were performed, with a significance level of $\alpha = 0.05$.

The Ethics Committee of Baotou Medical College approved this study (Ethical number: Baotou Medical College Ethics Committee Approval [2023] No. 16). The Committee approved the waiver of informed consent from parents/guardians of the minors, due to the study being a retrospective analysis of clinical data and all methods were performed in accordance with the ethical guidelines.

RESULTS

Basic information

Among the 688 patients with TB in this study, 393 were newly diagnosed, whereas 295 were previously treated. The male-to-female ratio among these patients was 2.8:1. Most patients were hospitalized due to pulmonary TB.

Drug resistance testing results

By examining the resistance results to four anti-TB drugs – isoniazid, RIF, ethambutol and streptomycin – this study identified a drug resistance rate of 44.04% among the 688 isolates. Fifteen combinations of drug resistance were observed among these four drugs, with each combination exhibiting resistance. The ranking of monodrug resistance was as follows: ethambutol (8.14%), isoniazid (5.67%), RIF (5.52%), and streptomycin (1.45%). Among the multidrug resistance patterns, the highest resistance rates were observed for isoniazid + RIF + streptomycin and isoniazid + RIF + ethambutol + streptomycin, each at 4.51% (see Table 1).

Table 1. Drug resistance frequencies of various drug combinations in treatment-resistant patients with tuberculosis*

Combinations	n	Drug resistance rate (95% CI)	Composition ratio % (n/303)
Any drug resistance	—	—	—
H	166	24.13 (20.93–27.33)	—
R	165	23.98 (20.79–27.17)	—
E	79	11.48 (9.10–13.86)	—
S	164	23.84 (20.65–27.02)	—
Monodrug resistance	143	20.78 (17.75–23.82)	47.19 (143/303)
H	39	5.67 (3.94–7.4)	12.87 (39/303)
R	38	5.52 (3.82–7.23)	12.54 (38/303)
E	56	8.14 (6.1–10.18)	18.48 (56/303)
S	10	1.45 (0.56–2.35)	3.3 (10/303)
Multidrug resistance	99	14.39 (11.77–17.01)	32.67 (99/303)
H+R	26	3.78 (2.35–5.2)	8.58 (26/303)
H+R+E	11	1.6 (0.66–2.54)	3.63 (11/303)
H+R+S	31	4.51 (2.96–6.06)	10.23 (31/303)
H+R+E+S	31	4.51 (2.96–6.06)	10.23 (31/303)
Polydrug resistance	61	8.87 (6.74–10.99)	20.13 (61/303)
H+E	2	0.29 (0.11–0.69)	0.66 (2/303)
H+S	23	3.34 (2–4.69)	7.59 (23/303)
H+E+S	3	0.44 (0.06–0.93)	0.99 (3/303)
R+E	13	1.89 (0.87–2.91)	4.29 (13/303)
R+S	11	1.6 (0.66–2.54)	3.63 (11/303)
R+E+S	4	0.58 (0.01–1.15)	1.32 (4/303)
E+S	5	0.73 (0.09–1.36)	1.65 (5/303)
Total	303	44.04 (40.33–47.75)	100 (303/303)

H – isoniazid; R – rifampicin; E – ethambutol; S – streptomycin;
"—" indicates no corresponding value

Drug resistance distribution among patients with tuberculosis

Among the 688 patients, 385 were drug-sensitive, 143 were monoresistant, 61 were polyresistant and 99 were multidrug-resistant. At a significance level of $\alpha = 0.05$, a significant difference in the distribution of drug resistance was found between newly diagnosed and previously treated patients ($\chi^2 = 49.620$, $p < 0.001$). There were no significant differences in the composition ratios of the four drug resistance categories across sex, age groups, ethnicity, patient types or diagnostic outcome groups (all p-values > 0.05) (see Table 2).

Analysis of factors influencing drug resistance in patients with tuberculosis

The multivariate analysis of the logistic regression model showed that women had a lower risk of developing drug resistance than men (odds ratio [OR] = 0.68, $p < 0.05$). Patients aged 20–40 years had a higher risk of developing drug resistance than those aged 0–20 years (OR = 2.64, $p < 0.05$). Previously treated patients had a higher likelihood of developing drug resistance than newly diagnosed patients (OR = 2.34, $p < 0.05$). Hospitalized patients had a lower risk of developing drug resistance than outpatients (OR = 0.64, $p < 0.05$) (Figure 1).

DISCUSSION

The survey revealed that the prevalence of MDR-TB (14.39%) and overall drug resistance (44.04%) among patients in the Hulunbuir area of Inner Mongolia surpassed the rates reported in a drug resistance survey conducted among four provinces in northwest China between 2005 and 2011 (11.29% and 27.90%, respectively) and the national baseline survey (39.12% drug resistance rate) [7, 8]. These findings indicate a potentially higher drug resistance rate among patients with TB in the Hulunbuir area compared with other regions. The survey ranked the drug resistance rates of various anti-TB drugs as follows: isoniazid (24.13%), RIF (23.98%), streptomycin (23.84%), and ethambutol (11.48%). These rankings were highly similar to the reported drug resistance rates of acid-fast bacilli-positive samples in the Inner Mongolia region, according to Feng and He. [4]. The high resistance rate for streptomycin is notable, as TB treatment guidelines recommend its limited use. However, current guidelines that advocate for reduced use of streptomycin may unintentionally withhold a potentially lifesaving, inexpensive and readily available drug from certain patients with drug-resistant TB. Isoniazid and RIF are the most commonly used first-line oral anti-TB drugs, noted for their strong bactericidal effects, antimicrobial activity and low toxicity [9]. The appearance and progression of the resistant strain of Mtb depends largely on the selection of genetic mutations, which the *Mycobacterium* population exploits under selective pressure to favor antibiotic resistance. This study's OR analysis emphasizes the heightened risk of drug-resistant TB in patients aged 20–40 years and those undergoing retreatment, informing tailored clinical strategies. These findings provide actionable insights for healthcare providers to enhance treatment adherence and outcomes in these high-risk groups.

The virulence of Mtb, genetic factors of the host, HIV infection, and incomplete treatment of patients all contribute to the outbreak of drug-resistant TB [10]. Studies have revealed that risk factors for drug-resistant TB include monotherapy resulting from intermittent treatment, actual monotherapy due to irrational combination therapy, as well as insufficient drug concentrations leading to ineffective treatment [11]. Therefore, in the diagnosis and treatment of patients with TB, it is crucial to prioritize strict adherence to drug use principles and rational use of anti-TB drugs, particularly first-line medications.

In this survey, the proportions of monodrug resistance, MDR and polydrug resistance among newly diagnosed patients were 21.37%, 6.87%, and 7.12%, respectively, indicating possible transmission of drug resistance in the community [12]. Phenotypic DST is considered the gold standard for DST in China. However, TB laboratory tests in primary hospitals mainly consist of time-consuming methods, such as modified acid-fast staining and acid-fast culture [13, 14]. Current clinical practice relies on early anti-TB treatment principles similar to those for MDR-TB. However, these treatment regimens lack phenotypic DST results for

Table 2. Analysis of the distribution of drug resistance among patients with different characteristics

Characteristics	Drug resistance type				χ^2	p
	Sensitivity (%)	Monodrug resistance (%)	Polydrug resistance (%)	Multidrug resistance (%)		
Sex						
Male	269 (53.06)	111 (21.89)	49 (9.66)	78 (15.38)	7.049	0.070
Female	116 (64.09)	33 (18.23)	11 (6.08)	21 (11.6)		
Age (years)						
0–19	22 (75.86)	4 (13.79)	2 (6.9)	1 (3.45)	15.010	0.091
20–39	110 (52.38)	45 (21.43)	20 (9.52)	35 (16.67)		
40–59	165 (52.05)	73 (23.03)	29 (9.15)	50 (15.77)		
60–older	88 (66.67)	22 (16.67)	9 (6.82)	13 (9.85)		
Ethnic group						
Han	326 (56.5)	120 (20.8)	52 (9.01)	79 (13.69)	1.775	0.620
Mongolian	59 (53.15)	24 (21.62)	8 (7.21)	20 (18.02)		
New versus retreatment cases						
new	254 (64.63)	84 (21.37)	27 (6.87)	28 (7.12)	49.620	< 0.001
retreatment	131 (44.41)	60 (20.34)	33 (11.19)	71 (24.07)		
Type						
Outpatients	68 (50.37)	31 (22.96)	17 (12.59)	19 (14.07)	4.168	0.244
Inpatients	317 (57.32)	113 (20.43)	43 (7.78)	80 (14.47)		
Diagnosis						
Pulmonary tuberculosis	380 (55.96)	141 (20.77)	60 (8.84)	98 (14.43)	—	0.788
Extrapulmonary tuberculosis	5 (55.56)	3 (33.33)	0 (0)	1 (11.11)		

"—" represents the use of the Fisher exact probability method to calculate precise probabilities without calculating the χ^2 value

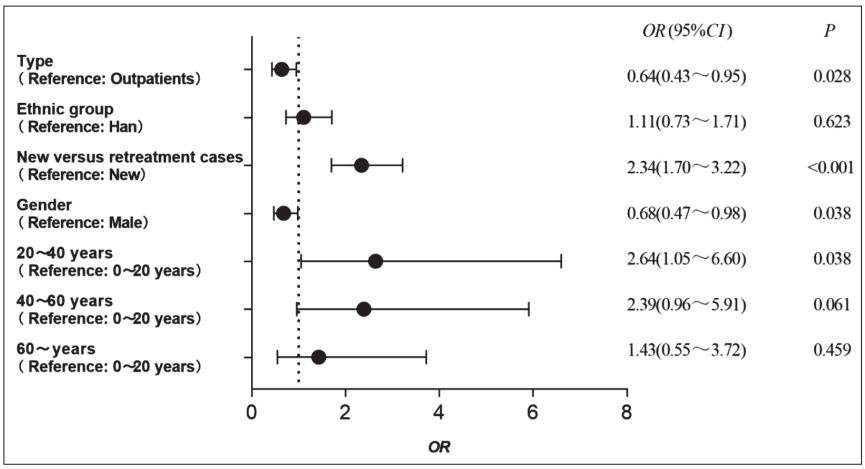

Figure 1. Multivariate analysis of a tuberculosis patient in a logistic regression model

other drugs. Consequently, when resistance to these drugs arises, it significantly impacts treatment efficacy and prognosis. Additionally, the results reveal that drug-resistant TB patients in the Hulunbuir area are distributed differently between newly diagnosed and previously treated patients. Several studies have demonstrated that drug resistance patterns vary between newly diagnosed and previously treated patients, potentially due to differences in drug exposure history and patient compliance [15]. Therefore, it is crucial to consider the characteristics of these patient populations in the clinical diagnosis and treatment process and select appropriate personalized treatment strategies based on specific situations, emphasizing the importance of drug guidance to enhance compliance.

Multivariate logistic regression analysis revealed several independent risk factors for drug-resistant TB, consistent with previous research findings [16]. Patients aged 20–40 had a 2.64 times higher risk of drug resistance compared with those aged 0–20. This may be attributed to higher levels of stress, social activities, and mobility among young and middle-aged patients, resulting in lower treatment compliance. Another reason is the relatively poor physical fitness to resist TB infection, which leads to prolonged disease [17]. This method was used for data collection in this study because of the high feasibility and validity of convenience sampling, but selection bias resulting from the use of convenience sampling is a possible explanation for this result. The risk of drug resistance is also higher in previously treated patients with pulmonary TB compared with newly diagnosed patients, potentially due to factors such as insufficient professional knowledge, improper medication, self-discontinuation of treatment or treatment failure in the initial stages [18]. Additionally, outpatients undergoing follow-up have a higher risk of drug resistance compared with hospitalized patients, which may be due to the non-adherence of patients to their 6-month therapy and/or a lack of physicians in therapy management. Another cause may be that some patients who require hospitalization may exhibit weaker willingness to partake in it, leading to poor compliance among outpatients. This emphasizes the importance of improving patient cooperation during outpatient follow-up.

Among newly diagnosed patients, male sex, age 20–40 years, and outpatient follow-up were identified as risk factors for drug resistance. In the retreatment group, risk factors included male sex, age 20–40 years and a prior history of treatment failure or relapse. These findings highlight the importance of tailoring interventions and management strategies based on each patient's treatment history and specific risk factors.

When compared to international data, our results are particularly striking. A global analysis by the World Health Organization (2023) reported a lower worldwide average for drug-resistant TB, indicating that Inner Mongolia, and particularly the Hulunbuir region, may be a hotspot for drug

resistance. The high resistance to isoniazid and rifampicin, observed in our study, mirrors the findings of Shabani et al. [9], who noted the increasing prevalence of resistance to these cornerstone anti-TB medications. In contrast, a study by Magotra et al. [13] reported lower resistance rates but noted the importance of personalized treatment strategies, which is also a key takeaway from our study. The need for tailored interventions is further emphasized by the high resistance rates to ethambutol, isoniazid, and rifampicin, which are consistent with the patterns reported by Feng et al. [15] in a study on drug sensitivity in Xi'an, China.

This study in Hulunbuir, Inner Mongolia, uniquely revealed high rates of drug resistance to ethambutol, isoniazid, and RIF among patients with TB, emphasizing the need for tailored treatment strategies in the region. Additionally, it identified social support and physical function as critical factors influencing resistance patterns, underscoring the importance of a holistic approach to combat drug-resistant TB. This study's findings prominently indicate that healthcare providers urgently need to adopt individualized treatment strategies that take into account the specific patterns of drug resistance observed in the region. This may include revised guidelines for the initial and ongoing treatment of TB, with a focus on more effective drug regimens and stricter monitoring of patient adherence. From a public health perspective, the findings highlight the importance of strengthening surveillance systems for drug resistance trends and of informing policy decisions. There is a clear need for targeted public health campaigns to raise awareness about the dangers of drug-resistant TB and the importance of completing the full course of medication.

Limitations

1. This analysis lacked detailed information on disease progression, treatment methods and certain laboratory test results, which warrants further research.
2. The molecular correlation could be further studied, and the evaluation index of patients should be improved.
3. Due to the use of pre-existing data, retrospective studies may lack certain specific information that was not fully documented in past records.

CONCLUSION

The drug resistance spectrum exhibited diversity and complexity, with variations in the distribution of drug resistance between newly diagnosed and patients who were treated again. Specifically, for male patients, especially those aged 20–40 years, and patients under outpatient follow-up, it is crucial to strengthen health education, improve healthcare insurance systems and enhance patient management models to improve compliance.

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Студија пресека о факторима који утичу на резистенцију на лекове клиничких узорака *Mycobacterium tuberculosis* у Хулунбуиру, Унутрашња Монголија

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САЖЕТАК

Увод/Циљ Циљ ове студије био је да побољша разумевање резистенције туберкулозе на лекове спровођењем ретроспективне анализе клиничких података болесника са туберкулозом у Хулунбуиру, Унутрашња Монголија, прикупљених између 2015. и 2017. године.

Методе Студија је укључивала ретроспективну анализу клиничких података болесника са туберкулозом. Подаци су коришћени за одређивање степена резистенције на појединачне лекове (укључујући етамбутол, изониазид, рифампицин и стрептомицин). Студија је такође испитивала стопе вишеструке резистенције на комбинације лекова, посебно изониазид + рифампицин + стрептомицин и изониазид + рифампицин + етамбутол + стрептомицин. Урађена је мултиваријантна логичка регресиона анализа да би се проценили фактори ризика повезани са резистенцијом на лекове, укључујући пол, статус хоспитализације, старост и претходна лечења.

Резултати Резултати су показали да су стопе резистенције на комбинације лекова, тј. вишеструке резистенције за обе комбинације, биле 4,51%, што је највећа стопа међу до сада анализираним комбинацијама. Етамбутол, изониазид и рифампицин имали су најучесталије три стопе резистенције међу свим изолатима. Изолати од болесника који су били подвргнути поновном лечењу показали су веће стопе резистенције на један лек, вишеструке резистенције и резистенцију на више лекова у поређењу са онима који су примали терапију први пут. Мултиваријантна логичка регресиона анализа је показала да су жене у мањем ризику од развоја резистенције на лекове од мушкараца. Поред тога, утврђено је да хоспитализовани болесници имају мањи ризик од појаве резистенције на лекове у поређењу са амбулантним болесницима. Студија је идентификовала старост између 20 и 40 година и поновну терапију као значајне факторе ризика за развој туберкулозе резистентне на лекове.

Закључак Ова студија је показала да је у региону Хулунбуир у Унутрашњој Монголији постојала приметна резистенција на лекове међу болесницима са туберкулозом, а са специфичним демографским факторима и факторима претходног лечења који доприносе развоју резистенције. Налази наглашавају важност разматрања ових фактора у развоју циљаних стратегија лечења и јавноздравствене политике за борбу против туберкулозе резистентне на лекове.

Кључне речи: *Mycobacterium tuberculosis*; резистенција на лекове; фактори од значаја

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Brucella-induced activation of AIM2 inflammasome and Caspase-1 enhances Interleukin-18 secretion in THP-1 cells

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SUMMARY

Introduction/Objective The purpose was to investigate the role of the inflammasome absent in melanoma 2 (AIM2) in *Brucella*-induced inflammatory responses in macrophages.

Methods A cell model of *Brucella* infection was established using a human macrophage cell line. Real-time quantitative polymerase chain reaction (RT-qPCR) and enzyme-linked immunosorbent assay (ELISA) kits were employed to measure the expression of the inflammasome AIM2 and the inflammatory cytokine Interleukin-18 (IL-18) at different time points post-infection, as well as to analyze the correlation between AIM2 and IL-18. Experiments involving the activation and inhibition of AIM2 were conducted to verify its effect on the production of the cytokine IL-18. Western blotting was conducted to determine the expression of AIM2 and IL-18.

Results It is found that IL-18 mRNA and concentration levels increased post-infection, peaking at 48 hours before decreasing by using RT-qPCR and ELISA ($p < 0.05$). Western blot analysis confirmed a similar temporal pattern for IL-18 protein expression ($p < 0.05$). Additionally, AIM2 and Caspase mRNA and protein expression were evaluated, showing a peak at 48 hours ($p < 0.05$), indicating inflammasome activation. The critical role of the AIM2 inflammasome and caspase-1 in IL-18 secretion was further demonstrated with AIM2 activators, which increased IL-18 mRNA and protein levels, and AIM2 inhibitors or Caspase-1 knockout, which reduced them ($p < 0.05$).

Conclusion *Brucella* infection induces the activation of the AIM2 inflammasome and caspase-1, leading to the secretion of the pro-inflammatory cytokine IL-18 in THP-1 cells.

Keywords: brucellosis; macrophage; AIM2; caspase-1; IL-18

INTRODUCTION

Human brucellosis is a zoonotic infectious disease caused by *Brucella* infection, which is widespread globally, with more than 500,000 new cases of human brucellosis reported by the World Health Organization annually [1, 2]. Human brucellosis is characterized by early nonspecific flu-like symptoms. Without timely antibiotic treatment, chronic infection may follow, leading to debilitating sequelae, including recurrent fever, osteomyelitis, arthritis, neurologic symptoms, and endocarditis [3]. The interaction between *Brucella* infection and the host immune response is key to the pathogenesis of the disease. Clarifying the pathogenic mechanisms of *Brucella* is crucial for the effective prevention and control of brucellosis; however, these mechanisms have not been well characterized.

The genus *Brucella* comprises gram-negative bacteria that cause disease in the host by surviving and replicating inside cells, primarily targeting macrophages, dendritic cells, and trophoblasts [4]. Macrophages are the first cellular barrier against external danger signals to the body. Studies have shown that the survival of *Brucella* within macrophages is due to its ability to modulate innate and adaptive immune signaling pathways. Macrophages employ various defense mechanisms, including phagocytosis, antigen presentation to immune effector cells, and inflammasome activation, and play a pivotal role in both inflammatory and chronic diseases. Macrophages are described as the primary cells expressing genes for inflammasomes, whose activation contributes not only to the secretion of pro-inflammatory cytokines but also to the induction of pyroptosis [5–8].

The innate immune system is the body's first line of defense [9]. It can recognize various endogenous and exogenous stimuli in the body through pattern recognition receptors, including pathogen-associated molecular patterns and danger-associated molecular patterns, and activate further immune responses [10, 11]. Inflammasomes are multi-protein complexes formed by the participation of pattern recognition receptors [12]. The absent in melanoma 2 (AIM2) inflammasome is one of the most studied and best described groups, acting as a cytosolic dsDNA sensor [13]. Upon host infection by bacteria, viruses, fungi, and parasites, the AIM2 inflammasome is typically activated. As an important cellular macromolecular signaling platform, the inflammasome converts the

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precursor of caspase-1 into active caspase-1, promoting the maturation and secretion of inflammatory cytokines: interleukin-1 β (IL-1β) and IL-18, while the activators also participate in the formation and cleavage of Gasdermin D, directly mediating pyroptosis [14, 15, 16]. Although the AIM2 inflammasome plays a critical protective role when the body is infected with viruses or bacteria, studies have shown that its overactivation causes damage to normal cells. The body has negative regulatory mechanisms for the activation of the AIM2 inflammasome that keep cells in a dormant state when not stimulated. Therefore, regulating the activation of the AIM2 inflammasome is key to controlling inflammatory responses and pyroptosis, enhancing the body's defenses, and reducing harm to the body. In recent years, the impact of AIM2 inflammasome-mediated pyroptosis on disease has received increasing attention, with scant research in the context of brucellosis. Thus, we undertook this work, using human-derived macrophage lines to explore the regulation of the AIM2 inflammasome and inflammatory cytokines during *Brucella* infection.

METHODS

Main materials and reagents

Human monocyte-derived macrophage line (THP-1 cells), *Brucella* suis strain M28, Roswell Park Memorial Institute (RPMI) medium or RPMI 1640, *Brucella* IL-18 enzyme-linked immunosorbent assay (ELISA) kit, *Brucella* total RNA extraction kit.

Establishment of the *Brucella* cell infection model

The *Brucella* cell infection model was established in a P3 laboratory. Human monocyte-derived THP-1 cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 1 mM pyruvate, 1% glutamine, 1% sodium pyruvate, and 1% penicillin/streptomycin. Cells were seeded at a density of 5×10^5 cells/well in a six-well plate and maintained at 37°C in a humidified 5% CO_2 incubator. Human-derived macrophages (THP-1) were infected with *Brucella* strains at various multiplicity of infections and cells were harvested at different time points (0, 24, 48, 72 hours).

Real-time quantitative polymerase chain reaction (RT-qPCR) for cytokine and inflammasome AIM2 expression levels

First, total RNA was extracted and purified from THP-1 cells using a total RNA extraction kit according to the manufacturer's instructions. RNA concentration and purity were measured with a spectrophotometer, and RNA integrity was assessed by agarose gel electrophoresis. RNA samples that passed quality control were used immediately for reverse transcription or stored at -80°C. Second, qualified RNA samples were reverse transcribed into cDNA using reverse transcriptase according to the manufacturer's instructions. Next, RT-qPCR reaction was performed. PCR primers were designed based on the published human AIM2 and caspase-1 gene sequences using Primer software. For the RT-qPCR reaction, cDNA was mixed with PCR master mix and aliquoted into tubes containing the primers. The final reaction volume was 20 μL, including 10 μL of PCR master mix, 0.8 μL of forward primer (10 μM), 0.8 μL of reverse primer (10 μM), 0.4 μL of ROX reference dye, and 2 μL of cDNA solution. Each gene in each sample was tested in triplicate. Tubes were gently mixed and briefly centrifuged to ensure that all components were at the bottom. The reaction mixture was then placed in RT-qPCR instrument for amplification according to the manufacturer's protocol. Finally, the relative mRNA expression levels were calculated. Ct values were automatically generated by the RT-qPCR instruction analysis software. Relative expression of mRNA levels was calculated using the 2-ΔΔCt method. The PCR primers involved in this study are listed in Table 1.

Table 1. Primer sequences of genes detected by RT-qPCR

Gene	Primer sequences
AIM2	F-TATCGGCACAGTGGTTTCTTAGAGG
	R-GGGCTGAGTTTGAAGCGTGTTG
Caspase-1	F-CCCACATCCTCAGGCTCAGAAG
	R-TGCGGCTTGACTTGTCCATTATTG
IL-18	F-TGGCTGCTGAACCAGTAGAAGAC
	R-GAGGCCGATTTCCTTGGTCAATG
GAPDH	F-TGCACCACCAACTGCTTAGC
	R-GGCATGGACTGTGGTCATGAG

RT-qPCR – real-time quantitative polymerase chain reaction; AIM2 – absent in melanoma 2; IL-18 – interleukin-18; GAPDH – glyceraldehyde 3-phosphate dehydrogenase

ELISA for IL-18 secretion cytokine levels

To quantify secreted cytokines, supernatants were collected from the 6-well plates and stored at -80°C. An ELISA kit was used to measure cytokine levels according to the manufacturer's instructions. After the reaction, a standard curve was plotted using a spectrophotometer, and the absorbance was measured to determine the level of the cytokine IL-18 in the serum samples.

Western blotting

Total protein of each cell group was extracted with RIPA lysate, and protein concentration was quantified using BCA protein concentration assay kit. Take 50 μg protein samples were separated by SDS-PAGE electrophoresis, and then wet transferred to PVDF membrane, 5% skimmed milk powder was sealed at room temperature for two hours. Diluted primary antibody was added respectively and incubated at 4°C overnight. The next day, the membrane was incubated with horseradish peroxidase-labelled secondary antibody at room temperature for two hours. The membrane was washed three times with tris-buffered saline with 0.1% Tween® 20 detergent. The enhanced chemiluminescence ultrasensitive chemiluminescent solution was added for

exposure in a gel imaging analysis system, and the gray values of the bands were measured by Image J software (Bethesda, MD: U.S. National Institutes of Health).

Data statistics

Data was analyzed using GraphPad Prism (GraphPad Software, San Diego, CA, USA) and presented as mean ± standard deviation (mean ± SD). Comparisons between the two groups were made using the independent samples t-test and between multiple groups using one-way ANOVA, with $p < 0.05$ being considered a statistically significant difference.

This work was approved by the Biomedical Research Ethics of Inner Mongolia Medical University (YKD202401097).

RESULTS

IL-18 mRNA levels and concentrations at different time points after THP-1 cells were infected with *Brucella*

After THP-1 cells were inoculated with *Brucella*, RT-qPCR and Elisa were used to evaluate the mRNA expression of the cytokine IL-18 at different time points. As shown in Figure 1A, it was observed that the mRNA expression of the cytokine IL-18 gradually increased over time after bacterial inoculation but began to decrease after 48 hours ($p < 0.05$). Likewise, as shown in Figure 1B, ELISA testing of the supernatant revealed that the concentration of the cytokine IL-18 increased significantly until reaching a peak at 48 hours and then began to decrease ($p < 0.05$).

Differences in IL-18 protein expression at different time points after THP-1 cells were infected with *Brucella*

To clarify the expression of IL-18 protein in THP-1 cells caused by *Brucella* infection, Western blotting was used to detect the protein expression of IL-18 in THP-1 cells at different time points (0, 24, 48, and 72 hours). The results showed that the expression of IL-18 in HP-1 cells increased in a time-dependent manner. At 48 hours, the protein expression of IL-18 in HP-1 cells reached a maximum ($p < 0.05$), as shown in Figure 2.

Analysis of inflammasome AIM2-related gene expression at different time points after THP-1 cells were infected with *Brucella*

After inoculation with *Brucella*, RT-qPCR was used to measure the relative expression of AIM2 and Caspase-1 mRNA at different time points. As shown in Figure 3A, the mRNA expression of cytokines AIM2 and Caspase changed over time after inoculation with *Brucella*. gradually increased over time and reached a peak at 48 hours

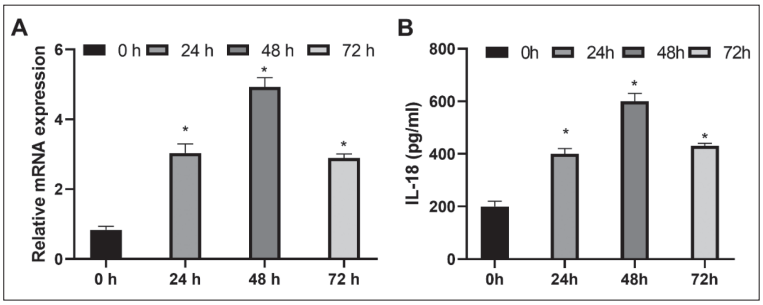

Figure 1. Expression of interleukin-18 (IL-18) at different time points after human monocyte-derived macrophage line (THP-1 cells) were infected with *Brucella*; A – relative expression of IL-18 mRNA in THP-1 cells after infection with Brucella; B – concentration of IL-18 in THP-1 cells after infection with *Brucella*;
compared to control $p < 0.05$

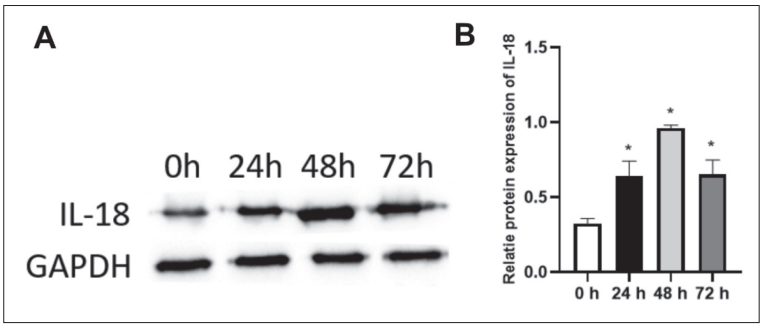

Figure 2. The secretion concentration of interleukin-18 (IL-18) at different time points after THP-1 cells were infected with *Brucella*; GAPDH – glyceraldehyde 3-phosphate dehydrogenase;
compared to control $p < 0.05$

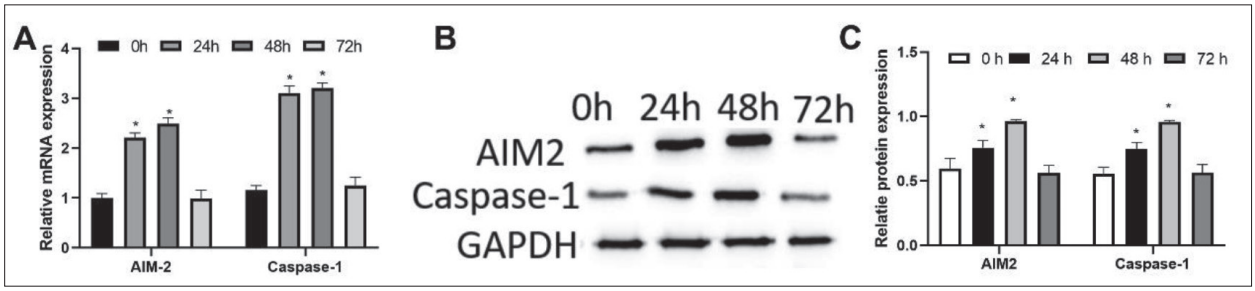

Figure 3. Relative levels of absent in melanoma 2 (AIM2) and Caspase-1 genes at different time points after human monocyte-derived macrophage line (THP-1 cells) were infected with Brucella; A – relative levels of AIM2 and Caspase-1 genes after THP-1 cells were infected with Brucella; B-C – relative protein expression of AIM2 and Caspase-1 in THP-1 cells after infection with Brucella; GAPDH – glyceraldehyde 3-phosphate dehydrogenase;
compared to control $p < 0.05$

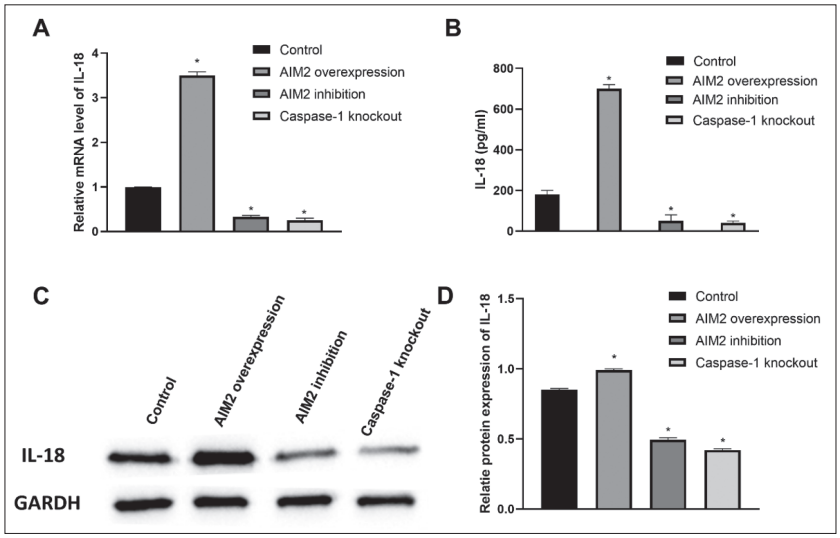

Figure 4. Absent in melanoma 2 (AIM2) and Caspase-1 mediate the secretion of cytokine interleukin-18 (IL-18); A – relative expression of IL-18 mRNA after interfering with the expression of AIM2 and Caspase-1; B – changes in IL-18 concentration after interfering with the expression of AIM2 and Caspase-1; C-D – changes in relative protein expression of IL-18 after interfering with the expression of AIM2 and Caspase-1; GAPDH – glyceraldehyde 3-phosphate dehydrogenase; compared to control $p < 0.05$

($p < 0.05$). As shown in Figure 3B–3C, Western blotting, after infection with *Brucella*, the relative protein expression of AIM2 and downstream caspase-1 genes in THP-1 cells reached a peak at 48 hours and then declined ($p < 0.05$). The results showed that the expression levels of AIM2 receptor and downstream caspase-1 activating genes increased early in infection, reached a peak after 48 hours, and gradually decreased.

Caspase-1 mediates the secretion of Cytokine IL-18

To confirm the role of inflammasome AIM2 in regulating cytokine secretion, THP-1 cells infected with *Brucella* were treated with AIM2 activators and inhibitors. Given the critical role of caspase-1 in classical inflammasome-mediated secretion of pro-inflammatory cytokines, we conducted gene knockout experiments to verify the role of caspase-1. RT-qPCR results found that treatment with AIM2 activator led to an increase in the relative expression of the cytokine IL-18 mRNA. On the contrary, after treatment with an AIM2 inhibitor and Caspase-1 knockout, the mRNA expression of IL-18 increased. The relative expression of mRNA decreased ($p < 0.05$), as shown in Figure 4 A. ELISA test found that the release concentration of cytokine IL-18 increased significantly after AIM2 activator treatment ($p < 0.05$), while after AIM2 inhibitor treatment and Caspase-1 knockout, the secretion level of cytokine IL-18 was decreased compared to the control group ($p < 0.05$), as shown in Figure 4 B. Western blotting results showed the same trend, as shown in Figures 4 C-D. The above results indicate that *Brucella* activates caspase-1 by activating inflammasome AIM2, thereby inducing the secretion of IL-18.

DISCUSSION

Inflammation plays a vital role in the pathogenesis and progression of brucellosis. Macrophages serve as the first line of defense against pathogens by sensing damage stimuli and producing various cytokines. The innate immune system includes pattern recognition receptors that can detect pathogen-associated molecular patterns such as lipopolysaccharides, peptidoglycans, or viruses [17]. The abnormal presence of these substances can activate the innate immune system, leading to the production of inflammatory cytokines to combat exogenous or endogenous threats. They also act as alarm signals to antigen-presenting cells, thereby initiating the adaptive immune response and serving as a bridge between innate and adaptive immunity. AIM2, a recently identified inflammasome receptor, is a member of the hematopoietic interferon-inducible HIN200 protein family. It binds to DNA through the HIN200 domain and forms a complex with ASC to initiate caspase-1, which promotes secretion of the cytokines IL-1β and IL-18 [18]. Studies have shown that the AIM2 inflammasome pathway is critical for host detection of stealth bacterial pathogens such as *Brucella*, which lack highly stimulatory ligands such as flagellin proteins and classical lipopolysaccharides. Due to the heterogeneity of tissue cells, the inflammatory cell necrosis induced by the inflammasome may have both promoting and inhibitory effects.

In present study, we characterized the role of the AIM2 inflammasome in induction of macrophage inflammatory responses to *Brucella* using human macrophage cell lines. By monitoring IL-18 production, we revealed the important role of AIM2 in the mediating of caspase-1 activation and IL-18 production in macrophages.

Currently, IL-18-mediated inflammation is mainly studied in animal models of bacterial, viral, parasitic, and fungal infections. The immune microenvironment shaped by IL-18 can further induce the differentiation of naive T cells into effector and memory T cells, activating the adaptive immune response and playing an important biological role in inflammation-related diseases. The study by Lin et al. [19] investigated the relationship between caspase-1-related inflammasome expression and serum inflammatory cytokine levels during acute brucellosis. In the acute phase of the disease, the inflammasomes are fully activated, inducing cytokines such as IFN-γ and IL-18, thereby triggering a cellular immune response. Our research also indicates that the production of IL-18 increases in the early stages of *Brucella* infection, which may be a crucial mechanism in the acute phase inflammatory response. We hypothesize that during the transition to the chronic phase of brucellosis, excessive production of IL-18 may lead to increased inflammatory burden and tissue damage, with deleterious consequences for the host. However, our results show that

the IL-18 secretion begins to decline 72 hours after *Brucella* infection. According to the 2019 *Brucella* guidelines, a diagnosis of chronic brucellosis is only confirmed after at least six months. Therefore, future experiments could extend the infection period to observe changes in IL-18 secretion to verify our hypothesis.

Studies have confirmed that the expression of the AIM2 inflammasome is increased in patients with systemic lupus nephritis and psoriasis, suggesting that the AIM2 inflammasome may be involved in autoimmune diseases [20, 21]. By measuring the expression of the AIM2 inflammasome at different time points during *Brucella* infection, we found a significant increase in AIM2 expression in the early stages of infection. We then treated *Brucella*-infected macrophages with AIM2 inflammasome activators and inhibitors. Compared to normal conditions, treatment with activators increased the release of the cytokine IL-18, while inhibitors significantly reduced it. These results confirm the important role of the AIM2 inflammasome in regulating the secretion of cytokine IL-18, and its involvement in the immune response to *Brucella*. By recognizing foreign or intracellular dsDNA, AIM2 triggers inflammasome activation, leading to the release of pro-inflammatory cytokines such as IL-1β, IL-18, and the induction of a heat metastasis response. In addition, AIM2 interacts with signaling pathways independent of inflammasome activation, such as AKT and NF-κB, to regulate cancer progression [22], which is consistent with our findings.

The upregulation of AIM2 and IL-18 expressions in the early stages of infection, followed by a downregulation after 72 hours, and the positive correlation between AIM2 expression and IL-18, may be attributable to the early recognition of *Brucella* by the AIM2 receptor. The pathogen is initially controlled through the generation of inflammatory cytokines. However, as the disease progresses into the chronic phase, *Brucella* may employ mechanisms to suppress or downregulate the AIM2 receptor response to evade clearance by the host immune system. In a research conducted by Su et al. [23] of patients with acute and chronic brucellosis, it was found that gene expression levels of the AIM2 receptor were higher in the acute-phase group than in the healthy control group, and lower in the chronic phase group, highlighting the significant role of the inflammasome AIM2 in the pathogenesis and development of brucellosis.

The inflammasome AIM2, upon binding to the precursor of caspase-1, facilitates the cleavage and activation of caspase-1. Activated caspase-1 promotes the production and secretion of IL-1β and IL-18, and can also cause cell membrane rupture, leading to the release of intracellular inflammatory cytokines and triggering a robust inflammatory response [24]. Using caspase-1 knockout experiments, we discovered that caspase-1 plays a critical role in the generation of IL-18, which is essential for the early innate immune response to *Brucella* infection. Fernanda and others have investigated the role of NLRP3 and AIM2 in inflammasome activation following *Brucella* infection and have shown that after *Brucella* infection of glial cells, it is predominantly the NLRP3 and AIM2 inflammasomes that coordinate to induce caspase-1 activation and cytokine secretion [25]. It is believed that the activation of the inflammasome AIM2 and caspase-1 plays an important role in the generation of the inflammatory response.

Limitations of the study

Based on our study findings, we hypothesize that the transition of brucellosis to its chronic phase may be due to the activation of AIM2, which triggers the overproduction of cytokines, leading to tissue damage. These insights and understandings pave the way for further investigation into the pathogenic mechanisms of *Brucella*, which will contribute to the development of novel therapeutic strategies. However, present research has its limitations; as a multifactorial disease, focusing solely on a single inflammasome within brucellosis does not provide a comprehensive picture, and a more holistic approach to the disease is warranted. It should also be noted that the role of AIM2 has been primarily studied in animals, and the impact on the immune capabilities of other human organs and tissues has not been thoroughly investigated. Emphasis on the local suppression of excessive and persistent activation of various inflammasomes may represent a future therapeutic direction for the treatment of brucellosis.

CONCLUSION

Brucella infection induces the activation of the AIM2 inflammasome and caspase-1, leading to the secretion of the pro-inflammatory cytokine IL-18 in THP-1 cells.

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Contribution of authors: We declare that this work was done by the authors named in present article. Li Yupu conceived and designed the study; Zhaojing Zhang, Pengfei Zhao and Pengfei Qiao collected and analyzed the data, while Yupu Li wrote the manuscript. All authors read and approved the manuscript.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest: None declared.

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Активација инфламазома *AIM*2 и каспазе-1 изазвана бруцелом повећава секрецију ИЛ-18 у *THP*-1 ћелијама

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САЖЕТАК

Увод/Циљ Циљ овог истраживања био је да се испита улога инфламазома одсутног у меланому 2 (*AIM*2) у инфламаторном одговору макрофага изазваном инфекцијом бруцелом.

Методе Модел инфекције ћелија бруцелом успостављен је коришћењем људске макрофагне ћелијске линије. Квантитативна полимеразна ланчана реакција (*RT-qPCR*) и ензимски повезани имуносорбентни тест (*ELISA*) коришћени су за мерење експресије инфламазома *AIM*2 и инфламаторног цитокина интерлеукина-18 (ИЛ-18) у различитим интервалима после инфекције, као и за анализу корелације између *AIM*2 и ИЛ-18. Спроведени су експерименти активације и инхибиције *AIM*2 како би се истражила његова улога у продукцији цитокина ИЛ-18. За одређивање експресије *AIM*2 и ИЛ-18 коришћена је метода Вестерн блот.

Резултати Утврђено је да су нивои мРНК ИЛ-18 и његова концентрација повећани после инфекције, достигавши максимум после 48 сати, пре него што су се смањили, што је потврђено *RT-qPCR* и *ELISA* тестом ($p < 0,05$). Анализом Вестерн блот потврђен је сличан временски образац експресије протеина ИЛ-18 ($p < 0,05$). Додатно, испитани су нивои мРНК и експресија протеина *AIM*2 и каспазе-1, који су достигли врхунац 48 сати после инфекције ($p < 0,05$), што указује на активацију инфламазома. Кључна улога инфламазома *AIM*2 и каспазе-1 у секрецији ИЛ-18 додатно је потврђена, јер је активација *AIM*2 довела до повећања нивоа мРНК и протеина ИЛ-18, док су инхибицијом *AIM*2 или инактивацијом каспазе-1 ти нивои смањени ($p < 0,05$).

Закључак Инфекција бруцелом изазива активацију инфламазома *AIM*2 и каспазе-1, што доводи до секреције проинфламаторног цитокина ИЛ-18 у *THP*-1 ћелијама.

Кључне речи: бруцелоза; макрофаг; *AIM*2; каспаза-1; ИЛ-18

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The predictive role of tumor infiltrating lymphocytes and pathohistological parameters for the occurrence of metastases in the clinical N0 neck of early-stage oral squamous cell carcinoma

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SUMMARY

Introduction/Objective With 337,173 registered cases worldwide in 2020, oral squamous cell carcinoma is the most common malignant tumor of the head and neck region. The status of lymph nodes in the neck is the most important isolated prognostic factor for the five-year survival of patients. This study aimed to determine the pathohistological predictors of the occurrence of occult neck metastases in early-stage oral squamous cell carcinoma.

Methods The study included 40 patients (mean age 62.8 ± 10.7) with early-stage oral squamous cell carcinoma and clinical N0 findings in the neck. All patients underwent radical transoral tumor ablation and elective neck lymphadenectomy. Based on pathohistological findings, the patients were divided into two groups: a group with occult metastases present and a group without occult metastases.

Results Occult metastases were present in 13 patients (32.5%). The results indicate a significant difference in the desmoplastic reaction of the stroma ($p < 0.001$), depth of invasion ($p < 0.001$), lymphocytic infiltration ($p < 0.001$), and lymphovascular invasion in univariate (OR 24, $p = 0.004$) and multivariate (OR 32.713, $p = 0.017$) logistic regression analyses in the group with occult metastases compared to the group without metastases.

Conclusion The values of the analyzed pathohistological predictors indicate a high degree of correlation with the occurrence of occult metastases in the neck. Their predictive significance strongly supports including these parameters as part of the standard pathohistological examination of oral squamous cell carcinoma.

Keywords: oral squamous cell carcinoma; occult metastases; pathohistological analysis; surgical treatment; observation

INTRODUCTION

With 337,713 registered cases worldwide in 2020, oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the head and neck region [1]. Given that it accounts for up to 90% of the total number of intraoral malignant tumors, it is by far the most common intraoral malignancy [2]. Aggressive behavior towards the surrounding anatomical structures and their rapid infiltration, early appearance of metastases in the neck, and frequent recurrence are some of the key clinical characteristics of OSCC. The five-year survival rate of early-stage OSCC patients varies from 80–90%, in contrast to advanced forms characterized by a five-year survival of 30–50% [3]. The fundamentals of OSCC treatment include radical tumor ablation, neck lymphadenectomy, adequate reconstruction of the resulting defect, and postoperative oncological therapy.

The status of lymph nodes in the neck (LNs) represents the most important isolated prognostic factor for the survival of patients with OSCC. The presence of metastases in the neck significantly reduces the five-year survival of patients to below 50% [4]. The treatment of N0 neck in the early stages of OSCC (pT1-N0-M0 and pT2-N0-M0) is still the subject of many debates in head and neck surgery without a clearly defined viewpoint for conducting elective neck lymphadenectomy (END) or the so-called watchful-waiting method, i.e., the observation of patients. The presence of occult metastases (OM) in the range of 16–36%, pathohistologically verified in postoperative dissections of the neck, significantly affects the five-year survival of patients [5]. Sentinel lymph node biopsy (SNB) is a routine surgical procedure for the detection of OM in the neck in OSCC. The use of SNB proves high sensitivity for detecting micrometastases and isolated tumor cells (ITC), a lower degree of morbidity of the surrounding tissue, and a considerably more positive economic aspect compared to END [6]. The sensitivity of SNB in the range

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of 75–87% for registering OM in the early stage of OSCC defines the method as safe and rational [7].

Literature data suggest that based on the pathohistological analysis of OSCC preparations, which includes depth of invasion (DOI), desmoplastic stromal reaction (DR), tumor budding, lymphovascular invasion (LVI), perineural invasion (PNI), and density of tumor-infiltrating lymphocytes (TILs), the occurrence of extranodal extension (ENE) of OSCC, which is directly related to the occurrence of metastases in the neck, can be predicted [8, 9].

This study aimed to determine whether the aforementioned pathohistological parameters can be reliable predictors for OM occurrence in OSCC stage pT1-N0-M0 and pT2-N0-M0.

METHODS

The study included 40 consecutive patients of both sexes treated at the Clinic for Maxillofacial Surgery in Niš from 2019 to 2022, in whom the presence of OSCC stage cT1-N0-M0 and cT2-N0-M0 was determined based on medical history, clinical examination, initial biopsy, and additional radiological examinations.

The criteria for excluding patients from the study included patients with higher T stages (cT3-cT4), recurrent OSCC that was in rT1-rT2 stage at the time of examination, radiologically confirmed the presence of secondary deposits (M1 stage), initially inoperable forms of OSCC turned into an operable state after oncological treatment, patients in whom the progression of the disease occurred from the verification of OSCC stage cT1-cT2 to the surgical procedure, and patients in whom, due to general condition or comorbidities, intervention in general endotracheal anesthesia was contraindicated.

The analysis of anamnestic data determined age, gender and risk factors in terms of alcohol consumption and tobacco smoking.

The clinical examination determined the precise location of the suspicious lesion in the oral cavity as well as its clinical form (exophytic or endophytic). The clinical examination also determined the Karnofsky score (Karnofsky Performance Status Scale – KPS) of the patients, which ranged 90–100 in all subjects (Figure 1).

After the anamnestic and clinical examination, punch biopsy of the suspicious lesion in the oral cavity was performed for the pathohistological verification of OSCC.

Radiological examinations included multi-slice computerized tomography of the head and neck, magnetic resonance imaging of the head and neck, lung imaging, and ultrasound of the neck and abdomen. Radiological examinations determined the precise diameter of the tumor, as well as its relation to the surrounding soft tissue and bone structures.

Surgical treatment of patients included radical transoral tumor ablation and END of neck levels I, II, and III (Figure 2).

The pathohistological analysis of postoperative OSCC preparations was done after the fixation of the preparations in a 10% solution of buffered formalin. The micromorphological analysis of histological sections made with the standard hematoxylin-eosin (H&E) method included the analysis of the status of resection margins, analysis of DOI, DR, LVI, PNI, tumor grade, and density of TILs. Immunohistochemical analyses were performed by determining the expression of pan-cytokeratin (pan-CK) (AE1/AE3) in suspicious lymph nodes of postoperative neck dissections to identify occult metastatic changes.

The statistical processing of data involved presentation in the form of arithmetic mean, standard deviation, minimal and maximal values, and ratio in the form of absolute and relative numbers. Numerical variables were compared using the t-test, whereas categorical characteristics were compared with the χ^2 test or Fisher's exact probability test. Logistic regression analysis was used to assess the

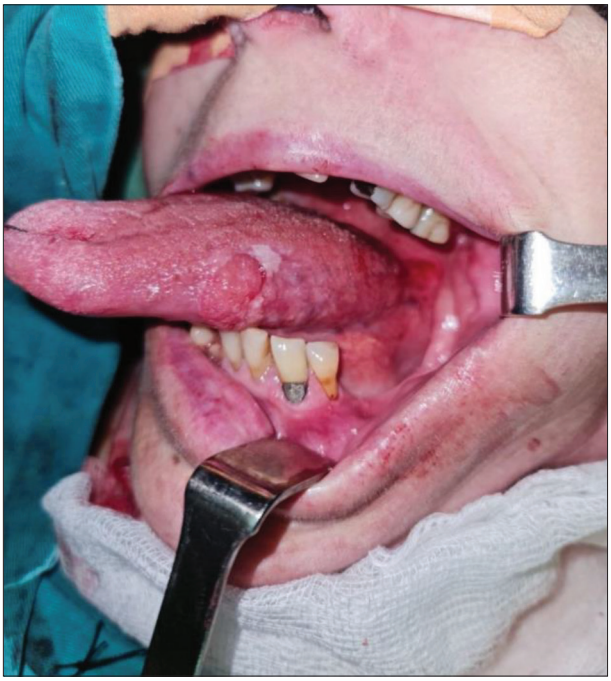

Figure 1. Oral squamous cell carcinoma of the tongue

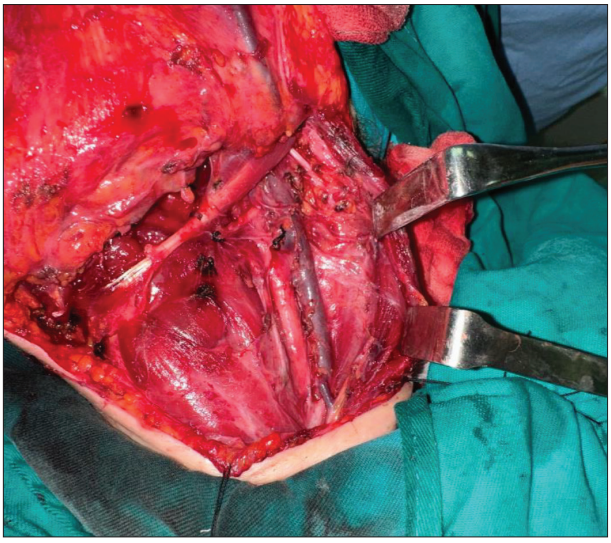

Figure 2. Elective neck lymphadenectomy of levels I, II, and III

Table 1. Demographic and clinical characteristics of patients regarding the presence of metastases

Characteristic		Total		Occult metastases				p[1]
				Yes		No		
Sex	male	25	62.5	9	69.2	16	59.3	0.794
	female	15	37.5	4	30.8	11	40.7	
Age (years)	< 55	10	25	3	23.1	7	25.9	1.000*
	55+	29	75	10	76.9	20	74.1	
Smoking		29	72.5	13	100	16	59.3	0.007*
Alcohol		22	55	9	69.2	13	48.1	0.312
Growth type	exophytic	21	52.5	5	38.5	16	59.3	0.370
	endophytic	19	47.5	8	61.5	11	40.7	
Tumor grade	I	12	30	1	7.7	11	40.7	0.114*
	II	20	50	9	69.2	11	40.7	
	III	8	20	3	23.1	5	18.5	
Tumor stage	T1	12	30	6	46.2	6	22.2	0.238
	T2	28	70	7	53.8	21	77.8	
Perineural invasion		25	62.5	7	53.8	18	66.7	0.663
Lymphovascular invasion		21	52.5	12	92.3	9	33.3	0.001*
Desmoplastic reaction	mature	15	37.5	0	0	15	55.6	< 0.001*
	intermediate	15	37.5	3	23.1	12	44.4	
	immature	10	25	10	76.9	0	0	
Depth of invasion (mm)	< 5	24	60	0	0	24	88.9	< 0.001*
	5+	16	40	13	100	3	11.1	
Lymphocytic infiltration	high	18	45	0	0	18	66.7	< 0.001*
	intermediate	13	32.5	4	30.8	9	33.3	
	low	9	22.5	9	69.2	0	0	

[1]χ^2 test;
*Fisher's exact probability test

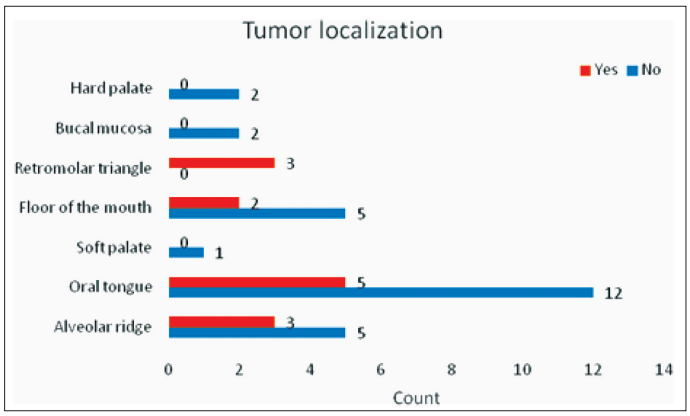

Figure 3. Tumor localization regarding the presence of occult metastases

relationship between the studied variables and the appearance of OM.

We excluded specific variables (DR, DOI, smoking, lymphocyte infiltration) from our logistic regression model due to perfect separation or multicollinearity, which was identified when these variables perfectly predicted the outcome, leading to model estimation issues such as non-convergence. This decision was made to ensure the stability and reliability of our model's parameter estimates.

The null hypothesis was tested with a significance threshold of $p < 0.05$. Statistical data processing was performed in the SPSS Statistics, Version 16.0 (SPSS Inc., Chicago, IL, USA) software package.

The study was approved by the Ethics Committee of the Dental Clinic in Niš (14/14-2019-4 EO) and the Ethics Committee of the Faculty of Medicine in Niš (12-16502/2-1).

RESULTS

The study included 40 patients (25 male and 15 female). The mean age of the studied population was 62.8 ± 10.7 (min 37 years, max 85 years). The majority of subjects were male (62.5%), older than 55 years (75.0%), smokers (72.5%), who regularly consumed alcohol (55.0%) and had exophytic tumor growth (52.5%).

There were 13 patients with OM in the group (32.5%). There was no statistically significant difference with respect to sex ($p = 0.794$), age ($p = 1.000$), alcohol consumption ($p = 0.312$), tumor growth type ($p = 0.370$) and tumor grade ($p = 0.114$) regarding the presence of OM (Table 1). Smoking was statistically significantly more common in patients with OM ($p = 0.007$).

PNI was present in 62.5% of patients and did not differ significantly in relation to the presence of metastases ($p = 0.663$). LVI was statistically significantly different in relation to the presence of metastases (92.3% *vs.* 33.3%, $p = 0.001$). DR differed statistically significantly in relation to the presence of metastases ($p < 0.001$), as well as DOI ($p < 0.001$). Lymphocytic infiltration was statistically significantly different in relation to the presence of metastases ($p < 0.001$). Statistical analysis does not show the existence of a significant correlation between tumor stage of OSCC (T1–T2) nor the presence of OM in the neck.

The lesions were localized on the tongue (42.5%), alveolar processes (20%), floor of the oral cavity (17.5%), hard palate (5%), retromolar triangle (7.5%), buccal mucosa (5%), and soft palate (5%) (Figure 3).

Univariate logistic regression analysis showed that LVI is a statistically significant risk

Table 2. Risk factors for the occurrence of occult metastases – univariate logistic regression analysis

Characteristic		Univariate logistic regression				Multivariate logistic regression			
		OR	95% CI		p	OR	95% CI		p
Female sex		0.646	0.158	2.637	0.543	2.126	0.041	109.137	0.707
Age		1.007	0.945	1.072	0.832	1.100	0.976	1.239	0.117
Alcohol		2.423	0.598	9.816	0.215	4.897	0.082	290.940	0.446
Growth type – exophytic		2.327	0.600	9.028	0.222	0.495	0.036	6.881	0.601
Tumor grade	I	ref group			0.114				0.244
	II	9.000	0.969	83.583	0.053	31.774	0.561	1798.965	0.093
	III	6.600	0.543	80.235	0.139	9.662	0.241	386.897	0.228
Perineural invasion		0.583	0.151	2.256	0.435	.069	0.004	1.266	0.072
Lymphovascular invasion		24.000	2.682	214.725	0.004	32.713	1.862	574.667	0.017
Constant						0.000			0.025

OR – odds ratio; 95% CI – 95% confidence interval; Hosmer–Lemeshow test $p = 0.135$

factor for OM (OR 24.0, p = 0.004). Moreover, it remained a statistically significant risk factor in the multivariate model (OR 32.713, p = 0.017) (Table 2).

DISCUSSION

The distribution of OSCC in the oral cavity varies at a global level and is primarily dependent on the geographic location that determines exposure to the most frequent etiological factors. The location of OSCC in the oral cavity is an important prognostic factor, as well as an implication of the patient's surgical treatment. Literature data indicate that the most common site of OSCC in Thailand, India and Southeast Asian countries is the buccal mucosa [10]. Available data from Nigeria and Germany highlight the gingiva of the alveolar processes of the lower and upper jaw as well as the floor of the oral cavity as predilection sites for OSCC [11, 12]. OSCC is most common in the tongue (41.7%), floor of the oral cavity and lips (16.5%, each), gingiva of the alveolar processes of the upper and lower jaw (10.6%), buccal mucosa (6.7%), retromolar triangle (5.6%), and hard and soft palate structures (2.3%) [13].

Alcohol and tobacco consumption are the most important etiological factors associated with the occurrence of OSCC. These factors are present in 70–80% of patients with OSCC [14]. Chemical carcinogens from alcohol and tobacco participate synergistically in generating mutations in tumor suppressor genes and thus promote carcinogenesis.

OSCC is considerably more frequent in males than in females. Literature data suggest that men make up 90–93% of patients with OSCC, whereas women represent only 7–10% of patients [15]. A marked discrepancy in the frequency of patients of both sexes is explained by more frequent exposure to etiological factors in men, in contrast to women. The male-female ratio differs primarily at the regional level, but the percentage of men with OSCC is almost always higher.

OSCC is significantly more common in patients older than 50 years [16]. The presented data on age vary by the region and the number of patients included in the study.

Pathohistological findings in postoperative neck dissections can be negative, indicating the presence of ITCs, micrometastases (MM) or macrometastases. Negative findings imply reactive cellular changes, sinus histiocytosis and benign cell inclusions. By definition, ITCs are small cell aggregates not larger than 0.2 mm, or non-confluent aggregates not exceeding 200 cells per section. MM represent individual tumor deposits with a 0.2–2 mm diameter, unlike macrometastases, which also represent individual tumor deposits, but larger than 2 mm. Considering that it is impossible to prove the existence of ITCs and MM by standard H&E examination, pan-cytokeratin (pan-CK) (AE1/AE3) has been introduced as a useful marker for determining the presence of OM. The detected presence of ITCs and MM in the value of 33.3% on preparations that were negative by H&E processing indicates that pan-CK (AE1/AE3) is a reliable marker for the detection of ITCs and MM [17]. DOI was defined as the distance from the normal basal membrane to the deepest point of tumor invasion, is an independent risk factor for the occurrence of OM in lymph nodes of the neck in patients with OSCC. Studying patients with early-stage OSCC, the threshold and increased value of DOI would represent an indicator for END [18]. The association between DOI over 5 mm and 36.25% of OM was presented in a study by Pakistani authors [19]. The study included 80 patients with pathohistologically proven OSCC. The authors concluded that patients with DOI +5 have a relative risk of occult neck nodes metastasis and that there is no significant relationship of occult neck node metastasis with age, gender, risk factors and tumor grade. LVI by definition refers to the presence of tumor cells in the lumen of a blood vessel or cellular adhesion to the vascular wall. The presence of LVI is interpreted as a negative prognostic factor for patients suffering from OSCC. In a study that included 442 patients with OSCC, Abigail E. Moore et al. [20] concluded that LVI is a significant pathohistological parameter for the occurrence of regional metastases. The same authors also reported the absence of correlation of LVI with tumor aggressiveness and local recurrence. Prakash Pandit et al. [21] conducted a study on 462 patients with OSCC and confirmed the link between LVI and metastases in the lymph nodes of the neck.

PNI represents the appearance of a tumor in close proximity to a nerve, involving at least 33% of its volume, or the presence of tumor cells within any of the three layers of the nerve sheath. The presence of PNI on pathohistological preparations of OSCC is associated with increased morbidity, poor prognosis and frequent local recurrences, but without a significant impact on the occurrence of regional metastases [22]. DR type is one of the key stromal characteristics of malignant tumors of the gastrointestinal tract [23]. Based on the morphology and arrangement of collagen fibers and myxoid stroma, DR is divided into mature, intermediate, and immature. The insight into available literature reveals works of numerous researchers who associate DR with the clinical characteristics of OSCC. In a study that included 308 patients with early-stage OSCC of the tongue, Almangush et al. [24] determined a relationship between the stromal reaction type and patient survival. However, the same authors did not find a relationship between the DR type and the appearance of metastases in the neck. Furthermore, Bittar et al. [25] conducted a study that included 157 patients with verified OSCC and a clinical N0 finding in the neck and determined an association between the appearance of OM in the neck and the type of DR. Noda et al. [8] suggested an association between immature tumor stroma and ENE. The aforementioned authors in a study that included 83 patients with pathohistologically verified OSCC indicate the existence of a significant correlation between immature tumor stroma and ENE. In addition, the results of our study indicate a significant correlation between the occurrence of OM and the immature tumor stroma of OSCC preparations.

TILs are a selected population of lymphocytes, predominantly of the T form, with a highly specific immune response to tumor cells. They represent reliable biomarkers for many solid tumors, including malignant tumors of the head and neck [26]. The method for the standardized assessment

of the presence of TILs using the H&E method published by the International Immuno-Oncology Biomarker Working Group is being very effectively applied in daily clinical practice [27]. Literature data indicate that high tumor infiltration by TILs represents a favorable prognostic sign for OSCC and other malignant tumors, in terms of an increased survival rate and less aggressiveness of the tumor [28]. The results of our study, which indicate a relationship between the appearance of OM in the neck, aggressiveness of the tumor and low tumor infiltration by TILs, are in correlation with the results of the aforementioned authors [8, 9, 29, 30].

CONCLUSION

The presence of OM in the 16–36% range in postoperative neck dissections undoubtedly has a negative effect on the five-year survival of OSCC patients, which varies 30–90%, depending on the stage of the tumor and the status of LNs.

The results of our study indicate a high degree of association of the analyzed pathohistological parameters with the occurrence of OM in the neck in patients with early-stage OSCC. The significance of predicting the risk of OM is a strong argument for the aforementioned parameters to be part of the standard pathohistological examination of OSCC.

We firmly believe that our idea of future inclusion of epigenetic examination of OSCC preparations in standard diagnostics will contribute to early detection of OM and thus an increase in the five-year survival of affected patients.

Conflict of interest: None declared.

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Предиктивна улога туморских инфилтрирајућих лимфоцита и патохистолошких параметара у настанку метастаза на клиничком *N*0 врату у раном стадијуму оралног сквамоцелуларног карцинома

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САЖЕТАК

Увод/Циљ Орални сквамоцелуларни карцином, са 337.713 регистрованих случајева у свету 2020. године, најучесталији је малигни тумор регије главе и врата. Статус лимфних нодуса врата представља најважнији изоловани прогностички фактор за петогодишње преживљавање болесника.
Циљ овог истраживања је утврдити да ли се на основу патохистолошке анализе препарата раног стадијума оралног сквамоцелуларног карцинома може предвидети појава окултних метастаза на врату.

Методе У студију је укључено 40 болесника са клиничким *N*0 налазима на врату у раном стадијуму оралног сквамоцелуларног карцинома. Свим болесницима је учињена радикална трансорална аблација тумора и елективна лимфаденектомија врата. На основу дефинитивног патохистолошког налаза болесници су подељени у групу са присуством и групу са одсуством окултних метастаза.

Резултати У испитиваној групи окултне метастазе су регистроване код 13 болесника (32,5%). Резултати студије указују на постојање значајне разлике параметара дезмопластичне реакције строме ($p < 0,001$), дубине инвазије ($p < 0,001$), лимфоцитне инфилтрације ($p < 0,001$) и лимфоваскуларне инвазије у универијантној (*OR* 24, $p = 0,004$) и мултиваријантној (*OR* 32,713, $p = 0,017$) логистичној регресионој анализи у групи болесника са окултним метастазама у односу на групу болесника без метастаза.

Закључак Резултати наше студије указују на директну повезаност појаве окултних метастаза на врату и појединих патохистолошких параметара. Сигнификантност у предикцији појаве окултних метастаза представља чврст аргумент да поменути параметри треба да биту стандардни део патохистолошког испитивања оралног сквамоцелуларног карцинома.

Кључне речи: орални сквамоцелуларни карцином; окултне метастазе; патохистолошка анализа; хируршко лечење; праћење

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Correlation of microRNAs-10b/21/34a expression levels with *IDH1*-mutation status in patients with glioblastoma

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SUMMARY

Introduction/Objective Isocitrate dehydrogenase (IDH) mutations play a significant role in gliomagenesis. Specific microRNAs, such as microRNA-10b and microRNA-21, act as oncogenic microRNAs, whereas microRNA-34a acts as a tumor suppressor in glioblastoma. Our study aimed to investigate whether the IDH1 mutation status correlates with microRNA-10b, -21, and -34a expression levels in patients with glioblastoma.

Methods The study included 43 patients diagnosed with glioblastoma. We examined microRNA-10b, -21, and -34a expression levels in peripheral blood mononuclear cells after surgery and prior to concurrent radiotherapy with temozolomide, as well as at the 15th and 30th fractions of radiotherapy with temozolomide. Data on IDH1 mutation status were gathered from medical histories and histopathology.

Results Two groups were created to assess the association of microRNA-10b, -21, and -34a expression levels: glioblastoma IDH1-wildtype and glioblastoma IDH1-mutant + not otherwise specified (NOS). The median microRNA-10b expression level before the initiation of concurrent radiotherapy with temozolomide was 130.44 (52.2–622.53) in the IDH1-wildtype group and 94.61 (2.13–816.89) in the IDH1-mutant + NOS group. The median microRNA-21 expression level was 57.16 (2.68–278.98) in the IDH1-wildtype group and 69.74 (4.6–825.43) in the IDH1-mutant + NOS group. The median microRNA-34a expression level was 13.52 (3.16–105.20) in the IDH1-wildtype group and 10.11 (1–210.55) in the IDH1-mutant + NOS group. The results showed no statistically significant difference in the expression levels of microRNA-10b, -21, or -34a between the two groups ($p > 0.05$).

Conclusion Our findings suggest that IDH1 mutation status may not be a critical factor for altered expression of microRNA-10b, -21, and -34a in glioblastoma patients.

Keywords: glioblastoma; microRNA; IDH mutation

INTRODUCTION

Over the past few years, a significant body of research has focused on the molecular and genetic profile of glioblastoma. This combined approach – defined by histopathology, molecular features, and genetic alterations in glioblastoma – led to changes in the World Health Organization (WHO) classification in 2016 and 2021 and a better understanding of tumor biology and clinical behavior of the disease [1, 2].

One of the most important features in the 2016 WHO classification of brain tumors was the inclusion of isocitrate dehydrogenase (IDH) mutation status in glioma classification. Since IDH1 is one of the most important enzymes for cell metabolism, alterations in IDH1 expression or gene mutations can impact enzyme activity and impair cellular metabolism [3]. IDH mutation is considered one of the initial occurrences in the development of astrocytomas and oligodendrogliomas [4]. In fact, research on the sequence of mutations in gliomas shows that IDH mutations occur even before *TP53* mutations in low-grade diffuse astrocytoma and secondary glioblastoma, but they are rare in primary glioblastoma [4]. According to the 2016 WHO classification, glioblastoma was divided into glioblastoma IDH-mutant, WHO grade IV, glioblastoma, IDH-wildtype WHO grade IV, and glioblastoma, Not Otherwise Specified (NOS) WHO grade IV [1]. In 2021, in addition to other parameters for the classification of glioblastoma, any astrocytoma with wildtype IDH is considered glioblastoma, IDH-wildtype, Central Nervous System (CNS) WHO grade IV [2].

MicroRNAs (miRNAs) are non-coding RNAs that play a critical role in gene expression regulation. They bind to incompletely complementary sequences on target messenger RNAs (mRNAs), leading to mRNA degradation or translation inhibition [5]. This mechanism of post-transcriptional regulation enables miRNAs to control various biological processes, such as development, differentiation, proliferation, and apoptosis, and they are among the key regulators of cell metabolism [6]. The expression of microRNAs can be altered by various mutations or regulated through promoter

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methylation [5]. MiRNAs can directly or indirectly target different genes, including IDH. Conversely, IDH mutations after the production of 2-hydroxyglutarate (2-HG) can influence or alter the expression of various miRNAs and regulate tumor development in gliomas [7].

In glioblastoma, specific miRNAs may have significant impact on tumorigenesis, invasiveness, and resistance to therapy. MiR-10b, miR-21, and miR-93 act as oncogenic miRNAs (oncomiRs), while miR-7, -34a, and -128 act as tumor suppressors in glioblastoma, and they target multiple genes [8]. Research has shown that miRNAs can be found in extracellular fluids. They are stable compared to cellular RNA, which is the reason why they can serve as potential biomarkers for various diseases, including cancer [9].

Since mutations in IDH play a role in gliomagenesis, and miRNAs-10b/21 act as oncomiRs and miR-34a acts as a tumor suppressor in glioblastoma, we aimed to investigate whether the IDH mutation status correlates with miRNA-10b/21/34a expression levels in patients with high-grade gliomas (HGGs – glioblastoma). A better understanding of this poorly understood feedback and regulatory mechanism between IDH mutation and miRNAs can yield additional valuable insights into the differing biological behaviors of IDH-mutant versus IDH-wildtype gliomas and possibly have therapeutic implications.

METHODS

This study examined miR-10b, -21, and -34a levels in peripheral blood mononuclear cells (PBMCs) from 43 glioblastoma patients. The 2016 WHO Classification of Tumors of the Central Nervous System was used. Blood samples were taken post-surgery and prior to treatment with concurrent radiotherapy (RT) and chemotherapy with temozolomide (TMZ), and at the 15th and 30th fractions of RT with concurrent TMZ. The study was conducted at the Clinic of Neurosurgery, University Clinical Center of Serbia, and the Institute for Oncology and Radiology of Serbia since October 2017, adhering to the ethical guidelines of the Declaration of Helsinki. The study protocol received approval from the Ethical Research Committee of the Faculty of Medicine, University of Belgrade (approval number 1322/X-39).

After surgery, patients received RT combined with TMZ, followed by adjuvant TMZ. RT began 4–6 weeks post-surgery, with 30 fractions of 2 Gy each, totaling 60 Gy, using either 3D conformal or VMAT technique (Figure 1). Concomitant therapy included 75 mg/m^2 TMZ daily during RT.

The data on IDH1 mutation status were gathered from medical history, histopathology, and immunohistochemistry results.

PBMCs were isolated from heparinized blood using Histopaque-1077 (Sigma-Aldrich, Burlington, MA, USA), and total RNA containing miRNAs was extracted using TRI Reagent(Sigma-Aldrich). RNA quality was assessed using a BioSpec-nano (Shimadzu Corporation, Kyoto, Japan) spectrophotometer, ensuring an A260/280 ratio of 1.7–2.1. Specific TaqMan® (Thermo Fisher Scientific Inc., Waltham, MA, USA) assays were employed to analyze miR-10b, -21, and -34a expression. The comparative delta-delta Ct method was used to calculate relative quantity values, normalizing to RNU6B and calibrating against the sample with the lowest RQ value.

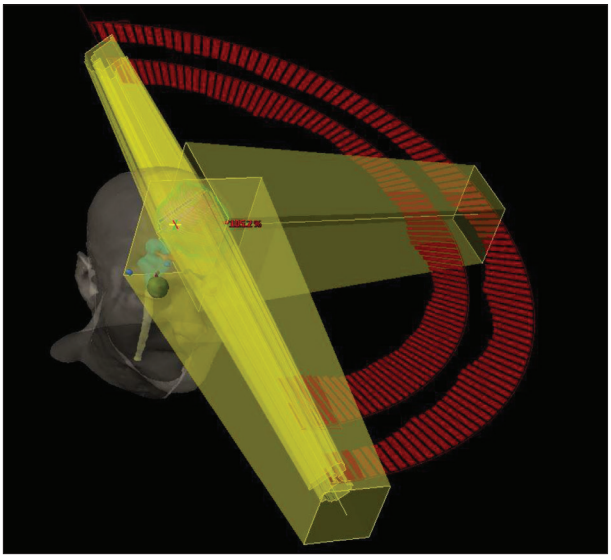

Figure 1. Volumetric modulated arc therapy (VMAT) technique of radiotherapy in a patient with glioblastoma (Institute for Oncology and Radiology of Serbia)

Statistical analysis

The Mann–Whitney U test was used to compare differences between two independent groups. For the analysis of the correlation of the level of expression of miRNA and IDH mutation status, Pearson's and Spearman's tests were used. Log-rank (Mantel–Cox test) was used to examine the significance of the differences. All statistical analyses were conducted using IBM SPSS Statistics, Version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Due to the unfavorable ratio of the number of outcomes to potential predictors, it was not possible to make comparisons among the three groups based on IDH1 status (22 patients had glioblastoma IDH1-wildtype, two patients had glioblastoma IDH1-mutant, and 19 patients had glioblastoma NOS).

According to data from the literature on the prognostic significance of the mutation's presence or absence, two groups were created to assess the association of miR-10b/21/34a expression levels: glioblastoma IDH1-wildtype and glioblastoma IDH1-mutant + NOS.

The median miRNA-10b expression level post-surgery and before the initiation of concomitant RT with TMZ was 130.44 (52.2–622.53) in the IDH1-wildtype glioblastoma group and 94.61 (2.13–816.89) in the IDH1-mutant + glioblastoma NOS group.

The median miRNA-21 expression level was 57.16 (2.68–278.98) in the IDH1-wildtype glioblastoma group and 69.74 (4.6–825.43) in the IDH1-mutant + glioblastoma NOS group.

The median miRNA-34a expression level was 13.52 (3.16–105.2) in the IDH1-wildtype glioblastoma group and 10.11 (1–210.55) in the IDH1-mutant + glioblastoma NOS group.

A complete overview of miRNA-10b/21/34a expression levels in relation to IDH1 mutation status is provided in Table 1.

Table 1. Correlation between miR-10b/21/34a expression levels and IDH1 mutation status

miR	*IDH1*-wild type GB	*IDH1-mutant* + NOS GB	p-value
miR-10b prior RT median (min-max)	130.44 (52.2–622.53)	94.61 (2.13–816.89)	0.234
miR-10b 15f + TMZ median (min-max)	83.35 (16.03–433.53)	100.7 (1–922.88)	0.451
miR-10b 30f + TMZ median (min-max)	131.75 (1.47–493.53)	102.96 (2.32–2751.5)	0.560
miR-21 prior RT median (min-max)	57.16 (2.68–278.98)	69.74 (4.6–825.43)	0.903
miR-21 15f + TMZ median (min-max)	30.53 (2.79–542.32)	70.57 (4.37–960.07)	0.451
miR-21 30f + TMZ median (min-max)	60.56 (1–410.72)	62.03 (3.11–1940.21)	0.981
miR-34a prior RT median (min-max)	13.52 (3.16–105.2)	10.11 (1–210.55)	0.662
miR-34a 15f + TMZ median (min-max)	34.48 (3.48–198.64)	41.93 (4.04–352.38)	0.504
miR-34a 30f +TMZ median (min-max)	51.42 (2.94–363.04)	88.52 (3.71–871.28)	0.644

f – fraction; miR – microRNA; IDH – isocitrate dehydrogenase; GB – glioblastoma; NOS – not otherwise specified; RT – radiotherapy; TMZ – temozolomide

The results showed no statistically significant difference in the expression levels of miR-10b/21/34a between the two groups ($p > 0.05$).

DISCUSSION

Given the established role of IDH mutations in glioblastoma and the overexpression of certain oncomiRs and tumor suppressor miRNAs in glioblastoma cells, we investigated the potential association between miR-10b/21/34a expression levels in PBMCs and IDH1 mutation status.

We hypothesized that expression levels of miR-10b, -21, and -34a would positively or negatively correlate with IDH1-mutation status in glioblastoma. Ji et al. [10] found that expression levels of miR-10b progressively rise with the advancement of WHO grades. Considering that miR-21 is a potent oncogene overexpressed in glioblastoma and that glioblastoma cells depend on miR-10b (with the ablation of the miR-10 gene being lethal for these cells) [11], we expected significantly higher expression of miR-10b/21 in the IDH1-wildtype glioblastoma group than in the IDH1-mutant + NOS group. However, our study did not find a statistically significant association between miR-10b/21 expression levels and IDH1 mutation status. On the other hand, Wang et al. [12] proposed an IDH1 mutation-specific miRNA signature. Precisely, in glioblastoma samples, the expression levels of 23 miRNAs varied by more than 1.5-fold between those with mutant IDH1 and those with wild-type IDH1, respectively [12]. One of the microRNAs with aberrant expression in IDH1 mutation glioblastoma is miR-34a. Similar to miR-10b/21 and IDH1-wildtype, we did not find significantly higher levels in the IDH1-mutant + NOS group compared to the IDH1-wildtype group. To check if there is a change in expression levels of miR-10b/21/34a during RT with TMZ in terms of IDH1 mutation status, we investigated and compared expression levels at the 15th and 30th fractions of RT with TMZ, but we did not find statistically significant results as well.

In low-grade glioma (LGG), IDH1/2 mutation status significantly influences miRNA expression [13]. The researchers developed a four-miRNA-based classifier (including miR-10b, -130b, -1304, and -302b) that effectively differentiated between high and low risk for poor prognosis in IDH1/2-mutant LGG [13]. Additionally, one study revealed a trio of miRNAs (miR-1-3p, miR-26a-1-3p, and miR-487b-3p) that showed differential expression in the serum of glioma patients, dependent on their IDH mutation status [14]. The expression and release of these miRNAs were lower in IDH-wildtype glioma cells compared to IDH-mutant cells [14].

Taking into account the previous data from the literature, we tried to understand the results we obtained and why they did not completely match the results from the literature. Despite some differences in the study's design, to the best of our knowledge, direct studies linking miR-10b/21/34a and IDH1 mutation status are limited. However, in the abstract published in 2014, Silber et al. [15] indicate that IDH mutations in gliomas lead to the repression of miR-34a, which is associated with enhanced platelet-derived growth factor (PDGF) signaling. Their findings suggest that miR-34a plays a crucial role in the cellular changes induced by IDH mutations, impacting tumor progression and potential therapeutic strategies [15].

MiRNAs play a vital role in complex regulatory networks that connect numerous genes and pathways, and their expression can be influenced by a variety of factors, making it difficult to establish a direct correlation with a specific mutation such as IDH1-mutation. Glioblastomas are highly heterogeneous tumors, meaning that different regions of the same tumor can have varying genetic and epigenetic profiles. The presence of an IDH1 mutation may trigger compensatory mechanisms within the tumor cells, which could mitigate the impact of the mutation on miRNA expression. Also, although IDH mutations may be the earliest steps in glioma genesis, it is highly likely that other simultaneous or subsequent molecular events are required for further tumor progression, primarily during the transformation of LGGs into HGGs [16]. The glioma microenvironment, various immune cells, stromal elements, and the cytoskeleton can trigger pathways and alter miRNA expression. It is important to emphasize that in our study, we collected samples for microRNA analysis after surgery and prior to starting RT, which may impact our findings. Additionally, the precision of these results might not match those obtained directly from glioblastoma or cerebrospinal fluid samples. Nevertheless, even with complete resection, in glioblastoma, there can be no real complete removal of all tumor cells due to its infiltrative behavior [17]. The tumor cells are considered to be located or migrated in the surrounding brain parenchyma after surgery [18], as well as glioma stem cells responsible for recurrence [19], which suggests that residual tumor cells can still express a spectrum of miRNAs.

It's also worth mentioning that miR-10b and -21 are not the only significant microRNAs in gliomagenesis. There is a spectrum of microRNAs with potential roles as oncomiRs or tumor suppressors. For example, Sippl et al. [20] suggested that miR-181a2 may serve as a prognostic marker for certain patients with IDH1-wildtype glioblastoma. Given that miR-181a2 regulates IDH1 expression in adipose tissue and considering the impact of IDH1 mutation on glioblastoma's clinical course and biological behavior, the researchers investigated the possible influence of miR-181a2 expression levels on IDH expression, the clinical course, and prognosis of GB patients [20]. More precisely, their findings suggest that low expression of miR-181a2 may positively influence the survival of glioblastoma patients through IDH1 regulation [20]. In LGG, Bondarev et al. [21] implied that certain miRNAs, such as miR-182, -455, and -891a, were generally increased in IDH-mutant gliomas, which are associated with a negative prognosis.

Besides the previously mentioned blood sampling after surgery we conducted, another difficulty that can be a limitation of the study is the number of patients included. Increasing the number of patients and samples for microRNA analysis could potentially show a different result. In our study, patients were classified according to the 2016 World Health Organization Classification of Tumors of the Central Nervous System, which in this particular study should not represent an obstacle. Despite a slight difference in prognosis, glioblastoma and diffuse astrocytoma grade IV have low survival rates, indicating that this design of the study could be applied to HGGs as well.

Results from this study are part of the doctoral dissertation of the first author, and represent a continuous work in the field of translational research in the field of radiobiology and a continuation of previously published work on miRNAs in glioblastoma [22, 23].

CONCLUSION

Our study did not confirm the significant correlation of microRNAs-10b/21/34a with IDH1 mutation status. Based on the results, it can be concluded that the expression levels of microRNAs miR-10b, miR-21, and miR-34a do not significantly differ between glioblastoma patients with IDH1-wildtype and those with IDH1-mutant + NOS. These results suggest that the IDH1 mutation status may not be a critical factor for altered expression of miRNA-10b/21/34a in glioblastoma patients. However, further research is encouraged. Identifying a possible association between specific miRNAs and IDH1 mutation status and other clinical and pathological parameters could refine our understanding of HGGs.

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Conflict of interest: None declared.

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Корелација нивоа експресије микроРНК-10б/21/34а са мутационим статусом *IDH1* код болесника са глиобластомом

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САЖЕТАК

Увод/Циљ Мутације изоцитрат дехидрогеназе (ИДХ) играју значајну улогу у глиомагенези. Поједине микроРНК као што су микроРНК-10б/21 делују као онкогене микроРНК, док микроРНК-34а делује као тумор супресор код глиобластома. Наша студија је имала за циљ да истражи потенцијалну корелацију статуса мутације ИДХ1 са нивоима експресије микроРНК-10б/21/34а код пацијената са глиобластомом.

Методе Ова студија је обухватила 43 пацијента који су имали постављену дијагнозу глиобластома. Испитивани су нивое експресије микроРНК-10б, микроРНК-21 и микроРНК-34а у мононуклеарним ћелијама периферне крви након операције, односно пре почетка лечења радиотерапијом са конкомитантним темозоломидом, као и на 15. и 30. фракцији радиотерапије са конкомитантним темозоломидом. Подаци о статусу мутације ИДХ1 прикупљени су из историје болести и дефинитивног хистопатолошког налаза.

Резултати Направљене су две групе за процену корелације нивоа експресије микроРНК-10б/21/34а у односу на ИДХ1 мутациони статус: глиобластом ИДХ1-дивљи тип и глиобластом ИДХ1-мутант + *Not Otherwise Specified* (*NOS*). Медијана експресије микроРНК-10б пре почетка конкоминатног лечења радиохемиотерапијом била је 130,44 (52,2–622,53) у групи ИДХ1-дивљег типа глиобластома и 94,61 (2,13–816,89) у групи ИДХ1-мутант + *NOS*. Медијана експресије микро РНК-21 била је 57,16 (2,68–278,98) у групи глиобластома ИДХ1 дивљег типа и 69,74 (4,6–825,43) у групи ИДХ1-мутант + *NOS*. Медијана експресије микроРНК-34а била је 13,52 (3,16–105,2) у групи глиобластома ИДХ1 дивљег типа и 10,11 (1–210,55) у групи ИДХ1-мутант + *NOS*. Није доказана статистички значајна разлика у нивоима експресије микроРНК-10б/21/34а између две посматране групе ($p > 0,05$).

Закључак. Наши резултати сугеришу да статус мутације ИДХ1 можда није кључни фактор за измењену експресију микроРНК-10б/21/34а код пацијената са глиобластомом.

Кључне речи: глиобластом; микроРНК; ИДХ мутација

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Male breast cancer – a single center experience

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SUMMARY

Introduction/Objective Male breast cancer is an exceptionally rare disease, accounting for only 0.5% of all male cancer cases, with an incidence of less than one case per 100,000 men annually. This study aims to present the experience of the Institute for Oncology and Radiology of Serbia (IORS) in managing male breast cancer.

Methods This retrospective study included all male patients treated at IORS for breast cancer during the period from 1997 to 2016. In total, 124 cases were included in this study and analyzed regarding demographic, clinical, and pathohistological characteristics, therapeutic approach, and treatment outcomes.

Results Most patients were in stages IIa (27.4%) and IIIb (33.9%). Modified Madden radical mastectomy was performed on 70% of patients. The most prevalent pathohistological tumor type was ductal invasive carcinoma, most frequently in the T2 stage. Most patients (92.1%) had a positive estrogen receptor (ER) and progesterone receptor status (92.1% and 82.4%, respectively), while human epidermal growth factor receptor 2 status was negative in 60% of the patients. The median overall survival was 121 months. Positive ER status was identified as the most important predictor of overall survival, while patients with initial stage IIIa/IIIb/IV disease had a greater risk of disease progression.

Conclusion Our research indicates that patients with ER-positive tumors, who are diagnosed with the disease early and do not have any distant or local metastases have significantly better overall survival rates.

Keywords: breast cancer; male; survival; stage; receptors

INTRODUCTION

Breast cancer (BC) in men is an exceptionally rare disease making up less than one case per 100,000 men annually and just 0.5% of all cancers in the male population [1, 2]. It is believed that men have a lower BC incidence than women because of their distinct hormonal status and volume of breast tissue [2, 3, 4]. Nonetheless, BC incidence is rising in both genders with an estimated 26% increase in men over the last 25 years [1, 2, 4].

Men are affected by most histological types of BC that afflict women; however, their incidence of occurrence varies. Roughly 90% of all BC in men are ductal, only 1% are lobular, and the remaining 9% accounts for rare BC subtypes like neuroendocrine, medullary, or tubular BCs [5]. When compared to female BCs, male BCs are more likely to express the estrogen receptors (ER), progesterone receptors (PR), and androgen receptors (AR), be hormonally responsive, have lower expression of human epidermal growth factor receptor 2 (HER2) receptor, and most often manifest as unilateral tumors [5].

This study aims to present the experience of the Institute for Oncology and Radiology of Serbia (IORS) in managing male BC, from 1997 to 2016, regarding demographic, clinical, and pathohistological characteristics, therapeutic approach, and treatment outcomes.

METHODS

This retrospective study included all male patients treated at IORS for BC from 1997 to 2016. For most patients, data were collected from archived and active medical histories; only after 2014 was part of the data accessed via the hospital's electronic medical records. The IORS review board approved the study, and informed consent for participation was obtained from all living patients with active medical histories. We analyzed demographic data, disease characteristics (stage of the disease, pathohistological and immunohistochemical tumor parameters), and treatment protocols. Some of the data could not have been retrieved due to inconsistent reporting in the archived medical records, especially when the initial part of the treatment had been done outside our cancer center. However, these patients have not been excluded from the series given that the disease is rare, and omitting could have potential implication on other insights gathered from the available data on these patients.

Numerical data are displayed by arithmetic mean and median, with standard deviation and percentiles. Attributive data are presented in absolute and relative frequencies. The Kolmogorov–Smirnov and Shapiro–Wilk tests were used to check the data normality. T-test, Mann–Whitney U, and χ^2 test were used to assess the significance of the difference. Cox proportional regression model was used for

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survival analysis. Survival curves were defined using the Kaplan–Meier method. In all analyses, $p < 0.05$ was considered statistically significant. Statistical analysis was performed using the statistical program IBM SPSS Statistics for Windows, v. 21.0 (IBM Corp., Armonk, NY, USA).

Ethical approval: The patients' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by competent ethics committee and conforms to the legal standards.

RESULTS

Between 1997 and 2016, IORS treated 124 male patients with BC, with an average age of 64.29 ± 11.18 years. All patients had a IORS multidisciplinary team – tumor board consisting of a medical oncologist, surgeon, and radiotherapist. Patients initially treated outside our cancer center, in general hospitals, did not undergo the same procedure and there were no multidisciplinary team decisions, complete evaluation and staging, and some of the data reporting was not uniform and standard.

The initial clinical disease stage was unknown in 20/124 (16%) as they had breast lump surgically removed in other institutions, without proper staging and data reporting. In the available data, most patients were in stages IIa (27.4%) and IIIb (33.9%), with 3.2% of patients in stage IV and just 8.9% in clinical stage I (Table 1). Surgical treatment was performed in 120/124 (97%) of patients; however, data regarding the type of surgery, based on standard surgical nomenclature, were unavailable for 15% (18/120) of patients. Modified Madden radical mastectomy was performed on 70% of patients (Table 2). Pathohistological data were unavailable for 18/124 (14.5%) of patients. The most prevalent pathohistological tumor type was ductal invasive carcinoma, present in 70% of patients (Table 3). Tumor grade II was the most frequently encountered in 88/106 (83%) patients, while grade I and II were evenly distributed in the population (8.1% and 8.2% of patients, respectively). The T2 tumor stage was most frequently encountered in surgically treated patients, followed by the T1 and T4 tumor stages (Table 4). There was an even distribution of patients with negative findings on ipsilateral axillary lymph nodes (N0, 50.6%) and those with metastases (N+, 49.4%). Data regarding tumor receptor expression was available only for 44/124 (35.5%) of patients. Most patients (92.1%) had a positive ER (92.1%) and PR (82.4%) status, while HER2 status was negative (0 or 1+) in 60% of the patients. None of the patients have been treated with neoadjuvant chemotherapy, probably since tumors have been previously surgically removed for the pathological verification, in the absence of non-surgical biopsies. Adjuvant chemotherapy was administered in 46/124 (37%) of patients and antiestrogen therapy in almost two-thirds of patients. Nearly half of the study population (61/124, 48.8%) received local radiotherapy. For the metastatic stage (3.2%) the systemic treatment has been administered based on the available protocols.

Table 1. Initial clinical disease stage in the study population

Initial clinical disease stage	N (%)*
I	11 (8.9)
IIa	34 (27.4)
IIb	8 (6.5)
IIIa	5 (4)
IIIb	42 (33.9)
IV	4 (3.2)
Unknown	20 (16.1)
Total	124 (100)

Table 2. Types of surgery performed in patients eligible for surgical treatment

Type of surgery	N (%)
Modified radical mastectomy – Madden	84 (70)
Simple mastectomy	5 (4.2)
Sparing surgery	13 (10.8)
Unknown	18 (15)
Total	120 (100)

Table 3. Pathohistological tumor types in our study population

Pathohistological tumor type	N (%)
Ductal invasive carcinoma	87 (70.2)
Lobular invasive carcinoma	8 (6.5)
Tubular carcinoma	1 (0.8)
Medullary carcinoma	1 (0.8)
Mixed ductal carcinoma + lobular carcinoma	4 (3.2)
Ductal carcinoma in situ	4 (3.2)
Multiple carcinomas	1 (0.8)
Unknown	18 (14.5)
Total	124 (100)

Table 4. Frequency of tumor sizes in our study population

Tumor size (pT)	N (%)
T1	34 (27.4)
T2	39 (31.5)
T3	3 (2.4)
T4	21 (16.9)
Unknown	27 (21.7)
Total	124 (100)

Follow-up and treatment outcomes

During the follow-up, one-third of the patients (43/124, 35%) had metastases. The most common were bone metastases (20/43, 47%), followed by visceral (13/43, 30%) and soft-tissue metastases (10/43, 23%). One-third of patients relapsed (40/124, 32.5%). The median overall survival was 121 months (95% CI: 58.1–183.9) (Figure 1). Median disease-free survival was not reached (Figure 2). Median survival until disease progression was 84 months (95% CI: 58.8–109.1) (Figure 3).

The median survival for patients with initial clinical disease stages I, IIa, and IIIb was not reached, while patients with initial disease stages IIIa, IIIb, and IV had a median survival of 39 months (Figure 4). Patients with T3/T4 tumors had significantly shorter overall survival than those with T1/T2 stages (73 *vs.* 121 months) (Figure 5). Patients with N+ status had a median survival of 84 months, while the median survival was not reached in patients with the N0 status (Figure 6).

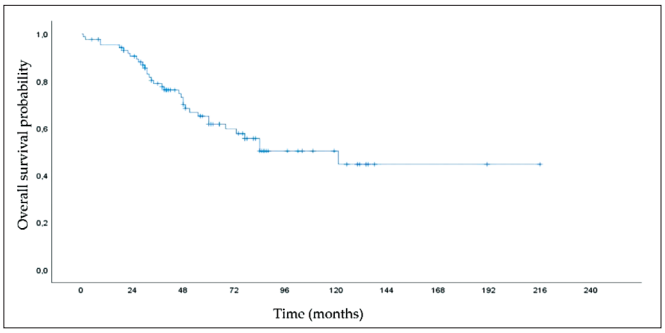

Figure 1. Overall patient survival in the study population

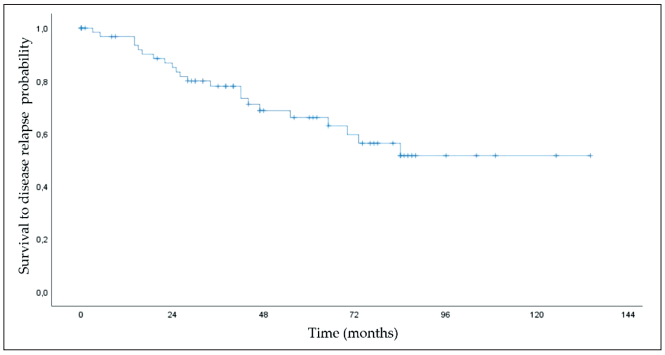

Figure 2. Survival to disease relapse in the study population

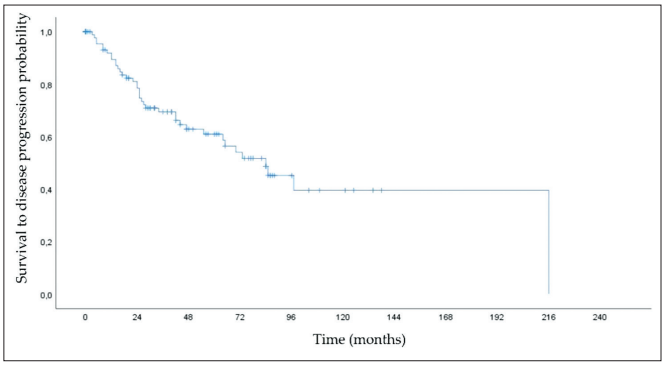

Figure 3. Survival to disease progression in the study population

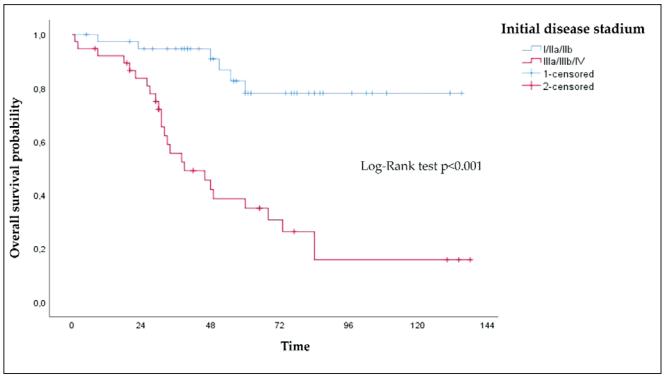

Figure 4. Kaplan–Meier curve of overall survival concerning disease stage

The following potential predictors for overall survival and survival until the disease progression were analyzed: age, initial disease stage (I/IIa/IIb vs. IIIa/IIIb/IV), T stage, N status, ER, PR, and HER2 status, adjuvant therapy (hormonal or systemic). The univariate and multivariate Cox regression analyses for overall survival and survival until the disease progression are shown in Tables 5 and 6,

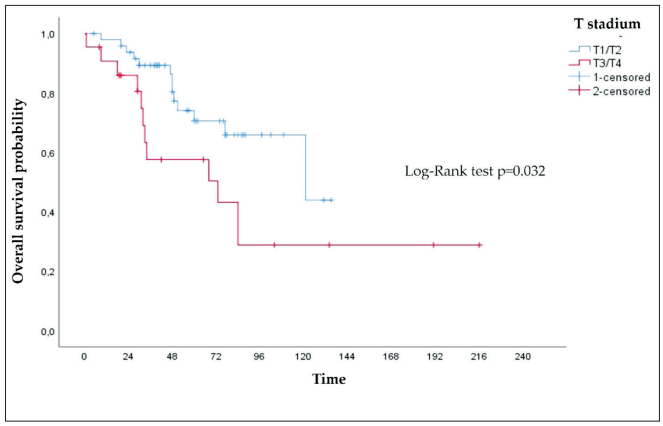

Figure 5. Kaplan–Meier curve of overall survival in relation to tumor size

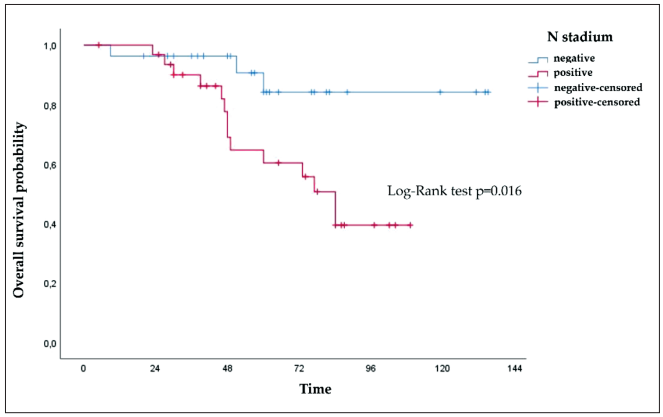

Figure 6. Kaplan–Meier curve of overall survival concerning axillary lymph node status

respectively. Positive ER status was identified as the most important and favorable predictor of overall survival. At the same time, the worse initial disease stadium (IIIa/IIIb/IV) was the most important predictor of disease progression.

Table 5. Univariate and multivariate Cox regression analysis of overall survival in male breast cancer patients

Univariate regression analysis	HR	95% CI	p
Initial disease stadium	6.367	2.603–15.573	< 0.001
Pathohistological T stadium	2.316	1.050–5.107	0.041
Pathohistological N stadium	1.012	1.001–1.022	0.027
Distant metastasis	3.447	1.646–7.217	0.001
Positive estrogen receptor status	0.097	0.025–0.375	0.001
Adjuvant therapy	0.417	0.210–0.828	0.012
Adjuvant hormonal therapy	0.413	0.208–0.820	0.012
Systemic therapy	3.899	1.671–0.099	0.002
Multivariate regression analysis			
Positive estrogen receptor status	0.058	0.005–0.650	0.021

Table 6. Univariate and multivariate Cox regression analysis of survival to disease progression in male breast cancer patients

Univariate regression analysis	HR	95% CI	p
Initial disease stadium	3.637	1.717–7.703	0.001
Positive estrogen receptor status	0.251	0.070–0.903	0.034
Adjuvant therapy	0.478	0.244–0.935	0.031
Multivariate regression analysis			
Initial disease stadium	3.620	1.008–13.003	0.049

DISCUSSION

In this study, we have shown the management experience of male BC at the Serbian referral facility for BC treatment (IORS). As male BC is an exceedingly uncommon disease, retrospective studies like this still provide most of our knowledge. In comparison to women, men are diagnosed with BC 100 times less frequently, with a peak incidence occurring at age 67, which is later than for women [1, 6]. The subjects' average age in our study was 64.29 ± 11.18 years, which is comparable to other studies [1, 3, 6, 7, 8]. Worldwide, the prevalence of BC in both men and women are rising [1, 5]. BC in females is detected more frequently in the asymptomatic phase, because of advanced screening programs [9]. However, as males are not screened for BC in any country in the world, the incidence of the disease in the male population remains obscure and it typically presents in more advanced stages [5]. This is supported by our study, which found that 75% of patients were diagnosed in more advanced clinical stages, and almost 50% of surgically treated patients had metastatic disease present in axillary lymph nodes. Similarly, in a recent study that included a larger population, 46.7% of male patients with BC had metastatic disease in axillary lymph nodes at the time of the diagnosis [6]. Contrary to other studies, however, most of our patients had axillary dissection rather than sentinel lymph node biopsy, a highly accurate technique that lowers surgical complications [10]. This is because sentinel lymph node biopsy is still not performed in most Serbian BC treatment facilities due to technical reasons.

Only 8.9% of the patients in our study had a stage I diagnosis while most patients were in stages II (33.9%) and III (37.3%). A comparable retrospective study conducted in Czechia between 2007 and 2017 found that more patients (37%) had been diagnosed with stage II, and fewer patients (26%) with stage III [7]. Some other studies showed a significantly higher proportion of patients in stage I of the disease (around 37%), which contradicts our findings [6, 11]. This can be explained by a generally lower level of health awareness in our population, the challenges associated with accessing healthcare, and the unavailability of modern diagnostic techniques before 2014. For example, preoperative core needle biopsy of suspicious breast lesions became available in IORS only after 2014.

Unlike women with BC, men with BC typically do not undergo sparing operations due to the smaller volume of breast tissue [12]. Although most patients in our study (70%) underwent a modified radical Madden mastectomy, a considerable portion of the patients (10.8%) underwent a sparing procedure. These patients had multiple comorbidities and were not suitable candidates for a more invasive surgical procedure.

Research indicates that male BCs are more likely to be of the ductal subtype and to express hormone receptors more frequently than HER2 receptors when compared to female BCs [5, 6, 13]. The results of this study are consistent with data from the literature showing that ductal invasive carcinoma was the most prevalent tumor type in our population (70.2% of cases). While only a small portion of the study population had data on BC receptor expression analysis available, over 80% of the analyzed BCs were hormone-dependent tumors, and 60% were HER2-negative. Similar results were obtained by Bielcikova et al. [7] as nearly 90% of their population had hormone-dependent and HER2-negative BCs.

Consistent with data from the literature, adjuvant chemotherapy was administered to almost one-third of our patients, two-thirds received adjuvant antiestrogen therapy, and roughly half underwent postoperative radiation [8, 13, 14].

Men with BC generally have a worse prognosis than women with BC [13, 15]. Many studies indicate that BC may be biologically different between sexes even though shorter survival in men may be, to some extent, explained by older age and later stage at diagnosis [1, 3, 13, 15]. In one of the largest studies that compared overall survival in male and female BC, Wang et al. [15] concluded that male patients had significantly higher mortality across all stages [15]. Namely, in men *vs.* women, the overall survival rate was 45.8% *vs*. 60.4%, while three-year and five-year survival rates were 86.4% *vs*. 91.7% and 77.6% *vs*.86.4%, respectively. Another recent study showed that males with BC had worse overall survival compared to females with BC when in stages III and IV, while overall survival was similar in early BC stages [16]. In our study, the patients' median overall survival was 121 months (95% CI: 58.1–183.9), and in one third (32.5%) disease relapsed. These data are comparable with the conclusions of other studies done in Europe [6, 7].

Patients with initial disease stage IIIa, IIIb, or IV had a median survival of 39 months, and this is the most important predictor for disease progression in our study. Patients with T3/T4 tumors had significantly shorter overall survival than those with T1/T2 stage (73 months *vs.* 121 months). These results are in accordance with the results of other studies [3, 7, 15, 16, 17].

In our analysis, positive ER status was the most significant favorable predictor of overall survival. The patients with positive ER status had a 94% lower chance of dying (HR: 0.058; 95% CI: 0.005–0.650, p = 0.021). However, in comparison to women, men with ER-positive BC were found to have higher mortality independently of tumor stage [15, 18]. Given that most male BCs express ER-beta whereas most female BCs express ER-alfa, one explanation could be that male BC has a different ER subtype than female BC [13]. ER status is an important predictor of overall survival in males with BC across the other studies [6, 15, 16, 18, 19], but surprisingly not in the research performed by Bielcikova et al. [7] where PR status was an independent predictor of overall survival in male BC patients. In our study, PR and HER2 status were insignificant predictors of the overall survival of our patients, which is comparable with the study of Yao et al. [18]. Nevertheless, only a small portion of the BCs in our research had an evaluation of hormone receptor expression. Modern diagnostic procedures should be more widely used in all Serbian centers treating male BC patients, given the effect of this information on overall survival and the decision

about the patient's subsequent care. Although the current guidelines recommend using similar algorithms for therapeutic decision-making in male as in female BC patients, there is widespread concern that only a fraction of male BC patients are currently treated with adjuvant hormonal and radiation therapy [6, 8, 10].

The retrospective nature of the research and the missing data are the limitations of our study. Hopefully implementing the computer system in all medical centers will improve medical research in our country by providing more detailed data.

CONCLUSION

Male BC is a rare disease, but its incidence is rising. In comparison to women, men are typically diagnosed later in life and with more advanced disease. As IORS is the referral center for BC treatment in Serbia, and the fact that there are no systematic registers of this disease in our country, this study mirrors important epidemiological and clinical facts regarding this rare disease in the Serbian population. Our research indicates that patients with ER-positive tumors, who are diagnosed with the disease early and do not have any distant or local metastases have significantly better overall survival rates. Although part of our population did not have access to advanced diagnostic techniques, as they were not available in Serbia until recently, overall, the results we obtained are in line with those of other European centers. It is imperative for all medical centers in Serbia that encounter males with BC to adhere to current oncological guidelines and adopt a customized, multidisciplinary management approach.

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Карцином дојке код мушкараца – искуство једног центра

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САЖЕТАК

Увод/Циљ Карциномом дојке код мушкараца је изузетно ретка болест, која има инциденцу од мање од једне особе на 100.000 људи и представља само 0,5% свих карцинома који се јављају код мушкараца. Ова студија има за циљ да представи искуство Института за онкологију и радиологију Србије у дијагностици и лечењу мушкараца са карциномом дојке.

Методе У ову ретроспективну студију су укључени сви мушкарци који су лечени у Институту за онкологију и радиологију Србије због карцинома дојке у периоду од 1997. године до 2016. године. Укупно 124 болесника анализирана су према демографским, клиничким и патохистолошким карактеристикама, терапеутском приступу и исходу лечења.

Резултати Већина болесника је иницијално била у стадијуму *IIa* (27,4%) и *IIIb* (33,9%). Код 70% болесника спроведена је модификована радикална мастектомија по Мадену. Дуктални инвазивни карцином, најчешће у стадијуму *T2*, био је најфреквентнији патохистолошки тип тумора. Већина болесника је имала позитиван статус естрогенских (92,1%) и прогестеронских (82,4%) рецептора, док је 60% болесника имало негативан статус рецептора за хумани епидермални фактор раста. Медијана укупног преживљавања је била 121 месец. Позитивни статус за естрогенски рецептор је идентификован као најважнији предиктор укупног преживљавања, док су болесници у иницијалном стадијуму болести *IIIa/IIIb/IV* имали већи ризик за прогресију болести.

Закључак Резултати нашег истраживања потврђују да мушкарци са карциномом дојке који испољава естрогенске рецепторе, чија је дијагноза постављена у раном стадијуму болести и који немају регионалне или удаљене метастазе, имају значајно боље преживљавање.

Кључне речи: карцином дојке; мушкарци; преживљавање; стадијум; рецептор

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Clinical application of traditional Chinese medicine eye-coating agents in the treatment of hordeola

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SUMMARY

Introduction/Objective This study aimed to investigate the therapeutic efficacy of combining traditional Chinese medicine (TCM) eye-coating agents with levofloxacin in treating patients with a hordeolum.

Methods Using convenience sampling, 110 patients with a hordeolum treated at Tianjin Eye Hospital between March and June 2018 were included in this study. Patients were randomly divided into three groups: a coating agents' group (coating agents + levofloxacin, n = 37), a wash group (eyelid wash + levofloxacin, n = 38) and a control group (levofloxacin alone, n = 35). Data on nodule size and visual analogue scale (VAS) scores for pain were collected before treatment and at three, five and seven days after treatment. Treatment efficacy was assessed after seven days, and comparisons were made between the three groups regarding nodule size, VAS scores and therapeutic outcomes.

Results Repeated-measures analysis of variance showed significant time effects, group effects and time-group interactions for both nodule size and VAS scores ($p < 0.05$). Over time, all groups exhibited a significant reduction in nodule size and VAS scores compared with the baseline. Pairwise comparisons revealed that on days three, five and seven, the order for nodule size and VAS scores was consistent across the groups: coating agents' group = wash group < control group. Treatment efficacy comparisons were statistically significant (94.59% *vs*. 86.84% *vs*. 71.43%, $\chi^2 = 7.622$, $p < 0.05$), with the order from highest to lowest efficacy being as follows: coating agents' group = wash group > control group.

Conclusion The combination of TCM eye coating agents with levofloxacin shows promise in treating a hordeolum, effectively reducing eyelid swelling and pain, shortening healing time and improving treatment success rates.

Keywords: Traditional Chinese medicine eye coating agents; hordeolum; levofloxacin; clinical outcomes

INTRODUCTION

A hordeolum refers to the acute purulent inflammation of the eyelid glands, often caused by *Staphylococcus aureus* infection [1]. This condition is a frequently occurring disease commonly diagnosed in ophthalmology outpatient clinics. A study identifies the presence of *Staphylococcus aureus* in the nasal cavity as a primary etiological factor in the onset of a hordeolum [2]. Clinically, it manifests as localized redness, swelling, heat and pain, with a palpable nodule at the affected area, known as a stye. If the hordeolum is near the external canthus, the pain is often more severe and may lead to reactive conjunctival oedema. It is imperative to avoid self-draining any formed abscess to prevent bacterial backflow into the cranial cavity, which could lead to severe complications, such as cavernous sinus thrombosis, which may be life-threatening.

In clinical practice, the treatment of a hordeolum is based on Western medicine, which typically involves the use of systemic and topical antibiotics to control inflammation, combined with heat applications to facilitate the resolution of the inflammatory process. When an abscess forms, incision and drainage should be performed [3]. Western medicine aims to alleviate symptoms, promote the discharge of pus and prevent spreading. However, the excessive use of Western eye drops can lead to drug resistance in the eyes, thereby reducing their efficacy in treating recurrent conditions, such as *herpes zoster ophthalmicus*. Moreover, surgery for hordeolum can cause psychological discomfort in patients and even the formation of eyelid scars [4]. The treatment duration usually lasts 5–10 days, severely disrupting patients' daily activities and occupational commitments. Frequent non-compliance due to work or academic obligations often results in an extended course of the disease and increases the likelihood of requiring surgical drainage [4].

In recent years, traditional Chinese medicine (TCM) has shown promise in treating hordeola and has been receiving increasing attention. TCM treats styes based on etiology and pathogenesis and is safer than Western medicine. Western medicine has large side effects in treating styes, and TCM can make up for the shortcomings of Western medicine with its

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mild efficacy and good conditioning. Many clinical studies have shown that TCM is effective in treating styes; it is safe and quick in effect. TCM has mild properties and little gastrointestinal irritation [5, 6, 7]. In TCM, a hordeolum is known by various names such as "needle eye," "subcutaneous canker," "soil ulcer" or "stolen needle." A hordeolum is described as a condition where the eye suddenly develops a vesicular rash and produces pus within three to five days [1]. The treatment philosophy in TCM has traditionally focused on clearing heat, detoxification and alleviating circulatory stasis [7, 8, 9]. Various methods derived from this philosophy, including herbal fumigation, ultrasonic herbal eye baths, direct current iontophoresis and herbal poultices, have demonstrated efficacy [7, 9, 10, 11]. TCM also places a significant emphasis on eyelid hygiene and anti-inflammatory treatment. The TCM eyelid wash has gained widespread usage due to its cost-effectiveness. It functions with the dual capabilities of cleansing and anti-inflammatory effects and exhibits high patient acceptability [10]. A specialized TCM eyelid wash formula has been developed in our institution, comprising key ingredients such as *Rheum rhabarbarum*, *Scutellaria baicalensis* root, *Coptis trifolia*, and *Phellodendron* bark. This formula has demonstrated efficacy in heat dissipation, swelling reduction, detoxification, and blood circulation promotion without any observed adverse reactions [12]. When applied as a wet compress, this eyelid wash can directly treat the affected area by targeting locally produced inflammatory mediators to reduce inflammation and promote blood circulation. This, in turn, alters the local tissue's oxygen supply environment and reduces local inflammatory responses [13].

However, the herbal decoctions in TCM are often cumbersome to prepare and store, leading to poor patient adherence. Therefore, to improve patient compliance without compromising therapeutic efficacy, there is a need for new, more convenient forms of application. Chinese herbal coating agents offer a promising alternative because they maintain effective drug concentrations, are easy to apply and store and can prolong the drug's residence time in the affected area [14]. The objective of this study is to evaluate the clinical effectiveness of a novel TCM topical agent in combination with levofloxacin in comparison with the established treatment protocol that involves the use of TCM eyelid wash and levofloxacin for the management of hordeola.

METHODS

Research participants

Using convenience sampling, data were collected from 110 cases of patients with a hordeolum admitted to Tianjin Eye Hospital between March 2018 and June 2018. If both eyes were affected, the more severe eye was studied. The patients were randomly divided into a coating agents' group (coating agents + levofloxacin) consisting of 37 cases, a wash group (eyelid wash + levofloxacin) with 38 cases and a control group (levofloxacin only) with 35 cases.

Inclusion criteria: (1) patients with a disease course within one week, no self-administered antibiotics, no use of steroid eye drops or ointment, no oral anti-inflammatory medication and no systemic symptoms, such as fever; (2) patients with good compliance, able to return for follow-up within seven days after the initiation of treatment.

Exclusion criteria: (1) patients with a disease course exceeding one week who had used relevant medications; (2) patients with localized pus or hordeolum ulceration; (3) patients with other severe eye diseases, such as glaucoma or retinal diseases; (4) patients allergic to the drug components, pregnant or breastfeeding women and children; (5) patients unable to comply with the treatment schedule or return to the clinic on time.

This study was approved by the hospital ethics committee (ethics number TJYYLL-2017-09). All participants or their legal guardians provided informed consent and signed written consent forms. In the case of underage participants, their guardians were ensured to fully understand the content of the study and signed informed consent forms.

Diagnostic criteria

The diagnostic criteria for a hordeolum are based on the guidelines edited by Loth et al. [15]. The condition is defined by localized redness, swelling, warmth, eyelid skin pain, induration, and significant tenderness upon palpation. In all patients, regularity of the eyelid margin, congestion, blockage of meibomian glands and the shifting area where the mucous membrane of the eyelid margin meets the eye skin are observed under slit-lamp microscopy.

Treatment protocols

Coating agents' group: 0.5% levofloxacin eye drops are applied to the affected eyelids four times daily, with 1–2 drops per application. Concurrently, an herbal coating agent made from the extracted components of the eyelid wash is used, which is evenly spread over the eyelids with a cotton swab twice daily for 15 minutes per application for a continuous six-day treatment period, as one course of therapy.

Wash group: 0.5% levofloxacin eye drops are applied to the affected eyelids four times daily, with 1–2 drops per application. Concurrently, a TCM eyelid wash solution is used, applied twice daily for 15 minutes each time over a continuous six-day treatment period to complete one course of therapy.

Control group: 0.5% levofloxacin eye drops are administered to the affected eyelids four times daily with 1–2 drops per application for a continuous six-day treatment period, constituting one course of therapy.

The eyelid wash consists of 10 g each of *Rheum rhabarbarum*, *Scutellaria*, *Coptis* and *Phellodendron*. The preparation process involves taking 10 doses of herbal pieces, totaling 400 g, and adding 4,800 ml of water. The mixture is soaked for 20 minutes and then decocted using an automated herbal decoction packaging machine for 90 minutes,

yielding a total of 3600 ml of herbal liquid. Each dose is 360 ml, and each sachet contains 180 ml. The liquid is stored at temperatures of 0–4°C and is applied to the eyes twice daily, with an approximate volume of 90 ml each time.

The herbal coating agent for the eye is prepared by first making an herbal extract concentrate from the eyelid wash using a rotary evaporator. According to the Pharmacopoeia of the People's Republic of China, 1 g of the extract corresponds to 2–5 g of raw material. In this experiment, the prepared extract corresponds to 3.48 g of raw material, which complies with the Pharmacopoeia standards. An appropriate amount of polyvinyl alcohol (PVA) is placed in beaker 1 and dissolved in distilled water at 85°C. An appropriate amount of extract is placed in beaker 2, added to distilled water and stirred until completely dissolved. The fully dissolved medicinal liquid from beaker 2 is poured into beaker 1 and mixed with PVA. Then, 95% ethanol is added dropwise while stirring, followed by the slow addition of acetone while continuing to stir. Finally, a small amount of nitrocellulose and menthol are added, and the mixture is allowed to cool naturally. The final product is stored at temperatures of 0°C–4°C.

The levofloxacin eye drops were purchased from Youfeng Pharmaceuticals (Yufeng International Group Co., Ltd., Wuxi, Jiangsu China) Ltd., Batch Number: J20100046.

Data collection

Before treatment, basic information, and clinical characteristics of the patients, such as gender, age, and lump location, were collected. The lump size and pain visual analogue scale (VAS) scores of the patients were collected before treatment and at third, fifth and seventh days after treatment. The treatment efficacy was evaluated after seven days of treatment.

Lump size measurement (mm): A calipers was used to measure the maximum diameter of the lump.

VAS score: This was utilized to assess the pain conditions before and after treatments for both groups. The score directly correlates with the intensity of the pain. A score of 0 indicates no pain, and the maximum score is ten. A score of ≤ 3 indicates mild pain, 4–6 indicates moderate pain and ≥ 7 indicates severe pain [16].

The efficacy evaluation was performed based on the criteria for blepharitis outlined in "Practical Ophthalmology" [17], as follows: (1) cured – redness, swelling, heat and pain symptoms disappear, conjunctiva shows no signs of edema or congestion and there is no tenderness upon palpation. Recovery is complete; (2) improved – reduced redness and swelling and alleviation of heat and pain symptoms. Mild discomfort still present; (3) ineffective – no alleviation of symptoms or worsening conditions, resulting in suppuration, rupture or the need for incision and drainage. In severe cases, cellulitis of the eyelid may occur, presenting systemic symptoms such as fever, chills, and headaches.

The overall effectiveness rate was calculated as Total Effective Cases = (Cured + Improved) Cases / Total Cases × 100%

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Data with normal distribution were expressed as x ± s. One-way analysis of variance (ANOVA) was used for comparing group means. Repeated measures ANOVA were used for repeated measures data. The least significant difference method was employed for pairwise comparisons between groups. Count data were represented by frequency or rate and analyzed using the χ^2 test. Pairwise comparisons for count data were performed using the χ^2 partitioning method. A value $p < 0.05$ was considered statistically significant.

Ethics: This study was conducted in accordance with the declaration of Helsinki, and with approval from the Ethics Committee of Tianjin Eye Hospital (NO.: TJYYLL-2017-09).

RESULTS

General patient information

A total of 110 patients were included in this study. The coating agents group consisted of 37 patients (16 men and 21 women) with an average age of 28.31 ± 10.32 years. The wash group had 38 patients (17 men and 21 women) with an average age of 29.76 ± 8.43 years. The control group had 35 patients (15 men and 20 women) with an average age of 28.44 ± 9.39 years. There were no statistically significant differences among the three groups in terms of gender ratio, age, lump size, VAS scores or lump location before the treatment ($p > 0.05$). The comparability among the groups was satisfactory (Table 1).

Table 1. Comparison of general patient characteristics

Variable		Coating agents' group (n = 37)	Wash group (n = 38)	Control group (n = 35)	χ^2/F value	p-value
Gender (male/female)		16/21	17/21	15/20	0.030	0.985
Age (years, x ± s)		28.31 ± 10.32	29.76 ± 8.43	28.44 ± 9.39	2.312	0.212
Lump Size (mm, x ± s)		9.13 ± 5.08	7.88 ± 4.47	8.58 ± 5.17	1.897	0.321
VAS Score (points, x ± s)		6.82 ± 2.01	7.02 ± 2.43	6.92 ± 1.83	2.110	0.254
Lump location	Right eye	20	20	15	1.063	0.588
	Left eye	17	18	20		

VAS – visual analog scale for pain

Table 2. Comparison of mass size and VAS score within seven days post-treatment

Variable	Time	Coating agents' group (n = 37)	Wash group (n = 38)	Control group (n = 35)	$F_{interaction}$ / $P_{interaction}$	F_{time} / P_{time}	$F_{treatment}$ / $P_{treatment}$
Lump size (mm, x ± s)	Baseline	9.13 ± 5.08	7.88 ± 4.47	8.58 ± 5.17	343.142 / 0.001	129.532 / 0.001	352.532 / 0.001
	Day 3[a]	5.23 ± 3.11	5.11 ± 2.89	6.10 ± 3.56			
	Day 5[a]	2.31 ± 1.28	2.28 ± 1.16	3.56 ± 2.19			
	Day 7[a]	1.02 ± 0.34	1.13 ± 0.28	2.34 ± 1.03			
VAS score (points, x ± s)	Baseline	6.82 ± 2.01	7.02 ± 2.43	6.92 ± 1.83	232.322 / 0.001	241.253 / 0.001	263.132 / 0.001
	Day 3[a]	3.51 ± 1.96	3.61 ± 2.13	4.78 ± 2.11			
	Day 5[a]	1.31 ± 0.93	1.45 ± 1.11	3.14 ± 1.22			
	Day 7[a]	0.43 ± 0.11	0.52 ± 0.12	2.11 ± 1.41			

VAS – visual analog scale for pain;
[a]statistically significant inter-group differences

Comparison of lump size and VAS scores within seven days post-treatment

A one-way repeated-measures ANOVA was employed to explore the effects of different treatment methods on lump size and VAS scores within seven days following treatment. According to the Shapiro–Wilk test, the data for each group followed an normal distribution ($p > 0.05$). Mauchly's sphericity test showed that the covariance matrices of each group were equal ($p > 0.05$). Data are represented as x ± s (Table 2).

In terms of lump size, the interaction between time and treatment was significant across the three groups ($F_{interaction} = 343.142$, $p < 0.001$). This finding suggests that the individual effects of different treatments on lump size varied at four different time points. Furthermore, the lump size in all three groups decreased over time ($F_{time} = 129.532$, $p < 0.001$). Different treatments had various effects on lump size ($F_{treatment} = 352.532$, $p < 0.001$). Pairwise comparisons on days three, five, and seven showed that the sequence of lump sizes remained consistent across the three groups: coating agents' group = wash group < control group.

In terms of VAS scores, there was significant interaction between time and treatment in the three groups ($F_{interaction} = 232.322$, $p < 0.001$). This finding shows that the impact of different treatment methods on VAS scores is different at different time points. As treatment progressed, the VAS scores of all groups decreased significantly ($F_{time} = 241.253$, $p < 0.001$), reflecting the positive effect of treatment on pain relief. In addition, the effects of different treatment measures on VAS scores also showed significant differences ($F_{treatment} = 263.132$, $p < 0.001$). Pairwise comparisons conducted on days three, five, and seven after treatment showed that the order of VAS scores among the three groups was consistent: coating agents' group = wash group < control group.

Comparison of efficacy rates among the three groups

The results indicated that in the coating agents' group, six patients were cured, 29 were effective and two were ineffective; in the wash group, two were cured, 31 were effective and five were ineffective; and in the control group, one was cured, 24 were effective and 10 were ineffective. A statistically significant difference was observed in the efficacy rates among the three groups (94.59% *vs.* 86.84% *vs.* 71.43%, $\chi^2 = 7.622$, $p < 0.05$). Further pairwise comparisons revealed that the efficacy rates among the three groups were ranked as follows: coating agents' group = wash group > control group (Table 3).

Table 3. Comparison of efficacy rates among the three groups

Group	Cured	Effective	Ineffective	Efficacy Rate
Coating agents' group (n = 37)	6	29	2	94.59%
Wash group (n = 38)	2	31	5	86.84%
Control group (n = 35)	1	24	10	71.43%
χ^2 value	7.622			
p-value	0.022			

DISCUSSION

In this study, both the coating agent and wash groups demonstrated superior effectiveness in treating the hordeolum size and alleviating pain compared with the control group. Moreover, patients in the coating agent and wash groups also exhibited quicker recovery rates than those in the control group. The overall effectiveness was also higher in the coating agent and wash groups compared with the control group. These findings indicate that the integration of TCM with levofloxacin-based treatment can significantly enhance therapeutic outcomes for hordeola. These results are consistent with previous studies, as many have shown that the combination of TCM and Western medicine in treating hordeola is more effective than using antibiotics or TCM alone [18, 19, 20]. For instance, one study [18] applied a topical formula combined with heat steaming and ofloxacin ointment for early stage hordeola and found that it reduced the rupture rate, showing better efficacy than antibiotics alone. In another study, Ji et al. [19] used a combination of levofloxacin eye drops and Wu Wei Xiao Du Yin (a TCM formula) to treat hordeola, which not only showed significant therapeutic effects but also reduced the recurrence rate. These findings further confirm our study's conclusion that the integration of TCM and Western medicine can significantly enhance the treatment efficacy of hordeola.

The prescription used in this study consisted of *Rheum rhabarbarum*, *Scutellaria baicalensis* (Huang Qin), *Coptis* (Huang Lian) and *Phellodendron* (Huang Bai), which are collectively known for their therapeutic effects of properties for heat-clearing, dispelling dampness, aiding detoxification, promoting blood circulation, and reducing swelling. These characteristics are crucial for explaining the superior outcomes observed in the coating agents' group and wash group compared with the control group. All four herbal ingredients inherently exhibit bitter and cold properties, used for treating various overheating syndromes. *Rheum rhabarbarum* is recognized for its heat-clearing, analgesic, blood-cooling and detoxifying properties. Its major components include both bound and free anthraquinones and anthrones. Modern pharmacological studies indicate that *Rheum rhabarbarum* possesses antibacterial activity [21, 22], and anti-inflammatory and antipyretic effects [23]. Research by Yong-Hai et al. [24] found that topical application of *Rheum rhabarbarum* vinegar could rapidly control inflammation and shorten the disease duration for a hordeolum. This is consistent with the study's observation of significant reductions in lump size and VAS scores in the coating agent group and wash group, indicating effective control of inflammation and pain. *Scutellaria baicalensis* is associated with the lung and stomach meridians and is particularly effective at clearing "real fire," a term in TCM that refers to acute inflammation or infection. Its decoction has been found to have a broad-spectrum antibacterial effect [25]. Further pharmacological investigations into the primary components of *Scutellaria* revealed that baicalein may exert its antipyretic effect by reducing levels of lump necrosis factor and interleukins [26]. Additionally, baicalein inhibits the expression of the *cyclooxygenase-2* gene and prevents the binding of the transcription factor CCAAT/enhancer-binding protein beta to DNA, thereby interfering with arachidonic acid metabolism and exerting an anti-inflammatory effect [27]. *Coptis* also displays a broad-spectrum antibacterial activity and significantly inhibits gram-positive bacteria, such as *Staphylococcus aureus* [25]. This is associated with the rapid alleviation of symptoms in the treatment groups. The primary medicinal components of *Coptis* and *Phellodendron* are alkaloids [28]. Specifically, berberine in *Coptis* can bind with single-stranded or double-stranded DNA to form complexes that impact DNA function [29]. Moreover, *Phellodendron* is known for its antimicrobial and bacteriostatic effects. *In vitro* studies have shown that it is most effective against *Chlamydia trachomatis* and *Pseudomonas aeruginosa* [30]. Leveraging the significant antimicrobial properties of these two ingredients, the coating agent can swiftly eliminate bacteria at the site of infection, thereby effectively alleviating the associated symptoms.

Nonetheless, this study has some limitations. First, the cases examined were exclusively sourced from outpatients of Tianjin Ophthalmology Hospital. The narrow scope of the patient pool may introduce certain biases in statistical interpretation due to the limited sample size. Second, due to time constraints inherent in the research process, the follow-up duration for patients was abbreviated, thus potentially compromising the assessment of long-term clinical efficacy. Additionally, our study did not employ a blinding method, which may have introduced performance and detection biases, as both patients and researchers were aware of the specific treatment assignments. This awareness could have influenced the reporting and interpretation of the results. Finally, the study falls short in its investigation into the mechanisms of treatment, especially in the context of TCM categorizations and theoretical elaborations. Accordingly, future research is called for, with an expanded sample size, scientifically rigorous and effective clinical efficacy studies, and well-designed randomized controlled trials. Further exploration into the scientific rationale and clinical evidence of treating styes using this methodology is warranted. Establishing stable animal models and deepening experimental and mechanistic research, especially in neurology, humoral biology, and histopathology, could be beneficial.

CONCLUSION

The combined application of topical TCM and levofloxacin demonstrated commendable effectiveness in treating styes. It successfully mitigated eyelid swelling and pain, expedited the healing process and increased the overall rate of effective treatment. Additionally, the established preparation methods for the eye wash contribute to its user-friendly nature, which is likely to enhance patient compliance.

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Клиничка примена средстава за премазивање очију традиционалне кинеске медицине у лечењу хордеолума (чмичка)

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САЖЕТАК

Увод/Циљ Циљ ове студије био је да истражи ефикасност препарата за очи традиционалне кинеске медицине у комбинацији са левофлоксацином у лечењу хордеолума (чмичка).

Методе Студија је обухватала 110 пацијената са хордеолумом који су лечени у офталмолошкој болници у Тјенцину од марта до јуна 2018. године. Пацијенти су насумично подељени у три групе: групу са средствима за премазивање (средство за премазивање + левофлоксацин, $n = 37$), групу за испирање капака (испирање капака + левофлоксацин, $n = 38$) и контролну групу (само левофлоксацин, $n = 35$). Подаци о величини нодула и резултати визуелне аналогне скале (ВАС) за бол прикупљени су пре лечења, као и трећег, петог и седмог дана након лечења. Ефикасност лечења је процењена седам дана касније, а упоређени су величина нодула, ВАС резултати и резултати лечења три групе.

Резултати Поновљена анализа варијансе показала је значајне временске ефекте, групне ефекте и интеракције временских група између величине нодула и ВАС резултата ($p < 0,05$). Током времена, величина нодула и ВАС резултати свих група значајно су смањени у поређењу са почетним стањем. Парно поређење показало је да су трећег, петог и седмог дана величина нодула и редослед ВАС резултата били исти између група: група са средствима за премазивање = група за испирање < контролна група. Упоређивање терапеутских ефеката било је статистички значајно (94,59% *vs.* 86,84% *vs.* 71,43%, $\chi^2 = 7,622$, $p < 0,05$), а редослед ефикасности лечења био је следећи: група са средствима за премазивање = група за испирање > контролна група.

Закључак Комбинација препарата за очи традиционалне кинеске медицине и левофлоксацина у лечењу хордеолума може ефикасно смањити оток и бол капка, скратити време зарастања и побољшати стопу успешности лечења.

Кључне речи: средства традиционалне кинеске медицине за премазивање очију; хордеолум; левофлоксацин; клинички исход

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The effect of three different acrylic intraocular lenses and capsulorhexis diameter on the posterior capsule opacification development

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SUMMARY

Introduction/Objective Cataract represents a blur of the crystalline lens. The only possible way of cataract treatment is the surgical one. One of the most common postoperative complications is the development of posterior capsule opacification (PCO). The aim of this study was to exam the effect of three different acrylic intraocular lenses (IOLs) and the capsulorhexis diameter on PCO development.

Methods The study included 92 patients with a diagnosis of senile cataract divided into three groups according to the IOL type. Every group was further divided into two subgroups depending on capsulorhexis size. PCO was measured in the first, sixth, 12th, 18th, and 24th month after the phacoemulsification.

Results The lowest PCO 24 months after phacoemulsification was measured in patients with three-piece hydrophobic IOL (0.3 ± 0.08). Capsulorhexis diameter less than 5 mm had a statistically significant effect in patients with single-piece hydrophilic (0.416 ± 0.187) and single-piece hydrophobic IOL (0.411 ± 0.082) for two years follow-up.

Conclusion PCO causes a decrease of visual acuity and can be a reason for patients' dissatisfaction in postoperative period. The only possible way for the treatment of developed PCO is the usage of YAG laser capsulotomy, a procedure which can be associated with serious complications. Thereby, the finest way for PCO treatment is its prevention. The main role in that prevention has a choice of adequate surgical technique and IOL.

Keywords: posterior capsule opacification; intraocular lens; phacoemulsification

INTRODUCTION

Cataract represents a blur of the crystalline lens. It is followed by the decrease of the visual acuity as the main symptom of the disease. Other symptoms include lental myopia, monocular diplopia, glare, decreased contrast sensitivity [1]. According to research from 2010, it is believed that over 90 million people in the world have some kind of visual impairment, and about 40 million are blind. Cataract is not only the most common lens disease, but it is also the leading cause of blindness in the world [2]. It is known that senile cataracts begin to develop in every patient who is over 65 years old. It develops due to agglomeration of proteins, influx of water into the lens or disorders of lens fiber differentiation. For this reason, we clinically distinguish the three most common types of cataracts: nuclear, cortical, and subcapsular [3]. Even though many investigators attempted to discover a substance which would be able to stop and reverse the process of cataract forming, the surgery remains the only possible way for treatment of developed cataract [4, 5]. Cataract surgery is the most performed surgical procedure in medicine worldwide [6].

For the last few decades phacoemulsification has been established as the most effective method in cataract surgery [7]. Using ultrasound energy, phaco probe aspirates the cataract. The probe contains a piezoelectric crystal, which vibrates with ultrasonic frequencies [8]. Among the many advantages is the creation of a relatively closed system during cataract surgery with a deeper and stable anterior chamber, which is associated with a reduced risk of intraoperative and postoperative complications [9]. Even though this technique has improved all aspects of cataract surgery, complications still occur. One of the most common postoperative complication is posterior capsule opacification (PCO) (Figure 1) [10]. By reducing postoperative best corrected visual acuity PCO could be a reason for patient's dissatisfaction in postoperative period. Good control of preoperative inflammation and glycemia, capsulorhexis diameter, enhanced hydrodissection, bimanual aspiration, choice of an adequate intraocular lens (IOL), postoperative anti-inflammatory therapy are some of the possibilities to reduce PCO incidence [11].

The aim of this study was to examine the effect of three different acrylic IOLs and

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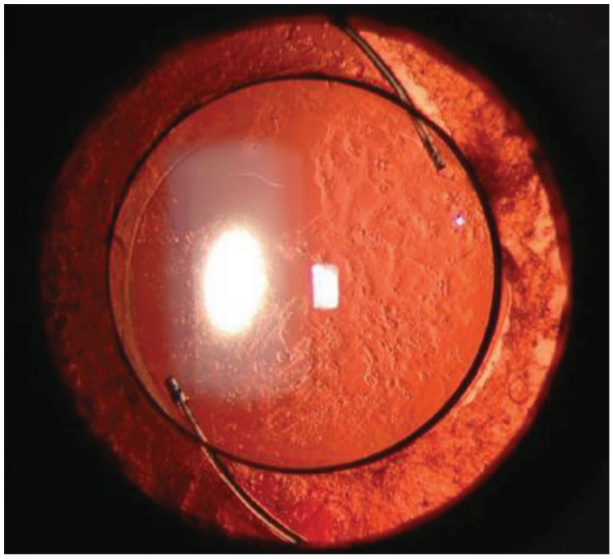

Figure 1. Posterior capsule opacification

capsulorhexis diameter on the PCO development in two years follow-up.

METHODS

The study was designed as a prospective, randomized study. It was conducted at the Clinic of ophthalmology, University Clinical Centre Kragujevac, Serbia. It included 92 patients with a diagnosis of senile cataract who were scheduled for cataract surgery. With the approval of the institutional Committee on Ethics (number 01/17/1829) and according to the tenets of the Declaration of Helsinki, the patients gave their written consent at the beginning of the study.

The main inclusion criteria were the presence of senile cataract. Patients under the age of 65 or those with other cataract types were excluded from the study. Patients with previous history of intraocular injuries or surgeries, as well as those who treated uveitis, glaucoma, retinal diseases or had zonular weakness were not able to participate in the study. Patients who were on chronic anti-inflammatory therapy were also excluded. The existence of pseudoexfoliation or pigment dispersive syndrome was also an exclusion criterion.

Before and after the surgery patients passed a complete ophthalmological examination including visual acuity measurement, Goldmann tonometry, slit lamp examination, ophthalmoscopy, ocular biometry and B scan ultrasonography. Before phacoemulsification, the patients were randomized into three groups according to the IOL which would be implanted:

First group (n = 31) – single-piece hydrophilic acrylic IOL (Eyecryl plus 600, Biotech Healthcare, Luzern, Switzerland);

Second group (n = 31) – single-piece hydrophobic acrylic IOL (AcrySof SA60AT, Alcon-Couvreur NV, Puurs, Belgium);

Third group (n = 30) – three-piece hydrophobic acrylic IOL (AcrySof MA60AC, Alcon-Couvreur NV).

All the surgeries were performed by an experienced surgeon under topical anesthesia. Phaco machine used in all surgeries was "Stellaris" (Bausch & Lomb, Laval, Quebec, Canada). Adequate preoperative mydriasis was achieved using topical application of phenylephrine and tropicamide (2.5% Phenylephrine®, 0.5% Tropicamide®, Zaječar Pharmacy, Zaječar, Serbia). Paracentesis at 2 and 10 o'clock were made and anterior chamber was fulfilled with 1% sodium hyaluronate viscoelastic (Bio-Hyalur, Biotech Healthcare). Central corneal incision and continuous curvilinear capsulorhexis were performed. Using a sterile ruler, under the microscope, capsulorhexis diameter was measured and recorded. A hydrodissection and nucleus rotation followed. When the nucleus was completely free, it was fragmented using "divide and conquer" technique. The remaining cortex was aspirated using bimanual aspiration and the capsular bag was fulfilled with cohesive viscoelastic. IOL was implanted in capsular bag. Viscoelastic was aspirated and an intracameral solution of cefuroxime with 1 mg / 0.1 ml balances salt solution (BSS) was injected. Corneal incisions were hydrated using a BSS. Postoperatively patients were administrated topical dexamethasone-tobramycin (Tobradex®, Alcon-Couvreur NV) six times a day for three weeks and nepafenac (Nevanac®, Alcon-Couvreur NV) four times a day for two weeks in the operated eye.

During patients' visits in postoperative periods a high-resolution image in retroillumination and maximal mydriasis were made at the biomicroscope. A PCO were measured using "Evaluation of Posterior Capsule Opacification 2000," a standard software program for PCO analysis [12]. PCO was measured five times in postoperative period: one, six, 12, 18, and 24 months after the cataract surgery. According to the capsulorhexis size every group was further divided into two subgroups: above and less of 5 mm. PCO was compared according to the IOL type and capsulorhexis diameter during two years of follow-up period.

IBM SPSS Statistics Version 22.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. For comparing PCO values among the groups and during the study period paired t-test and ANOVA were used ($p < 0.05$ and $p < 0.001$ were considered statistically significant).

RESULTS

The research included 92 patients who were divided according to the implanted IOL type into three groups. In all patients, cataract surgery was performed in only one eye, so the number of included eyes was equal to the number of patients (n = 92). In total, 48 were males (52.2%) and 44 females (47.8%). No statistically significant difference was recorded among sexes in the study, as well as in every group ($p > 0.05$).

Mean patients' age in the study was 73.5 ± 5.95 years (median 72, range 65–87 years). No statistically significant difference was recorded in patients' age depending on the type of implanted IOL ($p > 0.05$) (Table 1).

Table 1. Mean patients' age depending on the intraocular lens type

Intraocular lens (IOL)	n	Mean	Sd	Range
Single-piece hydrophilic IOL	31	72.94	6.12	65–86
Single-piece hydrophobic IOL	31	73.42	5.39	65–85
Three-piece hydrophobic IOL	30	74.03	6.44	65–87
Significance		p > 0.05		

Table 2. Posterior capsule opacification one month after phacoemulsification

Intraocular lens (IOL)	Mean	> 5 mm	< 5 mm
Single-piece hydrophilic IOL	0.004 ± 0.002	0.005 ± 0.001	0.002 ± 0.007
Single-piece hydrophobic IOL	0.003 ± 0.005	0.002 ± 0.005	0.002 ± 0.005
Three-piece hydrophobic IOL	0.003 ± 0.008	0.001 ± 0.005	0.005 ± 0.012
Significance	> 0.05	> 0.05	

Table 3. Posterior capsule opacification six months after phacoemulsification

Intraocular lens (IOL)	Mean	> 5 mm	< 5 mm
Single-piece hydrophilic IOL	0.041 ± 0.002	0.042 ± 0.001	0.034 ± 0.021
Single-piece hydrophobic IOL	0.031 ± 0.019	0.035 ± 0.017	0.027 ± 0.02
Three-piece hydrophobic IOL	0.03 ± 0.014	0.032 ± 0.013	0.027 ± 0.016
Significance	> 0.05	> 0.05	

Table 4. Posterior capsule opacification 12 months after phacoemulsification

Intraocular lens	Mean	> 5 mm	< 5 mm
Single-piece hydrophilic IOL	0.133 ± 0.027	0.147 ± 0.02	0.132 ± 0.03
Single-piece hydrophobic IOL	0.097 ± 0.02	0.1 ± 0.02	0.092 ± 0.02
Three-piece hydrophobic IOL	0.055 ± 0.009	0.061 ± 0.006	0.055 ± 0.012
Significance	< 0.001**	> 0.05	

**Highly statistically significant

Table 5. Posterior capsule opacification 18 months after phacoemulsification

Intraocular lens (IOL)	Mean	> 5 mm	< 5 mm
Single-piece hydrophilic IOL	0.316 ± 0.07	0.335 ± 0.057	0.311 ± 0.076
Single-piece hydrophobic IOL	0.305 ± 0.05	0.305 ± 0.047	0.292 ± 0.05
Three-piece hydrophobic IOL	0.154 ± 0.03	0.159 ± 0.022	0.148 ± 0.028
Significance	< 0.001**	< 0.05*	

*Statistically significant;
**highly statistically significant

Table 6. Posterior capsule opacification 24 months after phacoemulsification

Intraocular lens (IOL)	Mean	> 5 mm	< 5 mm
Single-piece hydrophilic IOL	0.445 ± 0.2	0.481 ± 0.219	0.416 ± 0.187
Single-piece hydrophobic IOL	0.446 ± 0.16	0.482 ± 0.21	0.411 ± 0.082
Three-piece hydrophobic IOL	0.3 ± 0.08	0.304 ± 0.07	0.293 ± 0.09
Significance	< 0.05*	< 0.05*	

*Statistically significant

In single-piece hydrophilic IOL and single-piece hydrophobic IOL groups 14 patients had capsulorhexis diameter above 5 mm and 17 patients capsulorhexis diameter less than 5 mm. In three-piece hydrophobic IOL group 16 patients had capsulorhexis diameter above 5 mm and 14 patients capsulorhexis diameter less than 5 mm.

One and six months after phacoemulsification, the highest mean PCO was measured in single-piece hydrophilic IOL group, but no statistical significance was noticed among the groups during these measurements ($p > 0.05$). Also, an analysis of the subgroups within each group did not determine the influence of the capsulorhexis diameter PCO development (Tables 2 and 3).

Intergroup analysis twelve months after phacoemulsification revealed the existence of high statistically significant difference among all groups ($p < 0.001$). The highest PCO was measured in single-piece hydrophilic IOL group, then single-piece hydrophobic IOL group and then three-piece hydrophobic IOL group. No significant difference was revealed according to the capsulorhexis size in all groups ($p > 0.05$) (Table 4).

PCO in patients with three-piece hydrophobic IOL group 18 months after the cataract surgery was 0.154 ± 0.03, which was significantly lower compared to single- IOLs groups ($p < 0.001$). PCO between patients with single-piece hydrophilic IOL and single-piece hydrophobic IOL was not significant ($p < 0.05$). Patients from single-piece hydrophilic IOL group and single-piece hydrophobic IOL group with capsulorhexis diameter less than 5 mm had significantly lower PCO compared with patients from the same groups but with capsulorhexis diameter above 5 mm ($p < 0.05$). No influence of capsulorhexis size was recorded in three-piece hydrophobic IOL group. (Table 5). The same trend of significance continued two years after phacoemulsification (Table 6).

DISCUSSION

Phacoemulsification reduced the incidence of PCO compared to the previously used extracapsular cataract extraction and intracapsular cataract extraction [13, 14]. Using phacoemulsification probe, as well as irrigation and aspiration it is possible to remove far more lens epithelial cells (LEC) during cataract surgery. But even this technique is not able to remove all LEC. In the postoperative period they undergo proliferation, migration and differentiation which is clinically manifested as PCO. It is known that postoperative inflammation has a key role in PCO development [15]. The incidence of PCO varies depending on ocular comorbidities, patients' age, used surgical technique, type of implanted IOL, length of the postoperative period. Many studies suggest incidence varies by 7–40% in patients with senile cataract, while in pediatric cataract PCO rate reaches 100%, due to high mitogenic potential of the remaining LECs [16, 17, 18]. The only possible treatment of developed PCO is YAG laser capsulotomy. This procedure could cause some serious side effects: iatrogenic IOL perforation ("pitting"), hyphema, corneal edema, intraocular pressure rise, retinal break, cystoid macular edema, chronic endophthalmitis. Therefore, research is unanimous that the best treatment of PCO is its prevention [19, 20].

Material and design of IOL have a huge effect in reducing PCO. Currently, the most used are IOLs made of acrylic material. Acrylic IOLs are associated with lower PCO compared to previously used silicone or hydrogel IOLs due to their great biocompatibility [21]. They are characterized by excellent optical performance, as well as the absence of an inflammatory response. Depending on the water content, acrylate IOLs can be hydrophobic containing less than 1% water, and hydrophilic containing

18–35% water. Considering design, acrylate IOLs can be single piece, made entirely of the same material, and three-piece with a haptics made of polymethyl methacrylate [22]. Researchers still do not agree which acrylate IOL is associated with the lowest PCO rate. The results are different depending on the IOL manufacturer, surgical technique, and duration of the follow-up. Analyzing all three groups in our study the first formation of PCO was recorded already one month after phacoemulsification. That indicates the process of proliferation, migration and differentiation of residual LECs began immediately after the cataract surgery. Until the end of the study, continuous progression of PCO was recorded in all groups. Six months after phacoemulsification the highest PCO was measured in single-piece hydrophilic IOL group, but without significance compared to other groups. At the 12th postoperative month, we observed a highly statistically significant difference among all groups. Again, the highest PCO was seen in single-piece hydrophilic IOL group (0.133 ± 0.027), then in the single-piece hydrophobic IOL group (0.097 ± 0.02) and finally in the three-piece hydrophobic IOL group (0.055 ± 0.009). That indicates material and design of the IOL had an influence in PCO. These results are similar with many previous studies [23, 24].

Ursell et al. [25] explained the possible reason for the lower PCO rate of hydrophobic acrylate IOLs. These IOLs have an adhesive surface on their back side, which binds tightly IOL to fibronectin and laminin contained in posterior lens capsule. In that way, a better barrier to the migration of residual LECs is created. Leydolt et al. [22] suggested that the higher PCO rate in hydrophilic IOLs may be in manner of its production. It is produced in a dehydrated form, only to be rehydrated afterwards. As a result of this process, the sharpness of the edges of the IOL may decrease, which facilitates the migration of LECs [22].

In our study, mean PCO in patients with implanted single-piece hydrophilic IOL and single-piece hydrophobic IOL 18 and 24 months after phacoemulsification was almost identical, while PCO in three-piece hydrophobic IOL group remained significantly lower. It can be concluded that in our study IOL material had no influence, while IOL design has shown to be a major factor in PCO reduction. The explanation of these results can be in different haptic-optic junctions in single-piece IOL and three-piece IOL. Haptics of single-piece IOL are made of the same material as optic and represent an extension of the optic. They are characterized by a notably wider root, which creates discontinuity in the capsular wrap around the IOL. That facilitates a migration of residual LECs [26]. The lower PCO incidence in three-piece IOL contributes to the presence of angulation between the optic and haptic, which is associated with a better positioning of the IOL inside the capsular bag. The angulation pushes the IOL towards the posterior lens capsule, significantly narrowing the space for LECs to migrate [27].

Capsulorhexis size could also have an influence on PCO development [28]. It is believed that when a capsulorhexis diameter is little less than IOL optic diameter the rest of anterior capsule and posterior capsule are ideally twisted around IOL creating an IOL – capsular bag complex. In some way, its content is protected from circulating pro-inflammatory mediators, as well as the complex narrows the space for LECs' migration [29]. In our study, significance was recorded in patients with single-piece hydrophilic IOL and single-piece hydrophobic IOL 18 and 24 months after phacoemulsification. Our results are in accordance with the results of Langwińska-Wośko et al. [30] who examined the influence of capsulorhexis size on the PCO occurrence on a sample of 297 eyes. Based on our results, according to the increase of significance during of the research, we can conclude that the influence of capsulorhexis diameter would achieve an even more intense impact in following years in PCO reduction.

CONCLUSION

We believe this research will be of great use in clinical practice knowing that PCO remains the most common postoperative complication of uneventful phacoemulsification. Knowing the possible complications of YAG laser capsulotomy, prevention of PCO development becomes even more important. Our study showed that PCO rate was very low in all groups, but if it is possible our results suggest the usage of three-piece IOL. If surgeon decides to implant single-piece IOL, we advocate him to make an extra effort and performs capsulorhexis less than 5 mm, so the reduced PCO rate is expected to be achieved in the years ahead.

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Ефекат три различита акрилна интраокуларна сочива и дијаметра капсулорексе на настанак опацификације задње капсуле сочива

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САЖЕТАК

Увод/Циљ Катаракта представља замућење кристалног сочива. Једини могући начин лечења катаракте је хируршки. Једна од најчешћих постоперативних компликација је развој опацификације задње капсуле.

Циљ ове студије је био да се испита ефекат три различита акрилна интраокуларна сочива и дијаметра капсулорексе на развој опацификације задње капсуле.

Методе Истраживањем су обухваћена 92 пацијента са дијагнозом сенилне катаракте подељена у три групе према типу интраокуларног сочива. Свака група је даље подељена у две подгрупе у зависности од дијаметра капсулорексе. Опацификација задње капсуле је мерена првог, шестог, 12, 18. и 24. месеца након факоемулзификације.

Резултати Најнижа опацификација задње капсуле сочива 24 месеца након факоемулзификације измерена је код пацијената са троделним хидрофобним интраокуларним сочивом (0,3 ± 0,08). Дијаметар капсулорексе мањи од 5 *mm* имао је статистички значајан ефекат код пацијената са једноделним хидрофилним интраокуларним сочивом (0,416 ± 0,187) и једноделним хидрофобним интраокуларним сочивом (0,411 ± 0,082) током две године праћења.

Закључак Опацификација задње капсуле изазива смањење видне оштрине и може представљати разлог незадовољства пацијената у постоперативном периоду. Једини могући начин лечења развијене опацификације задње капсуле је *YAG* ласерска капсулотомија, процедура која може бити праћена озбиљним компликацијама. Самим тим, најбољи третман опацификације задње капсуле је њена превенција. Главну улогу у тој превенцији има избор адекватне хируршке технике и интраокуларног сочива.

Кључне речи: опацификација задње капсуле; интраокуларно сочиво; факоемулзификација

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The impact of balneotherapy on IL-6 cytokine levels, disease activity, functional ability, fatigue and depression in patients with rheumatoid arthritis

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SUMMARY

Introduction/Objective The aim of this study was to determine the impact of balneotherapy (BT) on IL-6 cytokine levels, disease activity, functional capacity, fatigue, and depression in patients with rheumatoid arthritis (RA).

Methods The study included 46 patients with RA (16 with moderate, 16 with low disease activity, and 14 in remission) who underwent BT as part of a rheumatic disease treatment program at the Niška Banja Institute for a duration of three weeks. BT was administered in the form of radon mineral baths (lasting 15–20 minutes in the thermo-mineral waters of Niška Banja at a temperature of up to 37°C). To evaluate the effect of BT, the study utilized ELISA kits for measuring plasma cytokine IL-6 levels, DAS28 SE and Clinical Disease Activity Index (CDAI) for assessing and calculating disease activity indices, the HAQ questionnaire for measuring functional ability, the Beck Depression Inventory (BDI), and the FACIT-F questionnaire for assessing fatigue.

Results Our research results showed that the application of BT led to a statistically significant improvement ($p < 0.01$, $p < 0.001$) in the values of all examined parameters: IL-6, DAS28 SE, CDAI, HAQ, FACIT-F, and BDI. Comparing the improvement in these parameters among groups of patients with different disease activity levels (moderate, low, remission), the most pronounced anti-inflammatory effect of BT was observed in patients with moderate disease activity.

Conclusion BT improved almost all observed parameters. Alongside medication therapy, BT is a significant part of the comprehensive therapeutic approach for RA patients.

Keywords: balneotherapy; rheumatoid arthritis; cytokine IL-6; disease activity; functional ability; fatigue

INTRODUCTION

Since ancient times, thermal baths and mud have been used to treat rheumatic diseases and other musculoskeletal disorders. Balneotherapy (BT) is a complex therapeutic discipline that utilizes natural factors for treatment. Up until the mid-20th century, BT was the primary form of treatment for individuals suffering from locomotor system diseases.

It is a modality of physical therapy whose positive effects stem from the combination of the physical properties of water (due to immersion of parts of the body or the whole body in water) and/or the transfer of temperature and absorption of mineral substances through the skin [1, 2].

Its significance is evidenced by the fact that it is still actively used today alongside modern pharmacological therapy, as it affects all aspects of the disease, especially rheumatic conditions. Rheumatoid arthritis (RA) itself is a chronic, inflammatory, autoimmune disease of unknown etiology, characterized by pain, swelling, and destruction of synovial joints. If aggressive and inadequately treated, it can lead to significant disability and premature mortality [3, 4]. Studies have shown that BT can impact various symptoms of RA, including pain reduction, improved functional abilities, and decreased inflammation [5]. However, the exact mechanism by which BT, or immersion in mineral or thermal water, improves the condition of rheumatic diseases is not fully understood. The positive effects of this process are likely the result of a combination of factors, with mechanical, thermal, and chemical effects being the most prominent. According to some theories, pain reduction may be due to the pressure and temperature of the water on the skin (heat can reduce muscle spasm and increase the pain threshold). Therapy with thermal waters is believed to have an immunomodulatory effect. Recently, it has been proven that therapy with thermal waters also induces a reduction in the levels of prostaglandin E2, leukotriene B4, interleukin-1b, and tumor necrosis factor, important mediators of inflammation [6, 7].

However, despite certain findings and the widespread use of BT in the treatment of RA,

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its effects have not been fully investigated and explained in various aspects of rheumatic diseases.

The aim of this study was to determine the impact of BT on IL-6 cytokine levels, disease activity, functional capacity, fatigue, and depression in patients with RA.

METHODS

The study included a total of 46 patients (31 women and 15 men), with an average age of 61.32 ± 9.4 years and an average disease duration of 12.62 ± 6.42 years, diagnosed with RA based on the diagnostic criteria of the American College of Rheumatology and the European League Against Rheumatism 2010. According to disease activity, the patients were divided into three groups: 16 (34.78%) with moderate disease activity, 16 (34.78%) with low disease activity, and 14 (30.44%) in remission. Prior to inclusion in the study, all patients had been on stable therapy for the previous three months, specifically disease-modifying antirheumatic drugs (methotrexate at a dose of 15–20 mg per week), and on a stable dose of glucocorticoids for the past 6 weeks, with a maximum dose of prednisolone 5 mg per day.

During their treatment at the Niška Banja Institute, patients received BT for a duration of three weeks. BT was administered as radon mineral baths (lasting 15–20 minutes in the thermo-mineral waters of Niška Banja (radon waters) at a temperature of up to 37°C).

For this study, IL-6 cytokine levels, specifically its concentration in plasma, were measured using commercial ELISA kits, following the manufacturer's instructions, at the Biomedical Research Center of the Medical Faculty in Niš. ELISA kits manufactured by Elabscience were used, with a detection range of 1.56–100 pg/mL and a sensitivity of 0.94 pg/mL. In terms of disease activity assessment, a detailed clinical examination was conducted at the initial visit and after three weeks of BT. This included counting tender and swollen joints, analyzing 28 joints in typical regions affected by RA (shoulders, elbows, wrists, metacarpophalangeal, proximal interphalangeal joints of the hands, and knees). General health status was evaluated using a visual analog scale (VAS), consisting of a 10 cm line marked by the patients themselves, where 0 represents excellent health and 10 represents the worst condition. Disease activity was assessed before and after BT by calculating the Disease Activity Score DAS28 SE and the Clinical Disease Activity Index (CDAI). Functional ability was assessed using the Health Assessment Questionnaire (HAQ), which patients completed independently. Responses were scored 0–3, with higher HAQ values indicating a greater degree of disability and functional impairment. The HAQ consisted of 20 questions grouped into 8 main categories related to daily activities such as rising, walking, dressing, personal care, hygiene, reaching, gripping, and other activities.

Responses for each question were graded as follows:

0 – without difficulty;
1 – with some difficulty;
2 – with much difficulty;
3 – unable to do.

For assessing the impact of BT on depression, the Beck Depression Inventory (BDI) was used. This scale contains 21 questions, with four possible answers for each. Each question is graded from 0 to 3, so the minimum possible total score is 0 (indicating no depression) and the maximum is 63 (indicating severe depression). BDI values indicate the following:

1. 1–9 – minimal depression;
2. 10–16 – mild depression;
3. 17–29 – moderate depression;
4. 30–63 – severe depression.

For assessing fatigue, the Functional Assessment of Chronic Illness Therapy–Fatigue (FACIT–F) Scale version 4 was used. This is a short, easy-to-complete scale consisting of 13 questions measuring individual levels of fatigue during usual daily activities over the past week. Fatigue levels are measured on a Likert scale with responses: Not at all, A little bit, Somewhat, Quite a bit, Very much. The score ranges 0–52, with higher values indicating less fatigue.

The obtained results were statistically processed using IBM SPSS Statistics, Version 18.0 (IBM Corp., Armonk, NY, USA). The statistical analysis included the arithmetic mean, standard deviation, Student's t-test, and Wilcoxon test.

The authors declare that the article was written in accordance with the ethical standards of the Serbian Archives of Medicine and the ethical standards of the institutions associated with each author (Board of Ethics of the Niška Banja Institute for Treatment and Rehabilitation, Decision dated March 23, 2019).

RESULTS

The results of our study showed a significant reduction in disease activity following the application of BT. Specifically, the DAS28 SE was statistically significantly lower ($p < 0.01$) after BT compared to before BT. The mean disease activity measured by DAS28 SE for patients in remission was 2.16 ± 0.32 before BT and 1.82 ± 0.45 after. For patients with low disease activity, the DAS28 SE was 3 ± 0.17 before BT and 2.38 ± 0.3 after. Finally, for patients with moderate disease activity, the DAS28 SE decreased from 4.43 ± 0.56 before to 3.51 ± 0.57 after BT (Table 1).

Table 1. DAS28 SE and Clinical Disease Activity Index (CDAI) parameter values before and after balneotherapy

Disease activity	Parameter	Before	After	p
Remission I	DAS28 SE	2.16 ± 0.32	1.82 ± 0.45	0.002
	CDAI	3.74 ± 1.48	2.37 ± 1.01	0.002
LDA II	DAS28 SE	3 ± 0.17	2.38 ± 0.3	0.002
	CDAI	6.11 ± 1.49	3.39 ± 1.46	0.002
MDA III	DAS28 SE	4.43 ± 0.56	3.51 ± 0.57	0.001
	CDAI	15.84 ± 4.47	8.94 ± 3.05	0.001

LDA – low disease activity; MDA – moderate disease activity

A comparison of changes in DAS28 SE values between groups showed that the greatest reduction in DAS28 SE

was in the group with moderate disease activity compared to the group with low activity and remission (III *vs.* I: 0.92 ± 0.28 *vs.* 0.34 ± 0.18, p < 0.001; III *vs.* II: 0.92 ± 0.28 *vs.* 0.62 ± 0.21, p < 0.01).

The results also showed that the CDAI was statistically significantly lower (p < 0.01) after BT compared to before BT across all patient groups: patients in remission (CDAI before BT 3.74 ± 1.48, after BT 2.37 ± 1.01), patients with low disease activity (CDAI before BT 6.11 ± 1.49, after BT 3.39 ± 1.46), and patients with moderate disease activity (CDAI before BT 15.84 ± 4.47, after BT 8.94 ± 3.05) (Table 1). The greatest reduction in the CDAI index was recorded in the group with moderate disease activity (III *vs.* I: 6.9 ± 2.6 *vs.* 1.37 ± 1.23, p < 0.001; III *vs.* II: 6.9 ± 2.6 *vs.* 2.72 ± 1.34, p < 0.01).

When it comes to general health status, based on the use of the VAS, it was determined that there was an improvement in all observed groups of patients. The reduction in VAS parameter values was statistically significant (p < 0.01) (Table 2). The change in VAS values was statistically significantly greater in the group of patients with moderate disease activity compared to the group with low activity and remission (III *vs.* I: 1.44 ± 0.6 *vs.* 0.45 ± 0.3, p < 0.01; III *vs.* II: 1.44 ± 0.6 *vs.* 0.81 ± 0.37, p < 0.05).

The study results also showed an improvement in functional ability after BT in all participants, regardless of the level of disease activity (moderate disease activity, low disease activity, and remission). Analysis of the data obtained from the HAQ questionnaire revealed a statistically significant reduction in HAQ scores after BT (p < 0.01). Specifically, the average HAQ value before BT for patients in remission was 0.59 ± 0.36, which decreased to 0.39 ± 0.25 after. For patients with low disease activity, the HAQ value before BT was 0.92 ± 0.29, which decreased to 0.55 ± 0.2 after, and there was also a reduction for patients with moderate disease activity (HAQ before BT 1.25 ± 0.33, after therapy 0.88 ± 0.27) (Table 2). By comparing the changes in HAQ questionnaire values before and after the application of BT among the groups, the results showed that the greatest change occurred in the group with moderate and low disease activity (III *vs.* I: 0.37 ± 0.21 *vs.* 0.2 ± 0.09, p < 0.01; III *vs.* II: 0.37 ± 0.21 *vs.* 0.37 ± 0.16, ns).

The application of BT had a favorable effect on changes in IL-6 cytokine levels. All three examined groups showed a reduction in IL-6 cytokine levels, with these changes being statistically significant only in patients with moderate disease activity (p < 0.01). The change in IL-6 cytokine levels in patients in remission after BT was 3.43 (before BT 17.68 ± 13.12, after BT 14.25 ± 10.88), in patients with low disease activity it was 9.99 (before BT 35.45 ± 31.17, after BT 25.46 ± 27.08), and in patients with moderate disease activity it was 14.94 (before BT 55.76 ± 43.13, after BT 40.82 ± 33.38) (Table 3). The results showed that the change in IL-6 values was statistically significantly greater in the moderate disease activity group compared to the low disease activity and remission groups (III *vs.* I: 14.94 ± 6.35 *vs.* 3.43 ± 0.2.95, p < 0.001, III *vs.* II: 14.94 ± 6.25 *vs.* 9.99 ± 4.38, p < 0.01).

Table 2. Visual analog scale (VAS) and Health Assessment Questionnaire (HAQ) parameter values before and after balneotherapy

Disease activity	Parameter	Before	After	p
Remission I	VAS	1.32 ± 0.48	0.87 ± 0.33	0.003
	HAQ	0.59 ± 0.36	0.39 ± 0.25	0.004
LDA II	VAS	1.89 ± 0.53	1.08 ± 0.35	0.002
	HAQ	0.92 ± 0.29	0.55 ± 0.2	0.002
MDA III	VAS	3.39 ± 0.6	1.95 ± 0.44	0.001
	HAQ	1.25 ± 0.33	0.88 ± 0.27	0.002

LDA – low disease activity; MDA – moderate disease activity

Table 3. IL-6 parameter values before and after balneotherapy

Disease activity	Before	After	p
Remission I	17.68 ± 13.12	14.25 ± 10.88	0.016
LDA II	35.45 ± 31.17	25.46 ± 27.08	0.031
MDA III	55.76 ± 43.13	40.82 ± 33.38	0.004

LDA – low disease activity; MDA – moderate disease activity

Table 4. BDI and FACITF parameter values before and after balneotherapy

Disease activity	Parameter	Before	After	p
Remission I	BDI	2.89 ± 2.49	1.99 ± 1.65	0.008
	FACITF	42.21 ± 6.36	46.37 ± 4.03	0.008
LDA II	BDI	5.33 ± 3.27	3.02 ± 1.94	0.006
	FACITF	40 ± 6.05	45.56 ± 3.85	0.006
MDA III	BDI	13.48 ± 6.11	8.87 ± 3.84	0.003
	FACITF	28.26 ± 5.07	36.81 ± 4.6	0.002

LDA – low disease activity; MDA – moderate disease activity;
BDI – Beck Depression Inventory; FACIT-F – Functional Assessment of Chronic Illness Therapy–Fatigue Scale

The results of our study showed a positive effect of BT on depression in patients. Specifically, depression parameter values were statistically significantly lower (p < 0.01) after BT compared to values before BT. The mean depression value measured by BDI was 2.89 ± 2.49 in patients in remission before BT and 1.99 ± 1.65 after. For patients with low disease activity, the BDI value was 5.33 ± 3.27 before BT and 3.02 ± 1.94 after. There was also a reduction in BDI values in patients with moderate disease activity, from 13.48 ± 6.11 before BT to 8.87 ± 3.84 after BT (Table 4). By comparing the change in BDI values among the groups, the greatest change was recorded in the moderate disease activity group (III *vs.* I: 4.61 ± 2.84 *vs.* 0.9 ± 0.65, p < 0.001; III *vs.* II: 4.61 ± 2.84 *vs.* 2.31 ± 1.23, p < 0.01).

The average fatigue value measured by the FACIT–F questionnaire increased in all three examined groups after 3 weeks of BT, indicating that the application of BT had a favorable effect on the feeling of fatigue. The study results showed a statistically significant (p < 0.01) increase in the FACIT–F parameter. The values of this parameter increased in patients in remission (FACIT–F before BT 42.21 ± 6.36, after BT 46.37 ± 4.03), with low disease activity (FACIT–F before BT 40 ± 6.05, after BT 45.56 ± 3.85), and in patients with moderate disease activity (FACIT–F before BT 28.26 ± 5.07, after BT 36.81 ± 4.6) (Table 4). By comparing the change in FACIT–F values among the groups, the results showed that the change in FACIT–F values in the moderate disease activity group was statistically significantly greater compared to the low activity

and remission groups (III *vs.* I: 8.55 ± 3.84 *vs.* 4.16 ± 2.95, $p < 0.01$; III *vs.* II: 8.55 ± 3.84 *vs.* 5.56 ± 2.93, $p < 0.01$).

DISCUSSION

BT, which includes thermal waters, mineral mud, and other natural factors such as salts and gases, is an important process in alleviating disease symptoms and improving the quality of life for patients with RA. It significantly improves the general condition of patients, including reducing pain, improving joint mobility, and decreasing inflammatory markers. Thermal waters used in BT are rich in minerals (sulfates, calcium, magnesium, sulfur, etc.), which can have anti-inflammatory effects and contribute to the reduction of RA symptoms [5, 8, 9, 10].

In a clinical sense, BT should be regarded like any other medication. It has its indications and contraindications, and besides the desired effects, it can also have side effects. Dosage is strictly individual, and its application depends on the stage and activity of the disease, previous treatment, and the general condition of the patient. Prior medication therapy is very important, as it brings the patient into the "thermal phase" suitable for the application of BT. The best results of this therapy can be achieved in the early stage of the disease and/or in remission, while high disease activity is potentially a contraindication for the application of BT in RA. Additionally, when applying BT in the treatment of RA, the significance of psychotherapy and climatotherapy must also be considered as part of the complexity of treating this group of patients. The positive correlation between somatic improvement and the reduction of depression in patients with RA is well known. BT can have a positive impact on the psychological state of RA patients. The relaxing effects of thermal waters can lead to reduced stress levels and improved sleep quality, which is important given the often-present comorbid depression and insomnia in these patients [11, 12, 13].

In our study, we examined the impact of BT on the level of cytokine IL-6, disease activity, functional ability, fatigue, and depression in patients with RA who were hospitalized at the Niška Banja Institute for a three-week rehabilitation program.

The thermomineral waters of Niška Banja are classified as homeothermal, oligomineral, earth-alkaline, and mildly radioactive radon waters, used in baths lasting 15–20 minutes at temperatures up to 37°C, and applied to the body surface depending on the state of the cardiovascular system. The applied baths can lead to the normalization of synovial membrane permeability and influence the myogenic and metabolic regulation of blood flow to the synovial membrane, fibrous joint capsule, and muscles. On the other hand, similar positive effects can be expected from the radon contained in the peloid. It is prepared from radioactive bigar (radon-bearing limestone deposit) from the springs, having oligomineral, thermal, and radioactive effects, and is applied as compresses at temperatures 40–45°C, with a procedure duration of 15 minutes. The application of these radon mineral baths and peloids at the Niška Banja Institute has a long-standing tradition in treating patients with RA. Experiences indicate favorable effects of BT in terms of reducing pain, subjective complaints, morning stiffness, and objective clinical and biohumoral parameters. However, it is important to note that the success of BT does not represent a cure but rather a single link in the complex treatment chain [11, 14, 15].

Based on our research, a significant improvement in the observed parameters was determined after the application of BT, which supports the thesis of the effectiveness of this therapeutic method in the treatment of RA. The results indicate a positive effect of this therapy on disease activity, functional ability, reduction of the inflammatory process, and reduction of fatigue and depression, which aligns with the findings of other studies on this topic [16–22].

Firstly, regarding the reduction of disease activity after the application of BT, as registered through the decrease in DAS28 SE and CDAI indices, it can result from several factors, as previously mentioned. Primarily, the thermal waters and minerals present in BT may have anti-inflammatory effects, which can directly contribute to the reduction of pain and joint swelling. Secondly, the heat transferred through thermal waters can have multiple effects. On one hand, it can relax muscles, alleviate spasms, and reduce joint pain, while on the other hand, warm water can dilate blood vessels, improve blood and lymph flow, and help eliminate toxins and metabolic waste products [5, 8, 23].

All of the aforementioned factors can contribute to a greater or lesser extent to the reduction of disease activity, which in turn further enhances the quality of life.

In addition to reducing disease activity, as suggested by some authors and confirmed in our study, there is also an increase in functional ability. Specifically, there was a significant reduction in HAQ score values during a single treatment course [24, 25]. This suggests that BT is of great clinical importance as it can simultaneously reduce disease activity and increase patients' ability to perform daily activities without hindrance.

A particularly important finding from this study concerns the impact of BT on the level of cytokine IL-6, a key mediator of inflammation in RA, about which there is insufficient data in the professional literature. The study found that there was a significant reduction in IL-6 levels after BT, which was statistically significant. This may indicate that this type of therapy substantially contributes to reducing inflammatory processes at the molecular level or the reactivity of the immune system. It suggests that BT can modulate the immune response in patients with RA. Specifically, the warm water and relaxation associated with this therapy can reduce stress and anxiety levels in patients, which may indirectly affect the reduction of IL-6 production. This is very important, considering that stress is a significant factor that increases IL-6 levels through the activation of the neuroendocrine system [26, 27].

Finally, the study aimed to determine the impact of BT on feelings of fatigue and depression. The research found that BT leads to a reduction in fatigue and depression; the study results showed statistically significant changes in the values of the FACIT–F and BDI parameters. This

positive impact of BT on reducing fatigue and depression may primarily arise from the physiological effects of BT itself. The physical characteristics of mineral waters, such as temperature and mineral content, can contribute to muscle relaxation and pain reduction, which can indirectly improve mood and reduce stress, anxiety, and other symptoms of depression. Additionally, the mineral components of the water, such as magnesium, are known for their calming effects on the nervous system and can help regulate neurotransmitters associated with mood and fatigue [28, 29, 30].

CONCLUSION

BT, applied over three weeks in patients with RA, reduces the level of the pro-inflammatory cytokine interleukin-6, decreases disease activity, improves patients' functional ability, and reduces fatigue and depression in all RA patients, regardless of disease activity. The most pronounced anti-inflammatory effect of BT was observed in patients with moderate disease activity. Along with medication and physical therapy, BT is an important part of a comprehensive therapeutic approach for RA patients.

Conflict of interest: None declared.

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Утицај балнеотерапије на ниво цитокина ИЛ-6, активност болести, функционалне способности, замор и депресију код болесника са реуматоидним артритисом

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САЖЕТАК

Увод/Циљ Циљ овог истраживања је био да се утврди утицај балнеотерапије (БТ) на ниво цитокина ИЛ-6, активност болести, функционалну способност, замор и депресију код особа са реуматоидним артритисом.

Методе Истраживање је обухватило 46 болесника са реуматоидним артритисом (16 са умереном, 16 са ниском активношћу болести и 14 са ремисијом) код којих је у оквиру програма лечења реуматских болесника у Институту „Нишка Бања" примењена БТ у трајању од три недеље. БТ је примењена у виду радонских минералних купки (у трајању од 15 до 20 минута у термоминералним водама Нишке Бање на температури до 37°C). За процену ефекта БТ у истраживању су коришћени: *ELISA* китови за мерење концентрације нивоа цитокина ИЛ-6 у плазми, *DAS28SE* и *CDAI* за процену индекса активности болести, упитник за мерење функционалне способности – *HAQ*, Бекова скала депресивности – *BDI* и упитник за процену замора – *FACITF*.

Резултати Резултати нашег истраживања су показали да је применом БТ дошло до статистички значајног побољшања ($p < 0,01$, $p < 0,001$) вредности свих испитиваних параметара: *IL-6*, *DAS28SE*, *CDAI*, *HAQ*, *FACIT-F* и *BDI*. Поређењем побољшања вредности ових параметара међу групама болесника са различитом активношћу болести (умерена, ниска, ремисија), најизраженије антиинфламаторно дејство БТ регистровано је код болесника са умереном активношћу болести.

Закључак БТ је побољшала скоро све посматране параметре. Поред терапије лековима, БТ је значајан део свеобухватног терапијског приступа за пацијенте са реуматоидним артритисом.

Кључне речи: балнеотерапија; реуматоидни артритис; цитокин ИЛ-6; активност болести; функционална способност; замор

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Therapy of swallowing and speech problem in patients with progressive supranuclear palsy

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SUMMARY

Introduction Progressive supranuclear palsy (PSP) is a rare form of neurodegenerative extrapyramidal disease. In addition to symmetrical parkinsonism, early falls, and non-reactivity to dopaminomimetic therapy, the disease also manifests as swallowing problems with frequent choking and incomprehensible, difficult speech. In this paper, we present a case of a patient with PSP who exhibited severe swallowing and speech disorders in the clinical presentation of the disease. Appropriate therapy was applied, resulting in a positive response with partial relief of the mentioned symptoms.

Case outline A 68-year-old male patient was referred to a speech therapist by a neurologist due to difficulties with swallowing and speaking. The patient exhibited impairments in the preparatory and oral phases of swallowing, including insufficient labial occlusion and weakened tongue mobility. The patient underwent intensive speech therapy treatment for six months. The rehabilitation program led to improved swallowing function and partial improvement in speech.

Conclusion The treatment of patients with PSP should be approached seriously and interdisciplinary, given the absence of causal therapy and the reliance on symptomatic treatment for specific disabling conditions. It is essential to focus on the selection of rehabilitation programs that can improve speech and swallowing functions, as well as enhance the quality of life for patients.

Keywords: progressive supranuclear palsy; swallowing; speech; rehabilitation

INTRODUCTION

Progressive supranuclear palsy (PSP) is the second most common form of neurodegenerative parkinsonism, after idiopathic Parkinson's disease [1]. It is characterized by axial, levodopa-unresponsive parkinsonism with vertical gaze palsy, early postural instability leading to backward falls, and cognitive as well as behavioral changes [2]. Nevertheless, PSP is approximately 10 times less common than typical Parkinson's disease. Some studies point out that annual prevalence is five to seven cases per 100,000 population [3], while the annual incidence rate ranges between 0.16 and 2.6 per 100,000 population [4].

According to the dominant clinical features, more than 10 subtypes of PSP have been described in the literature. The most common type subtype is Richardson's syndrome, which is characterized by early the early onset of postural instability, vertical supranuclear gaze palsy, and cognitive dysfunction. It is also associated with a faster disease progression and a shorter survival time [5]. In contrast, the occurrence of other PSP subtypes is very rare [6].

The average age of onset for disease is 60–65 years, with a slight male predominance [7]. Some suggest it is evenly distributed between males and females [8]. The average life expectancy in this form of PSP is 5–8 years [2].

The diagnosis of PSP poses a significant challenge, especially in the early stages. In recent years, substantial progress has been made in clinical practice regarding the detection of this condition.

PSP is mainly levodopa-unresponsive and, to date, no treatment strategy has proven successful. Therefore, the main treatment approach is symptomatic therapy.

We present a patient diagnosed with PSP, who exhibited pronounced swallowing and speech symptoms and underwent speech therapy rehabilitation. The implemented rehabilitation program led to improvements in swallowing function and partially enhanced speech.

CASE REPORT

The patient is a 68-year-old retired male, with no significant health issues, aside from high blood pressure. He has no known allergies and no positive family history of serious neurological disorders. The first symptoms appeared at age 62, when he noticed difficulties with walking and speaking. Over time, these symptoms progressed and, at the time of examination, at age 68, he presented with severe postural instability characterized by frequent backward falls, as well as slurred speech and difficulty swallowing.

A neurological examination revealed vertical gaze palsy and hypomimia. The patient exhibited dysarthric speech (slow, slurred, and poorly articulated) along with dysphagia. Bilateral

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bradykinesia and rigidity in the arms and legs were also noted. The applause test was positive. The patient's gait was slow and unstable, requiring assistance. There were no issues with sphincter control.

Basic laboratory tests were performed (including CRP, folic acid, thyroid hormones, vitamin B12, vitamin D and immunological tests). All parameters were within normal limits, except for low vitamin D levels.

Magnetic resonance imaging of the brain showed a "hummingbird" sign and a subtly elevated signal of the tegmentum and tectum (Figure 1), as well as significant atrophy from the concave edge of the mesencephalon (Figure 2).

EEG findings were normal. Holter monitoring and an EKG showed no deviations from normal. Cardiologically, only hypertension was diagnosed, and appropriate therapy was started. There were no signs of orthostatic hypotension.

The patient was diagnosed with probable PSP due to the presence of mandatory criteria and key characteristics including ocular motor dysfunction and postural instability. Additionally, the MRI findings supported the diagnosis of PSP [7]. The patient continued treatment with the adjusted antiparkinsonian drugs.

Owing to his inability to control food and liquids in the oral cavity, difficulty transferring bites or sips from the oral cavity to the pharynx, frequent choking and coughing, and difficult, often incomprehensible speech, the patient was referred for rehabilitation of speech and swallowing.

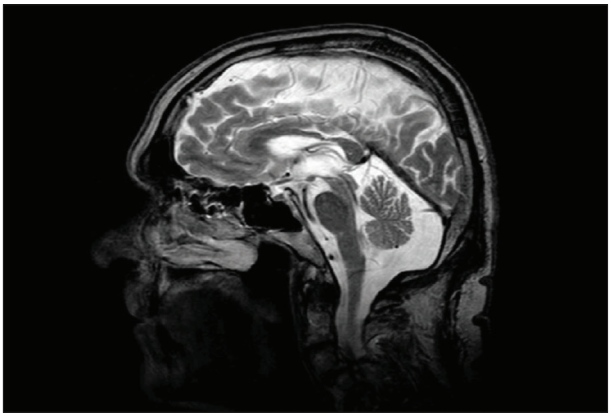

Figure 1. Magnetic resonance on the sagittal tomogram shows the so-called hummingbird sign and discretely elevated tegmentum and tectum signal; a lower ratio of the diameters of the pons and midbrain is present, as one of the differentiation factors in relation to Parkinson's disease

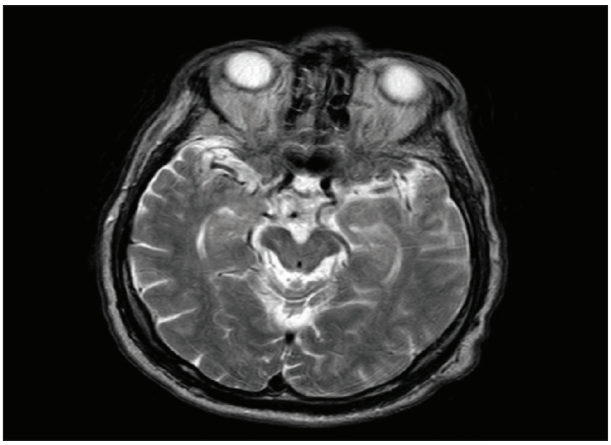

Figure 2. Magnetic resonance imaging shows significant atrophy with concave edges of the mesencephalon

Assessment of swallowing and speech abilities

To evaluate swallowing and speech, we used the Water Swallowing Test [9], the Oral Practice Test [10], and the Global Articulation Test [11].

Water Swallowing Test is a minimally invasive screening procedure used to identify oropharyngeal dysphagia. The patient is asked to sit and drink 30 ml of room-temperature water from a glass in his hand as he normally would. During the drinking process, we record the time taken to empty the glass and the drinking profile, which may indicate the need for further investigations. The profiles are as follows:

Profile 1 – the patient can swallow all the water in one gulp without choking;

Profile 2 – the patient can drink all the water in two or more sips without choking;

Profile 3 – the patient can drink all the water in one gulp with occasional choking;

Profile 4 – the patient can drink all the water in two or more sips with occasional choking;

Profile 5 – the patient frequently chokes and finds it difficult to drink all the water.

The Oral Practice Test assesses the voluntary muscle activity of the speech apparatus including the lips, cheeks, tongue, soft and hard palate and jaw. It evaluates the ability to perform specific voluntary movements of the orofacial muscles, which are controlled by certain cranial nerves (V, VII, IX, and XII). A dysfunction in any of these cranial nerves can lead to an impairment of the orofacial region, resulting in impaired articulation. The test consists of 21 tasks, ranging from simple actions (e.g., breathing in and out through the nose, extinguishing a match) to more complex tongue movements (e.g., creating a palatolingual groove). The subject is asked to perform each oral-motor pattern demonstrated by the examiner. For each successfully completed task, the subject receives one point, while no points are awarded for unsuccessful attempts.

The Global Articulation Test consists of 30 words and provides a detailed analysis of sounds, identifying both pathological sounds and those correctly pronounced. Correctly pronounced sounds are marked with a "+," distorted sounds with a "±," and omitted sounds with a "-."

The patient attended the speech therapy clinic twice a week for the first month, and once a week for the following five months. His daughter and wife were trained to help him carry out the exercises during daily activities. During therapy, we implemented oral practice exercises, voice articulation exercises using vowels in words and short sentences, as well as swallowing exercises to enhance the strength and mobility of the muscles essential for swallowing.

Outcome of applied treatment

Table 1 presents the results of the Water Swallowing Test and the Oral Practice Test conducted before speech therapy. In the Water Swallowing Test, which lasted 15 minutes, the patient obtained Profile 5 – he frequently choked and found it difficult to finish the water.

Table 1. Results of the Water Swallowing Test and oral practice before and after speech therapy

Test	Before therapy	After therapy
Water Swallowing Test	Profile 5	Profile 2
Oral practice test (task successfully completed / number of tasks)	3/21	6/21

After six months of continuous speech therapy, we re-evaluated the patient using the Water Swallowing Test, which lasted five minutes. He achieved Profile 2, meaning he could drink the water in two or more sips without choking. The patient experienced no choking episodes, as he continued to use the supraglottic swallowing technique.

On the 21-item Oral Practice Test, conducted before speech therapy, the patient scored three points for successfully completing three tasks, while he was unsuccessful in 18 tasks. Our results from an Oral Practice Test not previously administered to PSP patients, indicate notable weakness of the orofacial musculature. This reflects an inability to exert voluntary control, which is associated with impaired planning and programming of movements for speech, as well as chewing and swallowing food.

In the Oral Practice Test conducted after six months, the patient scored six points for successfully completing six tasks, while he was unsuccessful in 15 tasks. The results indicate minimal improvement, insufficient to significantly enhance the motor abilities of the oral structures, which is in line with other studies.

In Table 2, the Global Articulation Test conducted before speech therapy indicated impairment to all 30 phonemes, manifesting as distortion (9/±), substitution (15/±), and omission (6/-). This was especially pronounced in spontaneous speech, making it difficult to understand.

Table 2. Results of the Global Articulation Test before and after therapy

Time	Distorted and substitution voices	Omitted voices
Before therapy	24/30	6/30
After therapy	24/30	6/30

After six months, the patient distorted 10 phonemes (±), substituted 14 (±), and omitted 6 (-), indicating no major change in the patient's overall speech intelligibility.

The ethical commission of the Sveti Vračevi Public Health Institution Hospital in Bijeljina approved the study and consent was obtained from the patient and his family members. The research was conducted in accordance with the Declaration of Helsinki.

DISCUSSION

We present a patient diagnosed with PSP, who displayed pronounced swallowing and speech symptoms, and underwent speech therapy rehabilitation.

As the disease progresses, patients with PSP develop difficulties with swallowing and speech [12]. Dysphagia is the third most common symptom reported by PSP patients, with patients reporting a swallowing disorder three times more often than a speech disorder [13]. Some authors indicate that dysphagia in PSP occurs approximately three to four years after the onset of symptoms [14], which coincides with our patient's experience. In PSP, the oral/preparatory and oral phases of swallowing are most frequently impaired [15]. In our patient's manifestation, this likewise occurred. During the Water Swallowing Test, which lasted 15 minutes, the patient received Profile 5, reflecting frequent choking and difficulty finishing the water. The patient experienced issues with bolus preparation and transport, attributed to weakened labial occlusion, and restricted tongue mobility – essential for initiating swallowing and moving food across the base of the tongue, resulting in frequent choking.

In the treatment of patients with dysphagia, both direct and medical procedures are applied. In this case study, we utilized direct procedures (including some of the exercises for the preparation and oral phase of swallowing) along with oral motor exercises. Additionally, we implemented a supraglottic swallowing maneuver, a technique that helps reduce or control aspiration during the oral phase of swallowing, to voluntarily protect the airway.

The treatment of these patients involves exercises aimed at improving the coordination of the muscles necessary for swallowing and stimulating the swallowing reflex. Additionally, postural maneuvers (such as turning the head and tucking the chin position) are employed to redirect the flow of the bolus, helping to protect the airways and facilitate swallowing. Subsequently, the patient is advised to take small sips of water and bites of food, to cough and clear the throat after swallowing, and to modify the consistency of the food (soft/mixed/pureed) [16]. However, as the disease progresses and patients become unable to swallow, more invasive interventions are often required, such as a nasogastric tube or a percutaneous endoscopic gastrostomy [17].

Orofacial musculature plays an important role in feeding, chewing, swallowing, speaking, and facial expression. When oral musculature is weakened, patients often experience difficulties with chewing/swallowing food as well as speech/language difficulties [18], which is consistent with our case study.

A basic prerequisite for the correct pronunciation of sounds is a well-developed oral practice. This means that the muscles involved in speech can perform all the movements necessary for their proper articulation as well as for the process of chewing/swallowing food. In the Oral Practice Test conducted after six months, the subject scored six points for successfully performing six tasks, while he was unsuccessful in 15 tasks. The results indicate minimal improvement, which did not significantly

enhance the motor abilities of the oral structures – similar to results reported in other studies [19].

Good anatomy and mobility of the speech organs enable adequate speech. If one of these aspects is impaired to a lesser extent, it will have a negative impact on speech. Speech disorders in PSP manifest as weakness, incoordination, paralysis or paresis of the speech muscles, along with physiological impairments in speed, strength, sequencing, and accuracy of muscle movements, which is also characteristic of our case study [20]. Our patient exhibited dysarthria (slow, slurred, and poorly articulated speech) [21], characterized by reduced mobility, coordination, and precision of the orofacial musculature.

The Global Articulation Test revealed that numerous phonemes were distorted, substituted, or omitted, thus affecting overall intelligibility [22].

Good articulation is directly related to oral practice, meaning that the muscles involved in speech must perform well all the movements necessary for proper articulation. Speech rehabilitation in patients with PSP refers to exercises for the mobility of the speech organs, proper speech breathing, appropriate pace and rhythm of speech, and proper intonation of the voice. With these exercises, the functionality of the movement of the speech organs is achieved. According to the Global Articulation Test after conducted after six months, our patient's speech did not change significantly. Dysarthria remained present, along with impairments in a large number of phonemes due to distortion, substitution, and omission [22, 23], indicating the need for continued speech therapy rehabilitation.

Parkinsonism, vertical gaze palsy, postural instability accompanied by frequent backward falls and cognitive deficits can significantly impair or shorten the life expectancy of these patients. Therefore, it is essential to provide increased support to both the patients and their family members in the future [24, 25].

Swallowing and speech rehabilitation should be recommended as soon as symptoms become apparent, with the goal of preserving function and quality of life for as long as possible.

Conflict of interest: None declared.

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Терапија проблема гутања и говора код болесника са прогресивном супрануклеарном парализом

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САЖЕТАК

Увод Прогресивна супрануклеарна парализа је редак облик неуродегенеративне екстрапирамидне болести. Поред симетричног паркинсонизма, раних падова, нереактивности на допаминомиметичку терапију, болест се манифестује и проблемима гутања са честим загрцњивањем и неразумљивим, отежаним говором. У овом раду приказујемо болесника са прогресивном супрануклеарном парализом који у клиничкој презентацији болести има тежак поремећај гутања и говора. Примењена је одговарајућа терапија, која је резултовала позитивним одговором са делимичним олакшањем наведених симптома.

Приказ болесника Болесник мушког пола, старости 68 година, упућен је логопеду од стране неуролога због тешкоћа са гутањем и говором. Код болесника је била нарушена припремна и орална фаза гутања, са недовољном лабијалном оклузијом и ослабљеном покретљивошћу језика. Болесник је био укључен у интензиван логопедски третман у трајању од шест месеци. Примењени рехабилитациони програм резултирао је побољшању функције гутања и делимичном побољшању говора.

Закључак Лечењу болесника са прогресивном супрануклеарном парализом треба приступити озбиљно и интердисциплинарно, с обзиром на то да не постоји каузална терапија, те да је једина опција симптоматско лечење специфичних онеспособљавајућих стања. Неопходно је обратити пажњу на одабир рехабилитацијских програма који могу побољшати функције говора и гутања и квалитет живота болесника.

Кључне речи: прогресивна супрануклеарна парализа; гутање; говор; рехабилитација

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Autoimmune intestinal leiomyositis as a rare cause of chronic intestinal pseudo-obstruction in children – case report with literature review

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SUMMARY

Introduction Chronic intestinal pseudo-obstruction represents a group of rare disorders characterized by impaired gastrointestinal motility in the absence of mechanical bowel obstruction. These disorders can be primary or secondary, with autoimmune intestinal leiomyositis falling into the latter category. This condition is observed in adolescence and adulthood but is very rarely seen in children, especially in infancy.

Case outline A nine-month-old male infant was hospitalized due to persistent vomiting, abdominal bloating, and distension. After diagnostic evaluations and failure of conservative treatment measures, surgical formation of an ileostomy was performed. During the procedure, intestinal samples were obtained, revealing T lymphocyte infiltration of the intestine. Immunological blood analyses showed elevated serum immunoglobulins and smooth muscle autoantibodies. Combined with histological findings and elevated inflammatory markers, a diagnosis of autoimmune intestinal leiomyositis was established. Immunosuppressive therapy was initiated, leading to normalization of inflammatory markers and resolution of clinical symptoms. After four years of immunomodulatory therapy, the ileostomy was closed, and intestinal biopsies showed no inflammatory infiltrates. Five years later, the boy remains free of gastrointestinal symptoms, with normal growth and development.

Conclusion Although a rare condition, autoimmune intestinal leiomyositis is an important differential diagnostic entity in chronic intestinal pseudo-obstruction. Early disease recognition with intestinal biopsies, coupled with prompt and aggressive immunosuppressive therapy, enables favorable therapeutic outcomes.

Keywords: child; autoimmune intestinal leiomyositis; immunosuppression

INTRODUCTION

Chronic intestinal pseudo-obstruction (CIPO) refers to a group of rare, heterogeneous, and debilitating disorders marked by impaired gastrointestinal motility in the absence of mechanical blockage. The etiology may be primary (sporadic or familial), which is more prevalent in children, or secondary, associated with various metabolic, endocrine, toxic, infectious, or immune conditions [1]. There are only few studies, but suggested incidence is one per 40,000 live births in the United States [2].

The clinical presentation varies depending on the segment of the gastrointestinal tract involved, with common symptoms including abdominal distension, vomiting, and abdominal pain. The variability in the age of onset, disease severity, and underlying causes makes establishing clear diagnostic criteria challenging. To address this, an expert group led by The European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) published evidence- and consensus-based guidelines to standardize the diagnosis and management of pediatric patients with CIPO [3].

These guidelines served as a crucial reference in managing our patient, diagnosed with autoimmune enteric leiomyositis – a rare condition presenting in early infancy with CIPO. With only a handful of similar cases reported in the literature, this disorder is characterized by lymphocytic infiltration of the intestinal muscular layer causing intestinal inflammation with dysmotility and is often associated with poor prognosis.

Here, we presented the case with follow up of an infant with autoimmune intestinal leiomyositis, who demonstrated a favorable therapeutic response to an immunosuppressive regimen, achieving clinical, immunological, and histological remission.

CASE REPORT

A nine-month-old male presented to the emergency room with subocclusive symptoms, including abdominal distension, bloating, and vomiting. The patient's medical history was unremarkable except for chronic constipation, and there was no family history of chronic gastrointestinal or autoimmune diseases. On admission, laboratory findings revealed elevated inflammatory markers: erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)

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and hypoalbuminemia. Complete blood count and electrolytes were normal. Thyroid function tests were also normal. Cytomegalovirus and Epstein–Barr virus infections were ruled out using serological markers. Immunological evaluation demonstrated positivity for antinuclear antibodies and anti-smooth muscle antibodies (ASMA), accompanied by elevated gamma immunoglobulins (Table 1). Celiac serology was within the normal range. Imaging studies included a plain abdominal X-ray, which showed dilated small bowel loops with air-fluid levels, and an abdominal ultrasound, which revealed intestinal aperistalsis. A barium enema examination was normal.

Table 1. Laboratory and immunological panel at diagnosis and after immunosuppressive regimen when treating pediatric patient with autoimmune enteric leiomyositis

Parameters	Values at time of diagnosis	Values at ileostomy reversal	Normal values
CRP (mg/l)	77	< 0.5	< 3
ESR (mm/h)	55	5	≤ 10
Albumin (g/l)	21	35	22–47
IgG (g/l)	14.3	5.8	4.1–10.8
ANA	positive	negative	negative
ASMA	1/320	negative	negative

CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; IgG – gamma immunoglobulins; ANA – antinuclear antibodies; ASMA – anti-smooth muscle antibodies

Due to the absence of clinical improvement with conservative management, including antibiotics, prokinetic agents and enemas, the patient underwent emergency laparotomy. Intraoperative findings revealed massively dilated, aperistaltic small bowel with normal vascularization and no evidence of mechanical obstruction. The macroscopic appearance of the large bowel was normal. Full thickness of small and large bowel biopsies was obtained, and a diversion of ileostomy was performed.

Histological examination of the small and large bowel demonstrated dense lymphocytic infiltration (CD3+, CD4+ T lymphocytes, predominant and less prominent CD8+lymphocytes) in *lamina muscularis mucosae* and evidence of myenteric ganglionitis without fibrosis (Figure 1). Hirschsprung disease (Congenital intestinal aganglionosis) was excluded through histological analysis of large bowel samples (Figure 1). In the meantime, genetic testing (whole exome sequencing) was obtained and no pathological mutations associated with CIPO were found.

Based on the symptoms, histopathological findings and the immunological profile, treatment with methylprednisolone (1 mg/kg/day for 10 days, followed by prednisone tapering) and azathioprine (2.5 mg/kg/day) was initiated.

This therapeutic approach resulted in clinical remission with regular bowel movements and resolution of inflammatory syndrome. Steroids were tapered and discontinued after six months, while azathioprine was continued for maintenance. Despite stable clinical and laboratory remission, the patient experienced a recurrence of occlusive symptoms two years later, necessitating a second laparotomy. Intraoperative findings revealed extensive adhesions from the ligament of Treitz to the terminal ileum, described as "intestines adhered like glue." Surgical adhesiolysis was performed with intestinal biopsies. Histological analysis suggested mild fibrosis in the *lamina muscularis mucosae* secondary to inflammation without evidence of current lymphocytic inflammation. Steroids were introduced and tapered for a period of three months and azathioprine continued.

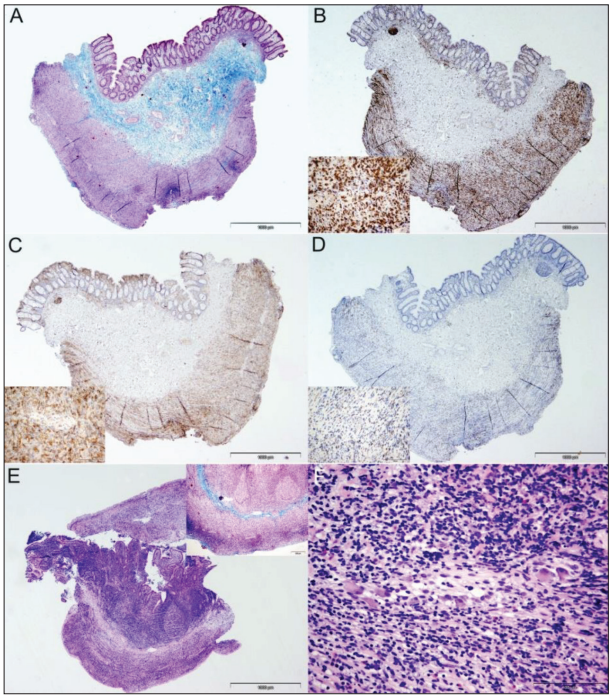

Figure 1. Histopathological evaluation of colon and ileal biopsies at diagnosis: A – biopsy of the colon showed no significant fibrosis (Masson-trichrome staining); B – CD3+ T lymphocytes infiltration of *lamina muscularis mucosae* and muscularispropria; C – CD4+ T lymphocytes infiltration of *lamina muscularis mucosae* and muscularispropria; D – CD8+T lymphocytes infiltration of *lamina muscularis mucosae* and muscularispropria; E and F – Terminal ileum biopsy showed similar histopathological features (H & E)

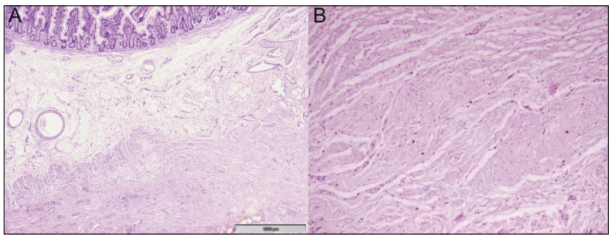

Figure 2. Ileal and colonic biopsy at ileostomy reversal; A – ileal biopsy adjacent to ileostomy revealed no inflammation in the intestinal wall (H & E); B – there were no signs of any pathological process in the muscular layer (H & E)

After a period of two years on azathioprine maintenance therapy, the patient remained clinically stable. Annual laboratory evaluations demonstrated normal inflammatory markers (CRP, ESR, and albumins), normalized gamma immunoglobulin levels and absence of histological inflammation on control intestinal biopsies. At the age of five, the ileostomy was successfully reversed. Intestinal biopsy revealed no inflammation in the intestinal wall (Figure 2). The patient is now a healthy, well-developed school-aged boy under regular medical follow-up.

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 all the subjects involved in the study.

DISCUSSION

CIPO is a rare and severe disorder characterized by episodes of bowel obstruction without a fixed, lumen-occlusive lesion. It can be categorized as congenital or acquired [1, 2, 4]. Acquired causes of CIPO may result from toxic exposure, various infections such as Cytomegalovirus, Epstein–Barr virus, and human polyomavirus virus [5], or autoimmune inflammatory processes. CIPO due to autoimmune enteric lymphocytic leiomyositis with an autoimmune reaction to smooth muscles or nerves has been reported only a handful patients in medical literature [6, 7, 8].

Most patients have a history of preceding gastroenteritis, after which abdominal distension and intestinal ileus occur. In three analyzed case reports from literature, gastroenteritis preceded the onset of CIPO, but this was not the case for our patient [8, 9, 10]. For this reason, it is hypothesized that molecular mimicry between pathogens and T lymphocytes on one side and smooth muscles in the intestinal wall on the other side may play a role in the pathogenesis of this disorder [5]. Anti-Yersinia pseudo tuberculosis antibodies were detected in one infant [11].

Since diagnosing this condition requires full-thickness bowel biopsy to detect changes across all layers, this is not yet possible via endoscopy in children and requires surgical bowel biopsy. Histological analysis reveals a dense T lymphocyte infiltrate in the *lamina muscularis mucosae*, while the mucosa and submucosa are typically spared [3].

When analyzing the age of onset of autoimmune intestinal leiomyositis, around 80% of children present with symptoms in early childhood, but majority of them were older than our patient [12]. The majority was two, three, and five years of age, with more cases reported in adolescents and young adults [8, 9, 10]. Only one child under one year of age with autoimmune intestinal leiomyositis has been reported so far, with a very unfavorable disease course and outcome [11]. The predominance of female patients may be attributed to the autoimmune nature of this disorder.

In the personal medical histories of reported cases, we observed that two children had concomitant autoimmune diseases: autoimmune hepatitis and pure red cell anemia [8, 9]. This was not the case for our patient, as clinical analysis and immunological screening excluded associated autoimmune diseases. This difference seems from the fact that patients with comorbid autoimmune diseases were older, aged two and 3.5 years, respectively [8, 9]. Additionally, there were some cases of CIPO in adult patients with various autoimmune diseases [13].

Conversely, most patients (six out of eight), including our patient, showed significantly elevated anti-smooth muscle antibody titers. Two reported patients, as well as our patient, exhibited elevated inflammatory markers [9, 10]. These findings further confirm the autoimmune nature of the disease and, alongside bowel wall biopsy, contribute significantly to accurate diagnosis.

The most critical diagnostic tool remains histological analysis of the full-thickness bowel wall. All literature reports describe marginal or absent inflammation in the mucosa and submucosa, which was confirmed in our case. In all reported cases, including the one presented in this study, there was a massive T lymphocyte infiltrate in the *lamina muscularis mucosae*, sometimes accompanied by signs of fibrosis [8]. This histological finding is crucial for diagnosing autoimmune intestinal leiomyositis and, alongside clinical signs of bowel obstruction and elevated immunological parameters, forms an essential part of the diagnostic algorithm recommended by the ESPGHAN expert group [3].

For this reason, treatment involves immunosuppressive medications, including steroids and immunomodulators. Previous reports have described the use of these immunosuppressive drugs with variable success [14]. The youngest patient, under one year of age, succumbed to the disease despite steroid therapy [11]. In contrast, most patients with autoimmune intestinal leiomyositis underwent prolonged steroid therapy with a relapsing disease course, requiring extended hospitalization [9, 10]. Additionally, there are abundant literature data about intestinal transplantation in CIPO patients, but autoimmune pathogenesis was not predominant etiology, so we cannot recommend this surgery as a therapy of choice [15, 16].

In our case, initial treatment began with steroids and azathioprine, with steroids discontinued after six months, while azathioprine maintenance therapy continued, as recommended by Oton et al. [6]. Our patient responded well to both initial immunosuppressive therapies and maintenance treatment with azathioprine. After two years, he experienced one relapse of intestinal pseudo-obstruction requiring surgical intervention (adhesiolysis) but subsequently achieved histological remission while on immunomodulator, confirmed at the time of ileostomy closure.

When comparing the clinical course of previously reported autoimmune intestinal leiomyositis cases with our patient, we observe a favorable course in our patient. He had fewer relapsing obstruction episodes, successful ileostomy closure, and achieved histological remission with minimal fibrotic tissue, due to the prolonged inflammatory process. This favorable course resulted from early, aggressive, and prolonged immunosuppressive treatment, as recommended by Ruuska et al. [9].

What stands out in our case is that our patient is the youngest reported with autoimmune intestinal leiomyositis who responded well to immunosuppressive therapy, leading to favorable clinical, laboratory, and histological outcomes.

This case highlights the critical role of combining steroids and azathioprine in managing very early-onset autoimmune leiomyositis effectively.

Although rare, autoimmune enteric leiomyositis should be an important consideration in the differential diagnosis of CIPO, regardless of the patient's age or sex. Accurate

diagnosis relies on a high index of clinical suspicion, supported by full-thickness biopsy findings of T-cell infiltration and ASMA positivity in most cases. Early recognition and timely initiation of therapy, before the development of fibrotic lesions, are critical for improving patient outcomes.

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Аутоимуни интестинални лејомиозитис као редак узрок хроничне интестиналне псеудоопструкције код деце – приказ болесника са прегледом литературе

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САЖЕТАК

Увод Хронична интестинална псеудоопструкција представља групу ретких обољења која се карактеришу поремећеним мотилитетом дигестивног тракта у одсуству механичке блокаде црева. Обољења могу бити примарна или секундарна, а аутоимуни интестинални лејомиозитис припада групи секундарних обољења. Овај поремећај се виђа у адолесценцији и одраслом добу, а врло ретко код деце, поготову у одојачком узрасту.

Приказ случаја Мушко одојче узраста девет месеци је хоспитализовано због упорног повраћања, надимања и дистензије трбуха. Након обављене дијагностике и неуспеха конзервативних терапијских мера извршено је хируршко формирање илеостоме. Том приликом су узети узорци црева, који су показали интестиналну инфилтрацију Т-лимфоцитима. Имунолошке анализе крви су показале повишење серумских имуноглобулина и антитела на глатке мишиће, па је уз хистолошки налаз и повишење упалних параметара постављена дијагноза аутоимуног интестиналног лејомиозитиса. Ординирана је имуносупресивна терапија, на коју је дете одреаговало нормализацијом упалних параметара и смиривањем клиничке слике. Након четири године примене имуномодулатора затворена је илеостома, а узете биопсије црева показале су одсуство запаљенског инфилтрата. Пет година након дијагнозе, дечак је без дигестивних тегоба, уредног раста и развоја.

Закључак Иако је аутоимуни интестинални лејомиозитис ретка болест, представља важан диференцијално-дијагностички ентитет код хроничне интестиналне псеудоопструкције. Рано препознавање болести са биопсијама црева, раном и агресивном применом имуносупресивне терапије омогућава повољан терапијски исход.

Кључне речи: дете; аутоимуни интестинални лејомиозитис; имуносупресија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Transglottic laryngeal melanoma presented as severe dyspnea

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SUMMARY

Introduction While mucosal melanoma of the head and neck remains an uncommon condition, its incidence has been on the rise in recent decades. Within the larynx, the supraglottis represents the most frequently affected subsite, followed by the glottis, with hoarseness being the predominant symptom. Given the tumor's aggressive behavior and its challenging location, it is often overlooked until it progresses to an advanced stage, which significantly worsens the prognosis.

Case report A 45-year-old male patient with stridorous breathing presented to the emergency room. An indirect laryngoscopy revealed a transglottic mass occupying the left piriform recess and the entire laryngeal inlet, leading to an exceptionally narrowed airway. Urgent tracheostomy was performed. Contrast-enhanced computed tomography of the neck identified an ulceroproliferative mass in the larynx, spanning the supraglottic, glottic, and subglottic regions, causing airway narrowing and extending into the left pyriform sinus. Laryngoscopy was performed, and biopsy of the laryngeal lesion confirmed a diagnosis of malignant melanoma. Unfortunately, the patient refused further surgical and oncological treatment, as well as additional diagnostic procedures. Four months after the initial diagnosis, the patient was lost to follow-up.

Conclusion Laryngeal melanoma is an uncommon condition that can necessitate an emergency tracheostomy. Since melanoma can be mistaken for other laryngeal malignancies, immunohistochemical analysis plays a crucial role in making an accurate diagnosis. Early detection of the disease is vital.

Keywords: melanoma; laryngeal tumor; stridor; tracheostomy

INTRODUCTION

Melanoma, which arises from the malignant transformation of melanocytes, is a type of cancer that is becoming increasingly common. Although head and neck mucosal melanoma (HNMM) is an uncommon malignancy, its incidence has been increasing in recent decades. [1, 2]. HNMM has an incidence of 1–4% of all melanomas and 0.03% of all cancer diagnoses [3]. The outlook for patients with this tumor is poor, as it tends to recur locally and metastasize to distant sites. Published five-year survival rates vary from 17.1 up to 40% [1, 4]. Mucosal melanomas affect males and females equally and are uncommon during the first three decades of life [4].

Mucosal melanoma can develop in various mucosal locations, but it is most detected in the head and neck region, as well as in the anogenital and visual tracts [5].

It is estimated that 40–60% of mucosal melanomas occur in the head and neck region. The most common sites of HNMM include the nasal cavities, paranasal sinuses, oral cavity, pharynx, and larynx [3, 6].

The most common subsite of mucosal melanoma in the larynx is supraglottis followed by glottis, with hoarseness of voice being the major symptom. A total of 60% of patients present with metastasis to neck lymph nodes, while another 60% develop distant metastases [7].

Symptoms of HNMM can be nonspecific, such as throat pain, hoarseness, or even asymptomatic. Due to the location and aggressive nature of this type of cancer, it often goes unnoticed until it has reached an advanced stage, resulting in a poor prognosis [3]. As a result, many patients are diagnosed late in the disease course when the cancer has already spread to other parts of the body.

Treating HNMM is an extremely arduous task because there is currently no therapeutic approach that has shown significant improvement in treatment outcomes [4].

Additionally, during the COVID-19 pandemic, it is expected that there will be delays in diagnosis and an increase in advanced cases of head and neck cancer due to disruptions in cancer screening and diagnosis [8]. The COVID-19 crisis has had a significant impact on every stage of the patient's journey from cancer diagnosis to treatment [9].

CASE REPORT

A 45-year-old male presented to the emergency room complaining of hoarseness, dysphagia, left-sided otalgia, and fatigue for more than

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four months. His medical history revealed prolonged consumption of tramadol tablets due to opiate withdrawal treatment and smoking one pack of cigarettes per day for 25 years. Upon examination, the patient was conscious but tachypneic with inspiratory stridor in a seated position, and oxygen saturation was 90% while breathing room air. Indirect laryngoscopy revealed a transglottic ulceroproliferative, reddish-colored mass occupying the left piriform recess and nearly obstructing the entire laryngeal inlet, resulting in a severely narrowed airway. Although no palpable neck nodes were detected, laryngeal malignancy was suspected, and the initial assessment indicated a high likelihood of difficult intubation. The patient was promptly taken to the operating room, where an awake tracheostomy was performed under local anesthesia using 25 mL of lidocaine (40 mg / 2 mL). A 10.0 mm tracheostomy cannula was successfully placed, securing the airway. The patient remained hemodynamically stable with an oxygen saturation of 98%.

Blood tests revealed an elevated white cell count (14.7×10^9/L) with neutrophilia (11.7×10^9/L), raised C-reactive protein (12.3 mg/L), and anemia (erythrocytes 4.01×10^{12}/L, hemoglobin 11.7 g/dL, hematocrit 0.357 L/L, mean cell hemoglobin concentration 328 g/L). Other blood tests, including liver function tests, were normal, and virology screening excluded HIV, hepatitis B virus, and hepatitis C infection.

Contrast-enhanced computed tomography (CT) of the neck revealed a laryngeal ulceroproliferative mass 35.7 mm × 31.3 mm × 63.4 mm in its diameter, involving the supraglottic, glottic, and subglottic regions, causing airway narrowing and extending into the left pyriform sinus (Figure 1). An additional high-resolution head CT scan demonstrated no signs of malignancy and involutional brain parenchymal volume loss. Chest radiography revealed normal findings, and no distant metastases were found on thorax CT and abdomen ultrasound. The patient underwent a laryngoscopy which confirmed clinical and CT findings. A massive laryngeal ulceroproliferative mass completely narrowed airway, occupying left hypopharynx with infiltration of the medial and anterior wall of the left pyriform sinus. The posterior wall of hypopharynx, postcricoid region and right pyriform sinus appeared without tumor infiltration. Biopsy was performed and a malignant melanoma was confirmed. Oesophagoscopy findings were normal, and an attentive intraoral examination revealed no notable findings. The histopathological examination revealed strong nuclear positivity for *SOX10* (Sry-related HMg-Box gene 10), a sensitive and specific marker of malignant melanoma, as well as weaker microphthalmia transcription factor (MITF) positivity (Figure 2). Considering all the performed diagnostics as well as clinical findings, our patient was staged as T4N0M0.

Unfortunately, the patient refused further surgical and oncological treatment, as well as additional diagnostic procedures. He was discharged from the hospital and regularly followed up for tracheostomy care but consistently refused further medical treatment. On the last control, he was in a normal state of consciousness, but his physical condition

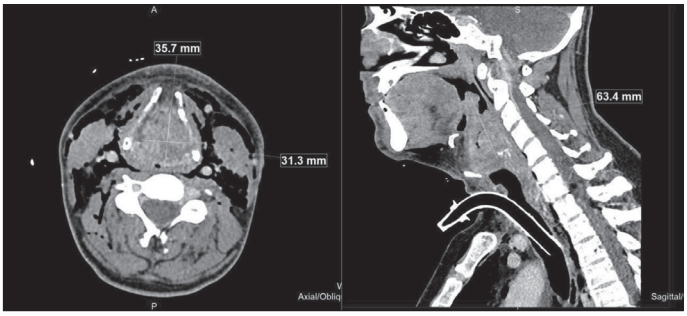

Figure 1. Contrast-enhanced computed tomography image of the neck reveals ulceroproliferative laryngeal mass with airway narrowing; tracheostomy tube is in the correct position

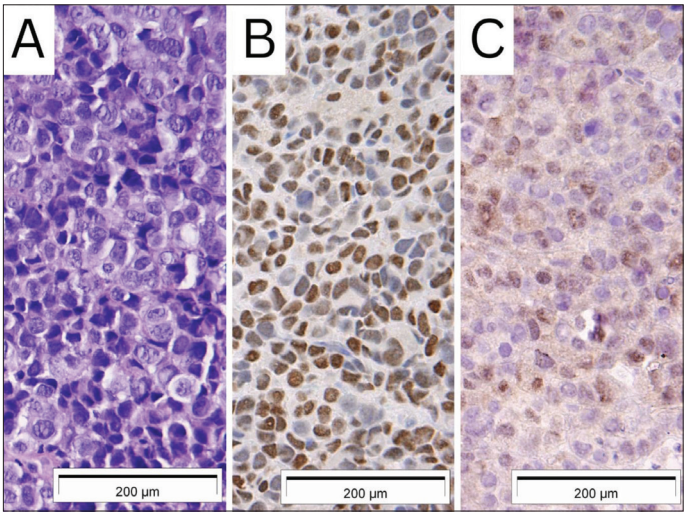

Figure 2. A – Tumor cells are large, epithelioid, with abundant eosinophilic cytoplasm and prominent nucleoli; hematoxylin and eosin stain, original magnification × 400; B – tumor cells show strong nuclear SOX10 positivity; C – tumor cells also show as weaker microphthalmia transcription factor positivity; original magnification × 400

was in rapid decline. Four months after the initial diagnosis, the patient was lost to follow-up.

All procedures were carried out in compliance with the institutional and/or national research committees' ethical standards, as well as the 1964 Helsinki Declaration and its revisions or similar ethical standards. The patient provided written permission to publish all shown material.

DISCUSSION

Mucosal melanoma of the larynx is an exceptionally rare malignancy, however, there has been a trend of rising incidence over the past few decades [3, 10].

The cause of HNMM remains unclear, but it is not believed to be linked to excessive UV exposure. Instead, contributing factors include poor-fitting dentures, physical injury, smoking, and a family history of the disease.

Our patient's family health history revealed no cases of melanoma, but he had a history of chronic heavy smoking. Mucosal melanoma is uncommon in patients under 65 years, however in the presented case, the patient was 45 years old [11].

The intricate anatomical arrangement of the head and neck coupled with the lack of early-stage symptoms present significant obstacles for both the diagnosis and treatment of malignant melanoma. Laryngeal melanoma may be presented as an unusual sensation in the patient's throat, hoarseness, or dyspnea [5]. Our patient had a significant clinical presentation with stridor and massive laryngeal infiltration.

The differential diagnosis of laryngeal masses, based on their clinical appearance, includes a variety of malignant and benign lesions, such as squamous cell carcinoma, neuroendocrine carcinoma, lymphoma, paraganglioma, and chronic granulomatous conditions of the larynx [12]. Laryngeal melanoma, as in our case, may arise as an amelanotic lesion. Hence, accurate diagnosis may be challenging, and priority must be placed on histologic assessment [3]. We underline that the clinical presentation of the tumor mass in the larynx in our patient did not arouse suspicion of mucosal melanoma. Histology analysis shows melanin abundant tumor cells altering from polyhedral to pleomorphic shapes with notable mitotic activity. Verification is performed with immunohistochemistry using S100 protein and melanocyte markers- MART1/Melan A, tyrosinase, HMB45, MITF. Protein S100 exhibits the highest sensitivity, while HMB45 shows the greatest specificity [13]. In the study of Suresh et al. [4], 90.2% of cases showed positivity both to S100 and HMB45. The absence of p16 has been reported in 74% of patients with mucosal melanoma.

Based on immunohistochemistry, it is not possible to distinguish between primary and metastatic melanoma [13]. Based on nodularity and primary submucosal localization, it is possible that malignant melanoma in this study is presented as metastasis. However, since a part of the tumor's surface was necrotic, it cannot be reliably ruled out that the larynx was the primary location.

Despite advancements in therapy modalities, HNMM still remains a rare oncologic condition with a dismal prognosis [12]. Primary mucosal melanoma is associated with an extremely poor prognosis due to its invasive growth and tendency to present at an advanced stage [7, 14]. As an outcome, no categorical treatment strategy was adopted for this entity.

Treatment for mucosal melanoma can include surgery, radiotherapy, chemotherapy and, latterly, target therapy and immunotherapy.

According to the study by Grant-Freemantle et al. [12], distant metastasis and local recurrence are the main contributors to mortality in HNMM. It was found that distant metastasis plays a more prominent role in mortality. The study also showed that survival rates were lower with primary radiotherapy compared to surgery alone, indicating that radiotherapy alone is less effective than surgery alone. However, radiotherapy can be highly effective in achieving long-term local control.

The application of postoperative radiotherapy, including its plan and dose, remains a topic of debate. In a study by Lu et al. [11], which included 288 patients with mucosal melanoma who underwent surgery, radiotherapy did not show an improvement in overall survival or disease-specific survival.

On the other hand, a systematic review conducted by Jarrom et al. [15] that focused on mucosal melanoma in the upper airways system determined that postoperative radiotherapy could enhance locoregional control of the disease. A study by Pincet et al. [2] also showed that primary or adjuvant radiotherapy provides a benefit on local control and overall survival.

The outcomes of recent treatment modalities, such as immunotherapies, have not brought about significant improvements in the prognosis of mucosal melanoma. However, these modalities provide new possibilities for the treatment of inoperable tumors or as adjuvant post-surgery treatments. Despite this, radical surgical procedures remain a fundamental component of mucosal melanoma treatment. It should be noted that targeted mutations vary between cutaneous and mucosal melanoma, which means that the treatment benefits are comparatively lesser. Also, response rates after surgical treatment are lower than those seen in patients with cutaneous melanoma (19% *vs*. 33%) [16, 17].

It is recommended that the initial and most effective treatment for mucosal melanoma is a complete resection with negative margins [16].

Managing mucosal melanoma is an extremely challenging task, and studies have not yet provided a clear statement regarding the role of radiotherapy and novel systemic treatments [17]. A study by Patel et al. [18] found that the only independent predictors of outcome in HNMM are clinical stage at presentation, tumor thickness greater than 5 mm, vascular invasion on histologic examination, and

Table 1. The brief literature review of glottic laryngeal mucosal melanoma in Europe

Cases	Author	Year	Country	Treatment	Follow-up
1	Cremonesi [19]	1956	Italy	Radiotherapy and neck dissection	No details
2	Lorentz [20]	1979	Germany	Partial laryngectomy and radiotherapy	No details
3	Hussain and Whitehead [21]	1989	United Kingdom (UK)	Radiotherapy	No recurrence identified at 24 months follow-up
4	Duwel and Michielssen [22]	1996	Belgium	Total laryngectomy	No details
5	Asare-Owusu et al. [23]	1999	UK	Radiotherapy	No recurrence identified at 24 months follow-up
6	Szmeja et al. [24]	2000	Poland	Total laryngectomy, neck dissection, and radiotherapy	No details
7	**Current case**	**2021**	**Serbia**	**None**	**No details**

the development of distant metastasis. In our case report, delayed presentation made urgency for tracheostomy. Total laryngectomy was advised, but the patient's refusal made the treatment impossible.

So far, literature review revealed only 60 cases of laryngeal melanoma and 18 of them were in glottic subregion [7]. In Europe, there have been six reported melanomas of the glottis, while the last one was recorded more than 20 years ago. Of all these cases, three patients had a combined treatment of radiotherapy and surgical treatment, while another three underwent monotherapy. Only two patients who were treated with radiotherapy attended regular examinations and it was noted that they did not have a recurrence of the disease during the two years follow-up (Table 1). It is evident that there is no categorical strategy for the treatment of these malignancies even in advanced European countries. It is also observed that most patients, four out of six, do not have a recorded history of follow-up after the treatment, just like in our case.

Laryngeal melanoma is a rare condition that may require emergency tracheostomy. Since melanoma can be mistaken for other laryngeal malignancies, immunohistochemical analysis plays a crucial role in making an accurate diagnosis. Early detection of the disease is vital. The recommended first-line treatment is radical surgery followed by postoperative radiotherapy for optimal locoregional control. Despite advances in treatment modalities, laryngeal melanoma still carries a poor prognosis with an exceptionally low five-year overall survival rate.

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Трансглотични меланом гркљана представљен отежаним дисањем

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САЖЕТАК

Увод Иако је меланом слузокоже главе и врата ретка болест, његова учесталост расте последњих деценија. Најчешћа локализација меланома ларинкса је супраглотис, затим глотис, а главни симптом је промуклост. Због локализације и агресивне природе ове врсте рака, често остаје асимптоматски све док не достигне узнапредовали стадијум болести, што резултира лошом прогнозом.

Приказ болесника Пацијент, стар 45 година, са отежаним дисањем, примљен је у хитну службу. Индиректна ларингоскопија открила је трансглотичну масу која заузима леви пириформни синус и скоро цео улаз у ларинкс, што је довело до изузетно суженог дисајног пута. Урађена је хитна трахеотомија. Контрастном компјутеризованом томографијом врата откривена је улцеропролиферативна маса ларинкса која обухвата супраглотис, глотис и субглотис са сужењем дисајних путева и ширењем у леви пириформни синус. Учињена је и директна ларингоскопија, а биопсијом је потврђен малигни меланом ларинкса. Нажалост, пацијент је одбио даље хируршко и онколошко лечење, као и додатне дијагностичке процедуре. Четири месеца након постављања дијагнозе пацијент је престао да долази на контроле.

Закључак Меланом ларинкса је ретко стање које може захтевати хитну трахеотомију. Пошто се меланом може заменити са другим малигнитетима ларинкса, имунохистохемијска анализа игра кључну улогу у постављању тачне дијагнозе. Рано откривање болести је од виталног значаја.

Кључне речи: меланом; тумор ларинкса; стридор; трахеотомија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Diffuse large B-cell type of the primary non-Hodgkin's lymphoma of the liver – a diagnostic problem

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SUMMARY

Introduction Primary Non-Hodgkin lymphoma of the liver is an extremely rare disease. It most often occurs as a diffuse large B-cell type.

Case outline We present the case of a 75-year-old patient who was admitted to our clinic with right-sided subcostal pain accompanied by malaise, weakness, and elevated body temperature. Laboratory analyzes were within normal limits. Ultrasonography and computed tomography findings showed a sharply marginated inhomogeneous lesion in the right liver lobe with central necrosis. Intraoperatively and pathohistologically, it was confirmed that it was diffuse large B-cell lymphoma of the liver. Surgery, chemotherapy, radiotherapy and their combination are the methods of treatment.

Conclusion Surgical treatment with chemotherapy allows for a significantly higher survival rate.

Keywords: Non-Hodgkin lymphoma; liver; diagnosis; treatment

INTRODUCTION

Lymphomas are a type of malignant disease that develops in lymph nodes and lymphatic tissue of other organs [1]. There are two main types of lymphoma: Hodgkin's disease and non-Hodgkin's lymphoma. Non-Hodgkin's lymphoma is divided into two types: diffuse large B-cell lymphoma (DLBCL) and follicular type [1, 2]. They are manifested by lymphadenopathy that can occur in any part of the body [2]. Lymph nodes are enlarged, hard in consistency and painless [3].

Extranodal forms of non-Hodgkin's lymphoma (about 40%) occur most often at the head and neck [3]. Primary non-Hodgkin lymphoma of the liver is an extremely rare disease. The tumor is most often solitary and located in the right lobe of the liver [4, 5]. The most common form of primary non-Hodgkin's lymphoma of the liver is the DLBCL [3, 4]. Secondary liver lymphomas are usually multiple and correspond to the progression of the primary nodal form of the disease (50%) [5, 6].

Middle-aged men are most often affected, especially those with underlining immunocompromising diseases [6, 7, 8]. Weight loss, elevated body temperature, sweating, dull abdominal pain in the upper part of the abdomen or epigastric discomfort, weakness, vague febrile state are the most common symptoms [6, 8] Physical examination does not provide a lot of information's and lymphadenopathy can be rarely detected [6]

Laboratory analyzes are within normal limits [4, 7]. Tumor markers (CEA, CA 19-9, AFP) and serology are normal. Hepatitis C (HCV) may be the basis for the development of primary liver lymphoma, although there are no real studies and results to confirm that [8, 9].

The diagnosis of primary liver lymphoma is considerably difficult [6, 9]. Ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) are the basic diagnostic procedures [9].

Surgery, chemotherapy and radiotherapy alone or in combination represent treatment modalities of choice [9, 10].

The prognosis of the disease is poor [10]. A small number of patients are treated operatively, but with mandatory postoperative chemotherapy with an increase in the survival rate [11].

Multidisciplinary approach is advised in treatment of primary liver lymphoma for optimal effect [10].

CASE REPORT

A 75-year-old patient was admitted to our clinic due to dull intermittent pain under the right rib cage, accompanied by malaise, weakness, and an occasional elevated body temperature of around 38 degreases for 3–4 months. Anamnesis data speak of cholecystectomy due to calculus 30 years ago, appendectomy and ovariectomy, as well as stable cardiological status – hypertension. Physical examination did not establish any pathological findings. Tumor markers (CEA, CA 19-9, AFP), serological

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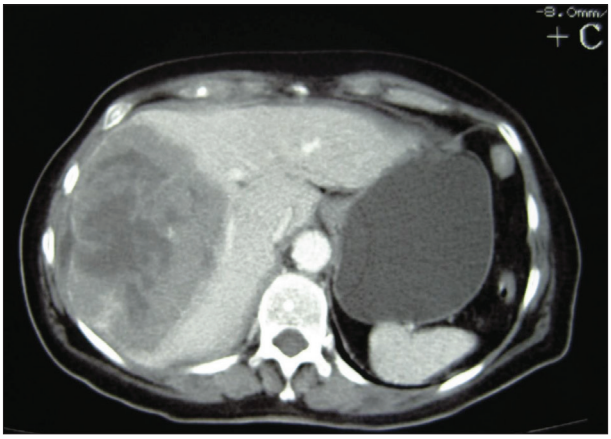

Figure 1. Computed tomography (CT) of the abdomen, arterial phase, axial section shows a predominantly hypodense, centrally necrotic lesion located in the right liver lobe, without CT signs of invasion of vascular structures

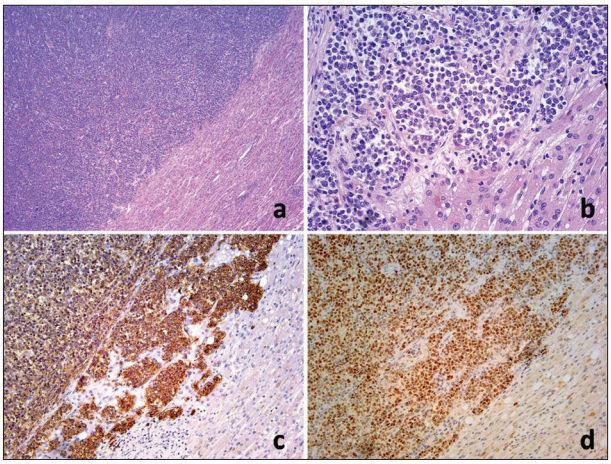

Figure 2. Histology of hepatic diffuse large B-cell lymphoma: a. mass forming lymphoma presented well defined boundaries towards hepatic parenchyma (HE, 10 ×), b. typical centroblastic cytomorphology (HE, 40 ×), c. strong immunoexpression of CD20 antibody (SAB+/DAB, 20 ×) and d. strong immunostaining with Bcl-6 antibody (SAB+/DAB, 20 ×) depicting GCB-type of this lymphoma

tests, laboratory analyzes ("hepatogram", complete blood count, leukocyte formula, bleeding times, electrolyte status, glycemia) were within normal limits except for SE 90, coagulation factor II 125, V 166, VII 128, IX 148. Esophagogastroduodenoscopy, colonoscopy, and radiography of the lungs where with in normal parameters for the patients age. Ultrasonographic and CT examination of the abdomen indicated a clearly demarcated inhomogeneous and predominantly hypodense lesion involving the greater part of the right lobe of the liver, diameter 165 × 130 × 110 mm with a zone of pronounced central necrosis (Figure 1).

After opening the abdomen by laparotomy, exploration of the abdomen verifies a large lesion with a diameter of about 15 cm, which completely destroys the V, VI, VII and part of the VIII segment of the liver with compression of the right hepatic vein. Through an anterior approach, a radiofrequency (RF) system resection of V, VI, VII, and part of segment VIII of the liver was performed with preservation of the remaining part of segment VIII and the medial hepatic vein. Other findings in the abdomen and liver were normal. The abdomen was drained with four drains. On the third postoperative day, a right sided pulmonary effusion was detected, which was treated conservatively. The patient started with oral intake on the fourth postoperative day, and the abdominal drains were removed on the sixth postoperative day. On the ninth postoperative day, the patient was discharged from the hospital in good shape and with proper laboratory analyses, X-ray findings of the lungs and ultrasound of the abdomen.

The definitive pathohistological finding with immunohistochemical analysis was: DLBCL of the liver: DLBCL, GCB-type with Ki-67 index of 70% and R1 resection (Figure 2). Tumors cells were small to intermediate in size, round, with scant cytoplasm, mostly vesicular nuclei, coarsely granulated chromatin, with central tumor necrosis and mucoid degeneration of the stroma.

One month after the operation, the patient had no complaints, laboratory tests, US of the abdomen and X-ray of the chest were normal. In the first three years, after the operation, the patient regularly attended periodic surgical examinations with laboratory analyzes and accompanying US or CT examination. During that period no relapse of the disease was detected. The patient declined chemotherapy treatment immediately after the operation, as suggested by the hematological oncology council.

This study was done in accordance with standards of the institutional Committee on Ethics. Patient's written consent was obtained.

DISCUSSION

Lymphomas are a heterogeneous type of malignant disease that develops from lymph nodes and lymphatic tissue of other organs. There are two main types of lymphoma: Hodgkin's disease (with four subtypes) and non-Hodgkin's lymphoma (25 subtypes) [1, 2]. The two most common subtypes of non-Hodgkin's lymphoma (over 50%), which make up about 5% of all malignancies, are: DLBCL and follicular type [1]. There are also transitional forms of these subtypes [2]. Most subtypes of both forms of lymphoma are manifested by lymphadenopathy that can occur in any part of the body [1]. One form is MALT lymphoma (mucosa-associated lymphoid tissue) that usually develops in the stomach (85%) to infectious and autoimmune agents [3]. Lymph nodes are enlarged, hard in consistency and painless. In the pathogenesis of the disease, there is the development of abnormal lymphocytes that multiply uncontrollably and accumulate in the lymphatic tissue [1].

Extranodal forms of non-Hodgkin's lymphoma (about 40%) occur most often on the head and neck [1, 2]. Primary non-Hodgkin lymphoma of the liver is an extremely rare disease [3]. The right lobe of the liver is more commonly affected than the left lobe, with an average size of the lesion about 11.5 cm [3, 4, 5]. Macroscopically the tumor is gray-white in color, soft in consistency, clearly demarcated, with lobulated contour, and with focal bleeding and necrosis [3, 6]. DLBCL is the most common form (0.016% of all non-Hodgkin's lymphomas or 0.4%

of extranodal non-Hodgkin's lymphomas) [4, 10]. It was first described in 1965 [5]. Secondary liver lymphoma is more frequent than primary form and corresponds to the progression of the primary nodal form of the disease [5]. Establishing a definitive diagnosis of the disease clinically and pathohistologically is difficult [6].

Middle-aged men are most often affected, although in some individual small studies, women over 60 years of age appear more often [4, 7, 12]. The disease occurs more often in people suffering from immunocompromising diseases [8].

The most common symptoms are weight loss, elevated body temperature, sweating, so-called "B symptoms" (37–86%), dull abdominal pain in the upper parts of the abdomen or epigastric discomfort, weakness are the most common symptoms [2, 4, 11]. Sometimes it occurs as a vague febrile condition or icterus (4%) [11].

Physical examination can be scanty in terms of hepatomegaly and always without lymphadenopathy [7].

Laboratory analyzes may be within the limits of normal values or with a slight "hepatogram" disorder, coagulation factor disorder, anemia, thrombocytopenia, sedimentation, lactate dehydrogenase [8, 13]. Tumor markers (CEA, CA 19-9, AFP) are normal. Serologically, the affected patient is usually HBsAg and HCV negative [3, 14]. In some cases (20–67%) patients can be HCV positive [14]. HCV can be the basis for the development of primary lymphoma of the liver, but there are no real studies to support that. Sometimes hypercalcemia can occur (parathyroid hormone-related peptide is elevated in lymphoma cells) [14].

Diagnostic modalities such as ultrasound examination of the abdomen, CT and MRI examination are highly sensitive in detecting these lesions, which are mostly solitary and hypovascular [15]. On cross-sectional imaging, they appear as hypodense or hypointense infiltrative lesions and are initially often misdiagnosed as primary tumors of hepatic origin [10]. Ultrasound examination detects a hypoechoic mass within the liver parenchyma, which in some cases, due to extensive necrosis (60%), may look like a cystically degraded structure [10, 15]. Color Doppler (CD) signal is present around the periphery of the lesion. CT examination can precisely localize the lesion and can shows the relationship with the vascular structures [15]. There is no pronounced post-contrast opacification of the lesion in the arterial phase of the examination nor the wash-out effect typical of hepatocellular carcinoma [12]. The tumor is predominantly hypodense due to hypovascularity with discretely higher density around the perimeter of the lesion [13]. Rarely, they can be complicated by intratumor hemorrhage, which can be seen on a CT scan as a zone of higher intralesional density. MRI characteristics of the lesion are lower signal intensity (IS) in T1 weighted image, higher IS in T2 weighted image, conspicuous restrictive diffusion without postcontrast viability and no uptake of liver specific contrast agent [15].

Very often a wrong diagnosis is made (non-specific symptomatology, normal laboratory values, difficult and unclear diagnostics, numerous subtypes of lymphoma) [10]. Differential diagnosis of primary liver lymphoma should include active form of chronic hepatitis, granulomatous cholangitis, inflammatory pseudotumor, anaplastic carcinoma, metastatic process [6, 7, 11].

Definitive diagnosis is made on the basis of pathohistological and immunohistochemical analysis while taking in to the account the remaining results [3, 10, 14, 15]. Therefore, it is necessary to exclude other lymphoproliferative, systemic, immunodeficient and other diseases [5, 11].

Surgery, chemotherapy and radiotherapy alone or in combination are treatment modalities of choice [4, 10]. Regardless of the type, the disease has a very aggressive course and the prognosis is usually poor. A massive liver tumor, high proliferative index, advanced age and liver cirrhosis are poor prognostic factors [5, 11]. Treatment with only chemotherapy, gives the patient prognosis of six months [14]. Monoclonal antibodies have great potential for the treatment of this disease [14]. If, over time, diagnostic procedures verify new liver lesions, it is necessary to change the chemotherapy. Each chemotherapy must be adapted to the age and general condition of the patient and the liver, stage of the disease, histopathological findings and phenotyping [10].

A relatively small number of patients were treated operatively, but with mandatory postoperative chemotherapy, after three weeks of surgery (cyclophosphamide, doxorubicin, vincristine, prednisone) [9, 12]. Survival of patients with primary liver lymphoma (DLBCL) increases from 54% to 80% with a combination of operative treatment and chemotherapy with an average survival of 39 months (5–124 months) [10, 13].

Multidisciplinary approach in treatment of primary liver lymphoma should be applied, if possible, because of its proven effects in overall patient average survival rate [5, 10].

In summary, primary liver lymphoma is a rare disease that poses challenges in both diagnosis and treatment. Advancements in diagnostic techniques, innovative treatment methods, and a collaborative multidisciplinary approach can help address the challenges of accurately diagnosing and promptly treating liver lymphoma, ultimately leading to improved average survival rates for patients.

Conflict of interest: None declared.

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Дифузни крупноћелијски Б тип примарног нехочкинског лимфома јетре – дијагностички проблем

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САЖЕТАК

Увод Примарни нехочкински лимфом јетре је изузетно ретко обољење. Најчешће се јавља као дифузни крупноћелијски Б тип.

Приказ болесника Приказан је случај болеснице старе 75 година која је примљена због болова испод десног ребарног лука праћених малаксалошћу, слабошћу и повишеном телесном температуром. Лабораторијске анализе биле су у границама нормалних вредности. Ултрасонографски и компјутеризованом томографијом се верификује јасно ограничена нехомогена туморска промена у десном лобусу јетре са централном зоном некрозе. Интраоперативно и патохистолошки потврђено је да се ради о дифузном крупноћелијском Б лимфому јетре. Хирургија, хемотерапија, радиотерапија и њихова комбинација су начини лечења.

Закључак Хируршки третман са хемотерапијом омогућава знатно вишу стопу преживљавања.

Кључне речи: нехочкински лимфом; јетра; дијагностика; лечење

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REVIEW ARTICLE / ПРЕГЛЕДНИ РАД

Vitamin D: a comprehensive review

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SUMMARY
Vitamin D (calciferol), i.e. its active metabolite calcitriol [1,25(OH)2D], apart from essential participation in calcium and phosphorus homeostasis, is an important factor in the regulation of cell proliferation, differentiation and apoptosis, angiogenesis, immune and hormonal activity and other processes in the human body. Hence, its optimal balance is extremely important for adequate prenatal and postnatal growth and development, as well as for the preservation of health in other phases of life. This article provides a brief overview of the natural sources of vitamin D, its metabolism and physiological role, as well as current recommendations related to the coverage of its optimal needs.
Keywords: vitamin D; physiological role; optimal need

INTRODUCTION

Vitamin D (calciferol), i.e. its active metabolite calcitriol [1,25(OH)2D] is an essential factor in ensuring calcium and phosphorus homeostasis and an important participant in the regulation of cell proliferation, differentiation and apoptosis, as well as immune, hormonal and other processes in the human organism [1–7]. Hence, its optimal balance in the body is extremely important for normal prenatal and later growth and development, as well as for maintaining health in other phases of life [8–14]. This article provides a brief overview of the natural sources of vitamin D, its metabolism and physiological role, as well as current guidelines related to meeting its optimal needs.

NATURAL SOURCES OF VITAMIN D

There are two major forms of vitamin D: vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol), which differ in origin and chemical structure. Both variants of vitamin D are the product of photolytic cleavage of the C9–C10 bond in the sterol B ring after exposure to ultraviolet-B (UV-B) rays of wavelengths 290–315 nm of the respective precursors, i.e. in the skin of man and most terrestrial vertebrates 7-dehydrocholesterol (7-DHC) to cholecalciferol and in yeast and other fungi ergosterol to ergocalciferol [1, 14, 15]. The origin of vitamin D in fish comes from zooplankton and microalgae in the food chain in water, while some land animals, such as dogs and cats, get it by consuming prey which stores vitamin D_3 in the liver and adipose tissue [16, 17]. Unlike cholecalciferol, the side chain of ergocalciferol has a double bond at the position C22–C23 and a methyl group at C24. A recent comparative analysis of the results of 20 studies showed that vitamin D_3, apart from more efficiently raising serum 25(OH)D concentration, has no advantage over vitamin D_2 [18].

Most of our vitamin D needs are met by production in the skin, while foods, excluding fish oil, fatty fish, liver, egg yolks, edible mushrooms treated with UV light, and fortified foods such as milk formulas, are poor sources of vitamin D (Table 1) [1, 7, 19, 20, 21].

Table 1. Vitamin D content in foods [20, 21]

Food	Content (IU/100 g)
Human milk	2.5–3
Standard milk formulae	40–60
Cow's milk	4
Chicken	15–20
Beef	30–50
Chicken liver	50
Hen egg yolk	125–215
Mushrooms, champignons*	75
Mushrooms, chanterelle*	210–570
Fish	200–990
Cod liver oil	8400–10,000

*Exposed to UV light

During photolysis of 7-DHC in epidermal keratocytes and dermal fibroblasts under the influence of sunlight, previtamin D_3 is formed, which is then isomerized to vitamin D_3 at skin temperature [1, 7, 13, 22]. The level of skin production of vitamin D depends on latitude and

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altitude, season, time of day, body surface area and length of exposure to the sun, amount of melanin in the skin, age and degree of protection from sunlight, and genetic factors [1, 7, 13, 23, 24]. Apart from staying in the shade and using sun protection creams, cloudy weather and air pollution significantly reduce the skin's production of vitamin D_3 [1, 7, 24]. It is important to point out that excessive exposure to sunlight, especially sunburn, increases the risk of skin cancer, so it should be avoided [14, 25]. Bearing in mind the cumulative nature of sun damage, this is additionally true for the pediatric age group, and especially for infants under six months of age, who should not be exposed to direct sunlight due to the developmental hypersensitivity of the skin [26].

Holick [14] suggests that exposing the arms and legs for 5–30 minutes (depending on the time of day, season, latitude, and skin pigmentation) between 10 a.m. and 3 p.m. twice a week is often enough to meet vitamin D needs. Due to photoisomerization into non-toxic metabolites (lumisterol, tachysterol, suprasterol I and II, and 5,6 trans cholecalciferol), the protective effect of melanin and skin desquamation, vitamin D intoxication through sun exposure is not possible [1, 7, 22, 25].

Vitamin D produced in the skin diffuses into the blood and, bound to vitamin D binding protein (DBP) and partly to albumin (10–15%), is transported to the liver, where it is hydroxylated into 25(OH)D (calcidiol), while the excess is deposited, mainly (75%) in fatty tissue, and the rest in the liver, muscles, and skeleton [1, 7, 22].

Vitamin D from food and supplements is absorbed in the small intestine by passive diffusion through micelles containing bile acids and then, incorporated into chylomicrons, exported from the enterocytes to the lymphatic system and transported to the systemic circulation [7]. The degree of intestinal absorption of vitamin D in a healthy person varies between 55% and 99% (on average about 80%), while in patients with fat malabsorption it is very low [7, 13, 22, 27]. After entering the circulation, vitamin D is released from chylomicrons under the action of lipoprotein lipase and follows the same path as the fraction formed in the skin [7, 22].

Intestinal absorption of calcidiol, which is found in drugs intended for patients with fat malabsorption, as well as in variable amounts in some foods of animal origin, such as meat (0.8–16.4 IU/100 g), liver (2.8–30.8 IU/100 g) and kidney (3.6–93.2 IU/100 g), since it is soluble in water, is independent of the presence of bile acids and the formation of micelles and is therefore significantly more effective (about 93%) [22, 28]. The active metabolite of vitamin D, 1,25(OH)2D (calcitriol), is found only in trace amounts in animal foods and does not contribute much to the biological activity of vitamin D [28]. Like other water-soluble substances, hydroxylated forms of vitamin D are absorbed directly from the proximal jejunum into the portal bloodstream and, bound to DBP and to a lesser extent to albumin, are distributed throughout the body [22, 28]. In contrast to vitamin D, which is mostly found in fat-tissue depots, 25(OH)D is more evenly distributed in the body (about 35% in fat tissue, 30% in blood, 20% in muscles, and 15% in other tissues) [22]. According to research conducted on submarine personnel, the half-life of 25(OH)D in circulation in the absence of cholecalciferol supplementation is about two months [29].

Fetal need for vitamin D is met by placental transfer of hydroxylated forms of vitamin D, i.e. 25(OH)D and 1,25(OH)2D [12]. At birth, human infants possess a small reserve of vitamin D in the form of 25(OH)D, which disappears during six to eight weeks of postnatal life [7].

ACTIVATION OF VITAMIN D

Since it is a biologically inert compound, in order to express its effect, vitamin D must go through two hydroxylation processes, the first in the hepatocyte microsomes and the second in the mitochondria of renal proximal tubular cells. The first hydroxylation is catalyzed by the 25-hydroxylase, resulting in 25(OH)D, the major circulating form of vitamin D, which is bound to DBP and to a lesser extent to albumin and distributed to all cells of the body. The second reaction is mediated by 1α-hydroxylase, which converts 25(OH)D to the biologically active hormone, 1,25(OH)2D. Apart from the kidney, 1α-hydroxylase is present in macrophages, monocytes and cells of the skeleton, teeth, breast, prostate, colon, pancreas, brain, adrenal glands, placenta and other tissues [1]. The half-life of 25(OH)D in circulation is about 15 days, while 1,25(OH)2D is inactivated in four hours [7, 30]. Therefore, serum 25(OH)D levels are used as a reliable indicator of vitamin D status in the body [7, 31]. Table 2 shows the criteria of the Institute of Medicine (IOM, since 2015 the National Academy of Medicine of the United States of America) regarding the level of 25(OH)D in serum and health [7]. Apart from the risk of a toxic effect of vitamin D, the IOM bases its recommendation regarding the upper reference value of the serum level of 25(OH)D on the absence of evidence to support that its level over 125 nmol/L results in additional health benefits [7]. The relevant associations of the Nordic and DACH countries, Australia and New Zealand, as well as the American Academy of Pediatrics and the Endocrine Society agree with the IOM guidelines regarding the lower limit of vitamin D adequacy based on serum 25(OH)D concentration, while the American Geriatrics Society, the International Foundation for Osteoporosis and some experts believe that it should be 75 nmol/L, respectively 100 nmol/L [30, 32, 33].

Table 2. Serum 25(OH)D concentrations and health [7]

nmol/L*	ng/mL*	Health status
< 30	< 12	Associated with vitamin D deficiency, which can lead to rickets in infants and children and osteomalacia in adults
30 to < 50	12 to < 20	Generally considered inadequate for bone and overall health in healthy individuals
≥ 50	≥ 20	Generally considered adequate for bone and overall health in healthy individuals
> 125	> 50	Linked to potential adverse effects, particularly at >150 nmol/L (> 60 ng/mL)

*One nmol/L = 0.4 ng/mL, and 1 ng/mL = 2.5 nmol/L

The activity of 1-alpha hydroxylase in the hydroxylation of 25(OH)D to 1,25(OH)2D in the kidneys is primarily stimulated by parathyroid hormone (PTH), but also by hypocalcemia, hypophosphatemia, growth hormone, sex hormones, prolactin, and low levels of serum 1,25(OH)2D, while the activity of this enzyme in extrarenal tissues is regulated by autochthonous factors, such as local growth factors, cytokines (gamma-interferon, tumor necrosis factor) and others [2, 5, 34]. A major suppressor of vitamin D activation is fibroblast growth factor-23 (FGF-23), a bone-derived hormone produced mainly by osteoblasts and osteocytes in response to increased extracellular phosphate and circulating 1,25(OH)2D [1, 35]. FGF-23 reduces renal 1,25(OH)2D synthesis by inhibiting the activity of 1α-hydroxylase, which transforms 25(OH)D into 1,25(OH)2D, and by stimulating 24-hydroxylase, which degrades both 25(OH)D and 1,25(OH)2D [1, 35, 36]. Therefore, adequate concentrations of 1,25(OH)2D in the body, in addition to regulating synthesis, are also achieved by controlling its inactivation. Inactivation of 1,25(OH)2D is carried out by hydroxylation at C24, not only in the kidneys, but also in the intestines, bones, cartilage, skin, prostate, placenta and other tissues, which results in the formation of inactive water-soluble products (calcitroic acid and 23-carboxylic derivatives) that are eliminated in bile and urine [2, 7, 22].

Due to efficient and unlimited intestinal absorption, high deposition in the body and extremely limited elimination, excessive oral intake of vitamin D leads to hypercalcemic heart rhythm disorders, the formation of renal stones, as well as soft tissue calcification and resultant renal and cardiovascular damage [7, 22, 37]. An identical problem occurs as part of a non-critically high parenteral administration of vitamin D. Likewise, excessive vitamin D intake during pregnancy, in addition to preterm birth, can cause excessive transplacental transfer of 25(OH)D and life-threatening hypercalcemia in the newborn [38].

PHYSIOLOGICAL EFFECTS OF VITAMIN D

The physiological effects of 1,25(OH)2D are mediated via the nuclear receptor (nVDR), i.e. by stimulating or inhibiting the transcription of more than 1000 human genes, and partly through membrane receptors (mVDRs) [1, 4, 13, 34, 39]. Both effects of 1,25(OH)2D work in synergy, although the genomic effect (i.e., mediated by nVDR) is much slower than the membrane effect [13]. By modulating gene expression, the synthesis of various proteins responsible for the classic (calcitropic) and non-classic (non-calcitropic) effects of vitamin D is regulated [1, 4, 35]. Membrane (non-genomic) effects of 1,25(OH)2D, also significant for cell function, are reflected in the regulation of cellular permeability to calcium and chlorine, as well as in raising the intracellular level of phospholipase C, cyclic guanosine monophosphate, protein kinase C and phosphoinositide metabolism [4, 34]. Through its main target tissues – the small intestine, kidneys and bones – vitamin D plays a key role in the regulation of calcium and phosphorus homeostasis [1, 4, 13]. In enterocytes and tubulocytes 1,25(OH)2D stimulates the synthesis of calcium channels, calbindin, Ca2+ATP-ase, 3Na+/Ca2+ ion exchanger and 2Na+/HPO42– cotransporter, thus enabling intestinal absorption and renal reabsorption of these ions and their transfer into the circulation [34]. The normal level of calcium and phosphorus in body fluids is of essential importance for numerous metabolic processes, neuromuscular function and mineralization of the skeleton and teeth [7]. In bone tissue, 1,25(OH)2D through nVDR and in cooperation with PTH induces the maturation of osteoclasts, which by remodeling bones release calcium and phosphorus into the circulation [1, 7, 34]. Although it represents, to a certain extent, a normal event, this process is particularly pronounced in conditions of insufficient intake, malabsorption or pathological loss of calcium [7]. In contrast, after the establishment of a normal serum calcium level, as well as during the period of growth and development, the genomic effect of vitamin D is primarily directed towards the maturation of osteoblasts and osteocytes [40]. In the renal tubule 1,25(OH)2D and PTH stimulate reabsorption of filtered calcium, thus contributing to the maintenance of its homeostasis [7]. Hence, the endocrine function of vitamin D is primarily aimed at increasing the absorption of calcium from food, while in conditions when this is not enough, at stimulating its renal reabsorption and mobilization from bones [1, 7].

In addition to calcitropic (classical), 1,25(OH)2D produced in the kidneys also has non-calcitropic (non-classical) effects, which are reflected in the modulation of T and B lymphocyte functions, reduction of renin expression, stimulation of insulin secretion and increased sensitivity of cells to its action, and participation in the control of the synthesis and release of thyrotropin hormone, atrial natriuretic peptide, osteocalcin and some other biomolecules [1, 5, 7, 11, 34]. The discovery that nVDR is present, not only in the cells of organs responsible for calcium and phosphorus metabolism but also in many other cells in the body, and that these cells contain the enzyme responsible for the hydroxylation of 25(OH)D into 1,25(OH)2D, as well as the 24-hydroxylase that deactivates it, led to the knowledge of autochthonously (locally) regulated non-classical, primarily non-calcitropic, effects of vitamin D [1, 34]. Optimal autocrine (intracrine) and paracrine production of 1,25(OH)2D, which is conditioned by the normal level of serum 25(OH)D, thanks to its antiproliferative, prodifferentiating, and proapoptotic effect, significantly reduces the risk of malignant alteration [1, 7]. In addition, the autocrine effects of vitamin D are reflected in anti-neoangiogenesis and differentiation of malignant cells, which slows down the spread of malignant tissue, as well as stimulating the function of macrophages/monocytes and other components of innate immunity [1, 5, 31].

The endocrine and autocrine-paracrine effects of vitamin D are fully expressed prenatally. In addition to ensuring the fetal needs in calcium and phosphorus, 1,25(OH)2D plays an important role in the development of the central nervous system, lungs, immunity and other systems [41, 42]. Hence, the optimal balance of vitamin D in a pregnant

woman is extremely important, not only for her health and the normal course of pregnancy, but also for the proper growth and development of the fetus [41, 42]. Covering the needs in vitamin D during lactation, due to the additional requirement in calcium and phosphorus, has an important role in the prevention of demineralization of the skeleton and teeth in nursing mothers [41, 43]. Apart from its essential role in proper prenatal and later growth and development, a normal balance of vitamin D is an extremely important factor in the prevention of various diseases, both in children and in adults and the elderly. This is supported by numerous epidemiological studies that, in addition to finding hypomineralization of bones and teeth, confirm an association between vitamin D deficiency and the appearance of some malignancies, especially those of the colon, prostate, breast, and ovaries, as well as autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, type 1 diabetes mellitus, and arterial hypertension, type 2 diabetes mellitus, and allergic, cardiovascular, and neuromuscular diseases [11, 13, 31, 39, 43]. In addition, vitamin D deficit during pregnancy, besides side effects on fetal development, carries the increased risk of gestational diabetes, pre-eclampsia, C-section, preterm delivery, postpartum depression, and other complications [1, 12, 41, 42, 43].

RECOMMENDED VITAMIN D INTAKES

Recommended dietary intakes of vitamin D for persons at risk for vitamin D deficiency, i.e. in the absence of optimal sun exposure, and upper-level intake according to the recommendation of the IOM are given in Table 3 [7]. The European Food Safety Authority fully agrees with these recommendations, as does the Endocrine Society (ES) [44, 45, 46]. Recommendations for dietary vitamin D intake for children and adolescents of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition, the American Academy of Pediatrics, and the Global Consensus on the Prevention of Nutritional Rickets are also in agreement with the IOM position [47, 48, 49]. The recommendations of other relevant associations are, to a greater or lesser extent, different [32, 50].

In contrast to the earlier position, the ES within the current Clinical Practice Guidelines on vitamin D recommends its routine supplementation for children and adolescents, pregnant women, adults with pre-diabetes and people older than 74 years, but not for healthy adults aged 19–74 years [31, 45, 46]. These recommendations do not apply to people whose physiology of vitamin D was disrupted due to health reasons or therapeutic procedures, as well as those who live in countries where food fortification with vitamin D is not standard, which must provide its entry in accordance with IOM dietary reference intakes [45]. Also, within the framework of vitamin D supplementation, intake of low daily doses is recommended, because high intermittent (weekly or monthly) application can lead to unwanted effects [45]. In addition, the ES within the previous recommendations, published in 2011, suggests that obese children and adults (BMI > 30 kg/m^2), due to the sequestration of vitamin D in adipose tissue, as well as patients taking anticonvulsant drugs, glucocorticoids, antifungals such as ketoconazole and AIDS drugs, due to the hypercatabolic effect on vitamin D, require at least two to three times higher vitamin D intake compared to the appropriate age group [31].

Table 3. Dietary reference intakes for vitamin D [7]

Life stage group (years)	Recommended dietary allowance (IU/day)	Upper-level intake (IU/day)
0–6 months	400*	1000
6–12 months	400*	1500
1–3 years	600	2500
4–8 years	600	3000
9–18 years	600	4000
19–70 years	600	4000
> 70 years	800	4000
Pregnant/nursing women	600	4000

*Adequate intake for infants is 400 IU/day for 0–12 months of age

CONCLUSION

Vitamin D is an essential component of numerous processes in the body and, accordingly, of adequate growth and development, as well as the preservation of health in all phases of life. It achieves its physiological effect in small and narrowly defined amounts. Therefore, its deficit and excess can lead to numerous, and in severe forms, very serious health problems. Cutaneous production, i.e. photolysis of 7-DHC under the influence of sunlight, is the main source of vitamin D, while food, excluding fish oil, marine fish, liver, egg yolks and milk formulas, contains little of it. In accordance with this, in the absence of optimal sun exposure, as well as insufficient intake of vitamin D in food, it is necessary to meet its need in the form of a supplement. Although the positions of the relevant international associations regarding optimal vitamin D intakes are not harmonized, the fact is that most of them fully or approximately align with the IOM recommendation from 2011.

Ethics: The authors declare that the article was written in accordance with ethical standards of the Serbian Archives of Medicine as well as ethical standards of medical facilities for each author involved.

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Витамин Д: свеобухватни преглед

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САЖЕТАК

Витамин Д (калциферол), односно његов активни метаболит калцитриол [1,25(*OH*)2*D*], осим есенцијалног учешћа у хомеостази калцијума и фосфора, битан је фактор у регулацији ћелијске пролиферације, диференцијације и апоптозе, ангиогенезе, имунске и хормонске активности и других процеса у људском организму. Отуда је његова оптимална равнотежа изузетно значајна за адекватан пренатални и постнатални раст и развој, као и за очување здравља у осталим фазама живота. У овом чланку дат је кратак преглед природног извора витамина Д, његовог метаболизма и физиолошке улоге, као и актуелних препорука у вези са обезбеђивањем његовог оптималног уноса.

Кључне речи: витамин Д; физиолошка улога; оптимални унос

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Public health aspects of vitamin D

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SUMMARY
In recent decades, the prevalence of known but insufficiently treated diseases and disorders has increased significantly. However, there is the manifestation of disorders whose causes were already known but were not sufficiently controlled. Public health problems are becoming more frequent and more current. Among the current public health problems, many symptoms and diseases are linked to certain vitamins, unhealthy lifestyles, and other contributing factors. Vitamin D is one of the current public health topics that has recently attracted increasing scientific attention. A biologically essential compound, vitamin D affects many functions in the human body, and the deficiency of this vitamin is widespread throughout the world. Vitamin D can be found in the form of dietary supplements, but it is also recognized as a registered medication in some contexts.
Keywords: disease prevention; public health; vitamin D

INTRODUCTION

The COVID-19 pandemic has highlighted numerous public health issues and significantly increased interest in preventive health measures, both at the personal and global level. This pandemic has prompted people to think about the importance of preventive measures in the occurrence of diseases [1]. One of the leading public health problems was vitamin D deficiency. It is a liposoluble, hydrophobic compound that can be found in two main forms: vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). The most common source of vitamin D is food of animal origin such as tuna, sardines, cod, and turkey. It is also found in plants, but it is scarcely bioavailable in these foods. We should not omit the fact that a large part of vitamin D is created endogenously through exposure to sunlight [2]. Vitamin D affects numerous organ systems, but lately its anti-cancer role and its great influence on the immune system have been highlighted. It also affects the cardiovascular, endocrine, and immune system, but it affects other organ systems as well, leading to a wide range of diseases. Moreover, numerous inflammatory bowel diseases, and some liver and lung diseases can also lead to a decrease in levels of vitamin D (Table 1) [3].

Table 1. General guidelines for preventing vitamin D deficiency and recommended daily intake [5]

Age (years)	Vitamin D recommended daily intake (IU)
< 1	≤ 400
1–18	≤ 1000
18–75	≤ 2000
> 75	≤ 4000

PUBLIC HEALTH REVIEW AND PUBLIC HEALTH PLANS AND RECOMMENDATIONS

Obesity is another condition associated with vitamin D deficiency, as shown by meta-analytical studies that included over 24,000 participants under the age of 18 [4]. These studies clearly indicate that deficiency in obese individuals is greater than in those who are optimally nourished [4]. The depot of vitamin D is mainly located in the liver, but it can also be stored in adipose tissue. This has critical implications, as achieving optimal vitamin D levels is more challenging in obese individuals due to its sequestration in fat stores [3]. It is important to point out that vitamin D_2 can be found in mushrooms, some plants, and yeast, while vitamin D_3 can be found in foods of animal origin and also via sun exposure [5]. All recommendations are based on a patient's age and vitamin D levels. The primary route of vitamin D elimination is via feces, along with loss through skin shedding [5]. When it was first reported that over 95% of children with rickets received vitamin D exclusively through breast milk, the American Pediatric Scientific Council recommended supplementation with vitamin D for newborns, infants, and children. Vitamin D has receptors in all cells in the body, but its best-known role is in the prevention of rickets [6]. (Sentence restructured below.) Dental caries represent an important public health problem. Studies have shown that insufficient vitamin D intake via breast milk, coupled with inadequate supplementation, can lead to degenerative changes in tooth enamel and other dental defects in children [8]. In a randomized controlled study conducted on school children,

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which lasted three years in Mongolia, the results clearly showed that supplementation with vitamin D in an adequate dose was effective in patients with a deficiency of this vitamin, but not effective in overall physical development of children [9]. A study conducted in China showed that vitamin D deficiency was more common in girls than in boys. However, the study included a pediatric population aged from birth to four years [10]. While vitamin D hypervitaminosis is rare, it is often caused by improper and inadequate use of this vitamin. In the United States, about three percent of people take more than the optimal dose of vitamin D, and it has been proven that there can be numerous consequences, namely the following: increased excretion of calcium in the urine, which can lead to nephrolithiasis, headache, nausea, and ataxia [11]. Some studies emphasize numerous advantages of supplementation with vitamin D_3 compared to vitamin D_2, as D_3 is more effective in achieving optimal serum levels and elicits a stronger biological response, which is obtained exclusively from dietary sources [12]. Vitamin D_3 is synthesized in the skin with sun exposure and has greater potency in activating vitamin D receptors [13]. A significant risk factor for the development of vitamin D deficiency is darker pigmentation of the skin, which filters sunlight and reduces the efficiency of vitamin D precursors in the skin [14]. Systematic meta-analytical studies support this, showing that children in Africa exhibit pronounced vitamin D deficiency and, consequently, a greater tendency to respiratory diseases such as asthma [15].

VITAMIN D DEFICIENCY AND INFECTIOUS DISEASES

Hospital-acquired infections caused by *Clostridioides difficile* have attracted the attention of the scientific public, especially in recent decades. It is known that only three antibiotics are effective against this bacterium. Recently, a new antibiotic from the group of macrolides, fidaxomicin, has demonstrated high efficacy. Retrospective four-year studies clearly show the severity of this infection in patients with comorbidities [16]. A randomized controlled trial further corroborates earlier findings, revealing that patients who received parenteral vitamin D_3 experienced significantly faster recovery. This outcome is attributed to cholecalciferol's dual role in modulating the immune system and positively influencing the intestinal microbiota [17]. The Epstein–Barr virus, which is a known and proven cause of sarcoma and cancer, also causes a major public health problem [18]. Vitamin D deficiency, as well as selenium deficiency, have a significant indirect effect on the occurrence of autoimmune diseases with comorbid Epstein–Barr virus infection [19]. Also, one of the most pressing public health problems in the last five years was the infection caused by the SARS-CoV-2 virus. An important role was played by liposoluble vitamins and their influence on cytokines, and a special role in an adequate immune response was played by vitamin D and vitamin E [20]. Vitamin E is the name for a group of compounds known as tocopherols. The most important biological function is played by alpha-tocopherol, which is responsible for preventing an exaggerated immune response [21].

INTERACTION OF VITAMIN D AND DRUGS

Vitamin D and its analogs have a strong synergistic effect with azacitidine and other anticancer drugs. Studies have shown that these compounds significantly enhance the efficacy of certain anticancer treatments compared to the effects of the drugs used independently [22]. Some drugs used in epilepsy, such as phenytoin, can induce cytochrome P450 and thereby accelerate the breakdown of vitamin D, and if used long-term can lead to severe deficiency of this vitamin. Therefore, patients should be closely monitored through regular check-ups to prevent the consequences of these effects [23]. Phenobarbital, carbamazepine, and primidone have a similar effect on vitamin D – these antiepileptic drugs also contribute to a decrease in vitamin D [24]. One of the strongest vitamin D antagonists that lowers serum vitamin D levels are corticosteroids. They impair vitamin D absorption in the intestines and stimulate the activity of hydroxylase enzymes, leading to a significant reduction in serum vitamin D levels [25].

CONSEQUENCES OF SELF-INITIATED VITAMIN D SUPPLEMENTATION

Limits for vitamin D deficiency (< 20 ng/ml) and insufficiency (20–30 ng/ml) to adequate levels of serum (30–80 ng/ml) are not completely scientifically based [26]. A randomized cohort study showed that taking vitamin D can result in severe hypercalcemia, but also that the optimal dose can prevent the occurrence of certain cancers [27]. Dexamethasone, a corticosteroid commonly used in various treatments, is a well-documented example of a drug that negatively impacts vitamin D metabolism [28]. Self-initiated intake of supplements and medicines, including vitamin D, can lead to serious health consequences if undertaken without consulting a healthcare professional. Both the dose and the duration of vitamin D supplementation can lead to serious consequences. A patient who used the prescribed therapy for an inadequate period of time subsequently developed severe complications, including vomiting, disorientation, and drowsiness. Laboratory analyses revealed hypercalcemia and acute kidney injury as a result of improper use [28].

Consequences of vitamin D hypovitaminosis

Apart from the key impact of vitamin D deficiency on the bone system, which results in osteopenia and osteoporosis, vitamin D has also been linked with diseases of nervous tissue, muscle, kidney, immune, and other organ systems [29]. Therefore, vitamin D deficiency is associated with the onset of malignant neoplasms of various types, hypertension, autoimmune diseases, type 2 diabetes, depression, and other diseases. The duration of vitamin D deficiency

also influences the disease course and impairment of health. A certain degree of deficiency and its duration are necessary for a disease to develop [30].

CONCLUSION

Preventing vitamin D deficiency requires a combination of proper dietary habits and proactive healthcare practices. Routine and timely visits to a physician are essential for early detection and prevention of hypovitaminosis. Routine medical check-ups can help identify symptoms of diseases associated with impaired vitamin D absorption, prompting targeted laboratory tests to assess serum vitamin D levels. Preventive and corrective measures for vitamin D deficiency can significantly reduce the risk of numerous associated diseases. Furthermore, it is crucial to consider the potential interactions between medications and vitamin D metabolism, as certain drugs can interfere with its absorption and function. Careful monitoring and adherence to professional guidance are vital to ensuring safe and effective vitamin D supplementation.

Ethics: This article was written in accordance with the ethical standards of the institutions and the journal.

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Јавноздравствени аспекти витамина Д

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САЖЕТАК

Последњих деценија све чешће се јављају познате али недовољно лечене болести и поремећаји. Такође, долази и до испољавања поремећаја чији су узроци одранније познати, али нису били довољно контролисани. Јавноздравствени проблеми постају све чешћи и значајнији. Међу њима, многи симптоми и болести повезани су са недостатком витамина, нездравим начином живота и другим узрочницима.

Витамин Д је једна од актуелних јавноздравствених тема која у последње време привлачи све већу научну пажњу. Ово биолошки важно једињење утиче на бројне функције у људском организму, а његов недостатак је чест у многим деловима света. Витамин Д се може наћи у облику суплемената, али може бити регистрован и као лек.

Кључне речи: превенција болести; јавно здравље; витамин Д

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HISTORY OF MEDICINE / ИСТОРИЈА МЕДИЦИНЕ

100 Years of the Clinic for Otorhinolaryngology and Maxillofacial Surgery at the University Clinical Center of Serbia (1924–2024)

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SUMMARY
The Clinic for Otorhinolaryngology and Maxillofacial Surgery at the University Clinical Center of Serbia celebrates its 100th anniversary in 2024. Established in 1924, the clinic has significantly contributed to the development of otorhinolaryngology in Serbia, keeping pace with European advancements. Its origins trace back to the early 1870s with Dr. Jovan Jovanović Zmaj, a physician who pioneered laryngoscopy in the country, and to Dr. Vladan Đorđević in 1871. Both were pioneers of laryngoscopy – Dr. Jovanović Zmaj in Novi Sad, and Dr. Đorđević in Belgrade. The clinic initially operated at the General State Hospital in Belgrade, before expanding and relocating multiple times. It has continually grown, introducing cutting-edge treatments and technologies, including endoscopic procedures, pediatric care, and advanced head and neck surgeries. Today, the clinic has 115 beds, six operating rooms, and specialized departments, offering comprehensive care in otorhinolaryngology and maxillofacial surgery. With a strong educational focus, it trains future medical professionals and continues to lead innovations in ENT and maxillofacial surgery. The clinic's strategic goals include modernizing equipment and expanding capacities, aiming to maintain its position as a leader in the field.
Keywords: otorhinolaryngology; maxillofacial surgery; medical history; Serbia; clinical education; health facility administration

INTRODUCTION

The origins of otorhinolaryngology date back to ancient Egypt, around 3500 BCE, where a physician is recorded to have "healed the king's nostrils" [1]. The first significant advancements in the field of otorhinolaryngology occurred in the 18th century, driven by an increasing understanding of anatomy, physiology, and pathology. Archaeological and textual sources examined by Bliquez indicate that specialized instruments and techniques for surgical procedures on the head and neck, such as cranial trepanation, already existed in Greco-Roman antiquity, and certain elements of these practices persisted into early medieval medical traditions [2]. The first surgical intervention in the field of ear, nose, and throat (ENT) was the catheterization of the Eustachian tube in 1724, followed shortly by the first attempts at mastoidectomy. Early in the 19th century, the first laryngoscopy was documented. The first university clinics specializing in otology and laryngology were established in Vienna in the year 1873 [3, 4]. With the merging of otology and rhinology in the early 20th century, the specialty of otorhinolaryngology was formed, giving rise to the medical branch as we know it today [3].

The development of Serbian otorhinolaryngology kept pace with European advancements, thanks to Dr. Jovan Jovanović Zmaj (1833–1904) [5], who, in addition to being a renowned writer, was one of the founders of otorhinolaryngology in Serbia. According to an advertisement first published in the newspaper "Zastava" on November 25, 1870, Dr. Jovan Jovanović Zmaj offered medical assistance, particularly for treating throat and larynx conditions, and promoted laryngoscopy, which he performed using a small mirror (Figure 1) [6]. Even today, indirect laryngoscopy remains a standard part of ENT examinations, performed using a small mirror. In the same period, Dr. Vladan Đorđević, who worked in the Principality of Serbia from 1871, applied laryngoscopy and rhinoscopy in his private practice and at the Military Hospital in Belgrade. By the end of 1872, he performed several ENT and maxillofacial surgeries, including nasal polyp removal, tonsillotomies, and excision of maxillary osteosarcoma and lower lip carcinoma. He also treated otitis, tympanic membrane perforation, deafness, congenital cleft lips, and laryngospasm with valerian tincture. In his notes, he described a rare case of curing esophageal stricture using bougies after nitric acid ingestion. Among major procedures, he highlighted the removal of cervical lymph nodes and neck phlegmon drainage [7].

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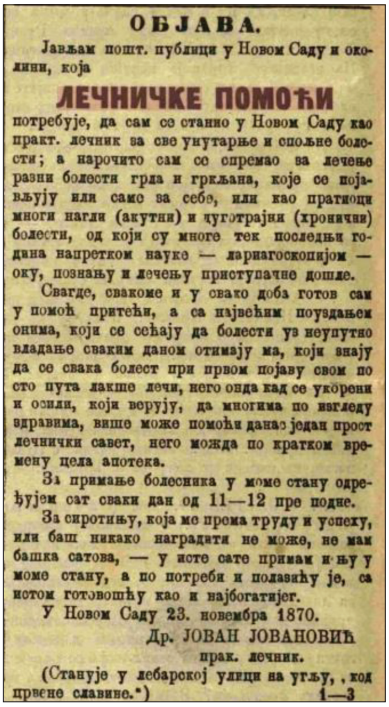

ОБЈАВА.

Јављам пошт. публици у Новом Саду и околини, која

ЛЕЧНИЧКЕ ПОМОЋИ

потребује, да сам се стално у Новом Саду као практ. лечник за све унутарње и спољне болести; а нарочито сам се спремао за лечење разни болести грла и гркљана, које се појављују или само за себе, или као пратиоци многи нагли (акутни) и чуготрајни (хронични) болести, од који су многе тек последњи година напретком науке — ларингоскопијом — оку, познању и лечењу приступачне дошле.

Свагде, свакоме и у свако доба готов сам у помоћ притећи, а са највећим поуздањем онима, који се сећају да болести уз неупутно владање сваким даном отимају ма, који знају да се свака болест при првом појаву свом по сто пута лакше лечи, него онда кад се укорени и осили, који верују, да многима по изгледу здравима, више може помоћи данас један прост лечнички савет, него можда по кратком времену цела апотека.

За примање болесника у моме стану одређујем сат сваки дан од 11—12 пре подне.

За сиротињу, која ме према труду и успеху, или баш никако наградити не може, не мам башка сатова, — у исте сате примам и њу у моме стану, а по потреби и полазим је, са истом готовошћу као и најбогатијег.

У Новом Саду 23. новембра 1870.

Др. ЈОВАН ЈОВАНОВИЋ
прак. лечник.

(Станује у лебарској улици на углу, , код црвене славине.*) 1—3

Figure 1. Advertisement of Dr. Jovan Jovanović Zmaj for medical practice after completing his medical studies; source: [6]

Figure 2. Current appearance of the building of the Serbian Medical Society, formerly the General State Hospital, at 19 George Washington Street; source: Korugić A, 2024, personal archive

DEVELOPMENT OF CLINIC FOR OTORHINOLARYNGOLOGY AND MAXILLOFACIAL SURGERY AT THE UNIVERSITY CLINICAL CENTER OF SERBIA

The first department for ear and throat diseases was opened in 1896 at the General State Hospital in Belgrade, which was established in 1881 and initially located at the site of the former Municipal Hospital in the Palilula district. The first generation of students enrolled in the ninth semester of the fifth year of studies in 1924 also attended courses in otorhinolaryngology. Following the decision made by the Faculty of Medicine in May 1924 to initiate teaching in the subject of otorhinolaryngology, work began on forming the corresponding clinic. The Clinic for ENT began operating shortly after the first lectures in otorhinolaryngology were held at the Faculty of Medicine, and by the end of December 1924, a substantial budget was approved for acquiring professional literature. The clinic was located on the premises of the Department of Ear, Nose, and Throat Diseases of the General State Hospital, 31 Vidinska Street – now George Washington Street – where the Serbian Medical Society is headquartered today (Figure 2).

The clinic had a lecture hall, a library, an outpatient unit, a waiting room, two patient rooms, and other auxiliary facilities. Due to the limited space available for teaching, professional, and scientific work, in 1927 a prefabricated wooden barrack with supporting walls, central heating, and a total of fourteen rooms was erected in the courtyard of the General State Hospital [8, 9]. Initially, the department had a capacity of 25 hospital beds, which was expanded to 35 beds by 1938 [10].

During the early years, over 400 surgeries were performed, including adenoidectomy, tonsillectomy, maxillary sinus surgery by Caldwell-Luc, nasal septum resection, radical mastoidectomy, antrotomy, juvenile fibroma surgery, and laryngofissure. By 1928, all classical ear surgeries were being performed, including surgeries for treatment of intracranial complications, surgeries of the paranasal sinuses, and some endonasal and endolaryngeal surgeries. Subsequently, neck surgery began to develop, with laryngectomies being performed, and in ear surgery, labyrinthectomies were carried out. Endoscopy also started to develop, but at that time, bronchoscopy and esophagoscopy were performed exclusively for the extraction of foreign bodies from the esophagus and lower respiratory tracts [8, 10].

In 1947, the Clinic moved to a building on Višegradska Street, expanding its capacity to 100 beds. The same year, the General State Hospital, the Clinical Hospital of the Faculty of Medicine, and the Infectious Diseases Hospital (now the Infectious Diseases Clinic) in Belgrade were merged as part of the creation of a unified clinical-hospital center during the post-World War II modernization of Serbia's healthcare system. This process was significantly accelerated after 1947 when various hospital functions were consolidated to improve the efficiency of medical services and the organization of medical education and research. Further expansion in 1955, including a pavilion previously used as a Midwifery School, increased the number of beds to 106, along with the modernization of operating rooms, increased endoscopic capabilities, and improvements to the outpatient units, pediatric department, library, and student exercise facilities [10].

Figure 3. The current building of the Clinic for Otorhinolaryngology and Maxillofacial Surgery on Pasterova Street; source: Arsović K, 2024, personal archive

After the formation of the Clinical Center, the Clinic relocated in 1983 from Višegradska Street to Pasterova Street, into the former building of the Otorhinolaryngology Clinic of the Military Hospital, which had been established in the year 1957 (Figure 3) [11, 12]. The outpatient section of the Clinic moved to a building on Deligradska Street, dating back to the Balkan Wars (built for the barracks of the Seventeenth Military Regiment), where the Military Academy was previously located until it was relocated to Banjica, leaving the Military Hospital behind [13]. At the time of its construction (1904–1909), this complex received high praise from domestic and foreign experts and was considered the most modern hospital in the Balkans. The building is now protected as a cultural heritage site bythe city of Belgrade [12].

The integration with the Rehabilitation Center of the Deaf Union of Yugoslavia in 1985 marked a step towards the formation of the Institute for Otorhinolaryngology and Maxillofacial Surgery of the Clinical Center of Serbia, which occurred in 1992.

IMPORTANT FIGURES

The first doctor to specialize in ear diseases in Serbia was Dr. Sigmund-Dragoljub Šraga (1866–1915), who was appointed as a medical assistant for ear diseases at the General State Hospital by the end of 1894. In 1896 he founded the Department for Ear and Throat Diseases at the General State Hospital and led it until 1906. The department was then headed by Dr. Milan Stefanović (1906–1912) and Dr. Ljubiša Vulović (1912–1924). After the department was reactivated following World War I, the Clinic for Ear, Nose, and Throat Diseases was established in 1924. Professor Dr. Ljubiša Vulović (1924–1945) was appointed as its part-time director after being elected as a lecturer at the Medical Faculty, thus continuing his leadership. Professor Vulović was the teacher of the first generations of Serbian otorhinolaryngologists, who laid the foundations of otorhinolaryngology in Serbia by establishing departments in Niš, Kragujevac, Novi Sad, and other cities [14, 15].

After World War I, there were very few otorhinolaryngologists left in Serbia, so by 1926, there were only 12 otorhinolaryngologists working in Serbia: Prof. Dr. Ljubiša Vulović, Dr. Bukus Alkalaj, Dr. Petar Zdravković, Dr. Sergej Popov, Dr. Nega Radojičić, Dr. Dobrivoje-Ćira Maksimović, Dr. Đoka Borisavljević, Dr. Isidor Vaskler, Dr. Pavle Abelcberg, Dr. Herc Kinstler, Dr. Josif Valčić, and Dr. Đorđe Mitrović [14].

Upon the retirement of Professor Vulović in 1945, Professor Milan Fotić was appointed director. In 1939, Professor Fotić performed the first total laryngectomy in Serbia [14]. In 1948, he founded the Otorhinolaryngology Section of the Serbian Medical Society [16]. He was unjustly retired in 1953 for political reasons, along with a group of professors from the Faculty of Medicine in Belgrade [14]. The development of the clinic continued through the generations. Professor Dr. Srećko Podvinec took over the leadership of the clinic in 1954, marking the beginning of its more intensive development. In 1963, the audiology, phoniatrics, and allergology departments were established [10].

From 1969 to 1978, the clinic was directed by Professor Dr. Dragoslav Savić, who received the Serbian Medical Society Award for scientific research and was also awarded the Order of Merit for the People. After him, the clinic was led by Professor Dr. Časlav Đoković from 1979 to 1983.

Professor Dr. Borivoje Krejović was the head of the Institute for ENT and MFS from 1983 to 2000, the first to promote functional reconstructive surgery for laryngeal cancers. After Professor Krejović retired in 2000, the Institute was led by Professor Dr. Gojko Stojičić. Professor Dr. Vojko Đukić managed the Institute for ENT and MFS from 2001 to 2013. The designation "Institute for ENT and MFS" was used until 2009, when it became the Clinic for Otorhinolaryngology and Maxillofacial Surgery.

Since 2013, the Clinic for ENT and MFS has been led by Professor Dr. Nenad Arsović, who continues to hold this position today [17].

STRUCTURE OF THE CLINIC

The Clinic for ENT and MFS is currently located in three buildings, and it employs 166 people. The main building, with four floors, contains six inpatient departments with 115 beds and six operating rooms.

The Endoscopy Department, with a day hospital, conducts laryngomicroscopic diagnostics and microsurgical endoscopic treatment of benign conditions and early malignant tumors of the larynx, esophagoscopy,

tracheobronchoscopy, removal of foreign bodies from the esophagus and lower airways, and laser interventions. The department is equipped with a modern microscope, laser, rigid endoscope, and flexible nasopharyngolaryngoscope.

The Pediatric Department, with intensive care, provides diagnosis and treatment of congenital anomalies, injuries, foreign bodies, inflammations, tumors, and other ENT and MFS conditions. The most common surgeries performed in pediatric patients are tonsillectomy, adenoidectomy, and the implantation of ventilation tubes in the ear. Esophagoscopy, tracheobronchoscopy, endoscopic sinus surgery, cochlear implantation, and surgical treatment of congenital anomalies are also performed.

The Intensive Care Department treats patients requiring constant medical supervision, continuous monitoring of vital parameters, and artificial ventilation. The department has 12 intensive care beds (level III) and six semi-intensive care beds.

The operating block consists of four operating rooms equipped with two surgical microscopes and a modern endoscopic surgery system. The rooms are equipped with a state-of-the-art navigation system for endoscopic sinus surgery as well as neuromonitoring equipment. The block also includes the Department of Anesthesiology, which is part of the Center for Anesthesiology at the University Clinical Center of Serbia (UKCS).

The Rhinology Department deals with congenital anomalies of the nose and septum, injuries and bleeding from the nose, nasal tumors, inflammations of the nose and paranasal sinuses, inflammations and tumors of the pharynx, and congenital anomalies, injuries, and inflammations of the larynx. The most frequently performed procedures include septoplasty, septorhinoplasty, conchoplasty, endoscopic and open sinus surgery, semi-amputations, and nasal amputations. The department has a total of 22 beds.

The Otology Department deals with congenital anomalies of the ear, injuries and inflammations of the external, middle, and inner ear, facial nerve function disorders, balance center disorders, and dizziness, as well as tumors of the external, middle, and inner ear. The surgeries performed include otoplasty, myringoplasty, tympanoplasty, mastoidectomy, middle ear exploration, ossiculoplasty, facial nerve decompression, stapedotomy, petrosectomy, cochlear implantation, as well as excision of ear tumors, semi-amputations, and auricular amputations. The Otology Department has 14 semi-intensive care beds and six general care beds.

The Maxillofacial Surgery Department deals with congenital anomalies and developmental deformities of the face and facial skeleton, injuries of the soft tissues of the face, oral cavity, and facial bones and jaws, inflammations of the soft tissues of the face, oral cavity, salivary glands, and dentogenic infections (abscesses and neck phlegmons). It also deals with surgical treatment of skin tumors of the face, oral cavity, salivary glands, facial bones and jaws, plastic and reconstructive surgery of the soft tissues and bones of the face, temporomandibular joint diseases, trigeminal neuralgia, and facial cosmetic surgery. The Maxillofacial Surgery Department has a total of 12 semi-intensive care beds.

The Oncology and Laryngology Department specializes in the diagnosis and therapy of benign and malignant tumors of the larynx, palliative care, and the treatment of oncological patients in the ENT region. The most frequently performed surgical procedures include tracheotomy, cordectomy, partial and total laryngectomies, neck dissections, lymph node extirpations, drainage of neck phlegmons and abscesses, and surgeries for tumors of the parapharyngeal space and congenital anomalies. The department has 20 semi-intensive care beds.

The clinic also has an emergency department that operates 24/7 to handle urgent ENT cases.

In the Polyclinic building, there are outpatient units for adults and children, an operating room for outpatient surgery, sections for audiovestibulology, phoniatrics, and rhinoallergology, a student exercise room, and an oncology consultation room. The third building is located at Slavija, housing the Center for Rehabilitation of Children with Hearing Impairments.

The Rhinoallergology Section specializes in the diagnosis and therapy of rhinological and immune-allergic pathological conditions of the upper respiratory tract and related regions. The Phoniatrics Section deals with the diagnosis, prevention, and treatment of all disorders of the spoken, sung, and professional voice, as well as all articulation disorders.

The Audiology and Vestibulology Section specializes in the diagnosis and treatment of all hearing and balance disorders. The Audiological Rehabilitation Section provides early rehabilitation for children with severe hearing impairments to facilitate hearing and speech rehabilitation and reduce psychomotor issues.

In 1954, Professor Srećko Podvinec and Professor Vladeta Popović founded the Tumor Council for Malignant Tumors of the Head and Neck, which is also the oldest council at the Faculty of Medicine. The council is still active today, comprising a multidisciplinary team of otorhinolaryngologists, maxillofacial surgeons, radiologists, oncologists, and pathologists.

Currently, the Clinic employs 24 ENT specialists, four maxillofacial surgery specialists, and one internal medicine specialist. As of August 2024, the Clinic also employs seven doctors specializing in otorhinolaryngology and maxillofacial surgery, and two clinical physicians. There are a total of 101 nurses and technicians, 73 with secondary education and 28 with higher education. Additionally, 12 healthcare associates and 16 non-medical workers are employed. The Clinic for ENT and MFS serves as a teaching base for the Department of Otorhinolaryngology and Maxillofacial Surgery, with three full professors, five associate professors, four assistant professors, and four clinical assistants. As part of the Program for Employment of the Most Successful Graduates of Medical Faculties and Secondary Medical Schools, sponsored by the Ministry of Health, the Clinic has employed the best students of the Faculty of Medicine in Belgrade since 2018. Through this program, 10 doctors have been employed at the Clinic to date [18].

Table 1. Report on the work of the Clinic for Otorhinolaryngology and Maxillofacial Surgery at the University Clinical Center of Serbia for the years 2013, 2018, 2022, and 2023

Parameter	2013	2018	2022	2023
Number of hospitalized patients	2887	3320	2914	3316
Number of beds	115	115	115	115
Number of hospital days	24,265	28,034	19,250	21,041
Average length of stay (days)	8.4	8.4	6.5	6.3
Average bed occupancy (%)	58	67	52	57
Number of outpatient examinations	60,459	64,663	60,302	69,167
Number of examinations per specialist	1840	2155	2412	2766
Number of surgeries	3341	4876	3664	3948
Number of surgeries per specialist	104	162	146	158

Figure 4. Reconstruction plan for the Clinic for Otorhinolaryngology and Maxillofacial Surgery at the University Clinical Center of Serbia; source: [19]

Based on Article 199 of the Law on Healthcare Protection and Article 18 of the Rulebook on Accreditation of Healthcare Institutions, Other Legal Entities, and Private Practice, a decision was made in December 2019 to accredit the Clinic for ENT and MFS at the UKCS for a period of seven years [14].

Table 1 presents statistical data on the work of the Clinic for ENT and MFS at the University Clinical Center of Serbia over four years as an illustration of the Clinic's capacity.

During the COVID-19 epidemic in Serbia from 2020 to 2023, the doctors and nurses of the Clinic for ENT and MFS were assigned to work in COVID departments within the UKCS and the COVID hospital in Batajnica after its opening. During those years, the Clinic operated in accordance with the decisions of the Ministry of Health, treating patients with ENT and MFS pathology who were not infected with the SARS-CoV-2 virus.

CONCLUSION

The Clinic's development strategy focuses on improving the current state through capacity expansion, equipment modernization, and quality service improvement. Through clearly defined goals that include space optimization, procurement of the most modern equipment, and implementation of enhanced procedures, we plan to achieve significant progress. The primary strategic goals are improving work quality parameters, patient satisfaction, staff motivation and education, the introduction of new technologies, and infrastructure development (Figure 4). We expect that this plan will bring visible benefits to patients and staff, thereby solidifying our position as a leader in providing top medical services in the fields of ENT and MFS.

Ethics: The authors declare that the article was written according to the ethical standards of the Serbian Archives of Medicine as well as ethical standards of institutions for each author involved.

Conflict of interest: None declared.

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Сто година Клинике за оториноларингологију и максилофацијалну хирургију Универзитетског клиничког центра Србије (1924–2024)

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САЖЕТАК

Клиника за оториноларингологију и максилофацијалну хирургију Универзитетског клиничког центра Србије обележава своју 100. годишњицу 2024. године. Основана 1924. године, Клиника је значајно допринела развоју оториноларингологије у Србији, пратећи европске трендове и напредак. Њени корени сежу до др Јована Јовановића Змаја почетком 1870. године, који је био пионир оториноларингологије у земљи, и др Владана Ђорђевића од 1871. године. Обојица су били пионири ларингоскопије, један у Новом Саду, други у Београду. Клиника је првобитно радила у Општој државној болници у Београду, пре него што се више пута проширивала и пресељавала. Током свог постојања константно је напредовала, уводећи најсавременије процедуре и технологије, укључујући ендоскопске процедуре, педијатријску негу и напредне операције главе и врата. Данас располаже са 115 кревета, шест операционих сала и специјализованим одељењима, пружајући свеобухватну негу у области оториноларингологије и максилофацијалне хирургије. С јаким фокусом на образовање, Клиника обучава будуће медицинске стручњаке и наставља да предводи иновације у оториноларингологији и максилофацијалној хирургији. Њени стратешки циљеви укључују модернизацију опреме и проширење капацитета, са циљем да задржи своју водећу позицију у овој области.

Кључне речи: оториноларингологија; максилофацијална хирургија; српска оториноларингологија; иновације; модернизација; образовање

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:
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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 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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