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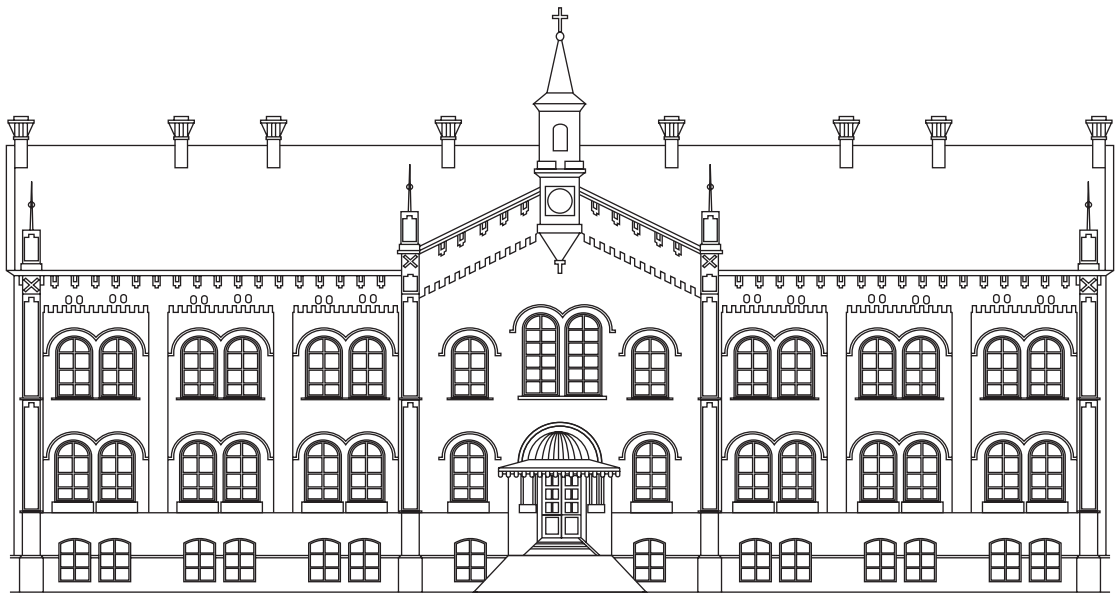

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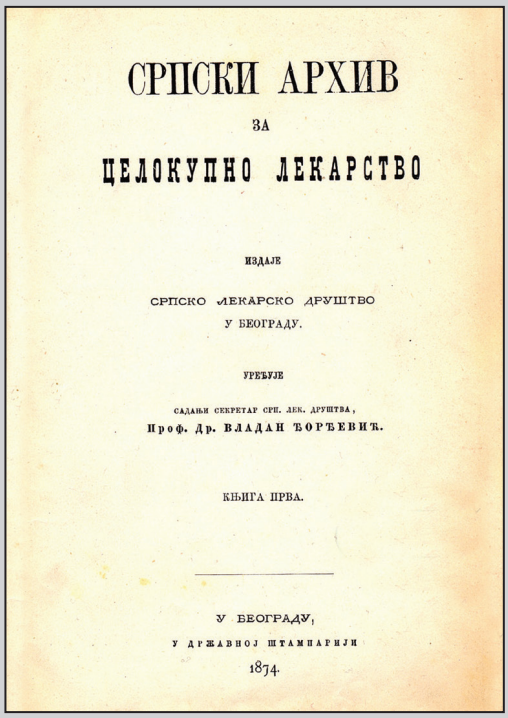

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ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

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У БЕОГРАДУ.

УРЕЂУЈЕ

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У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

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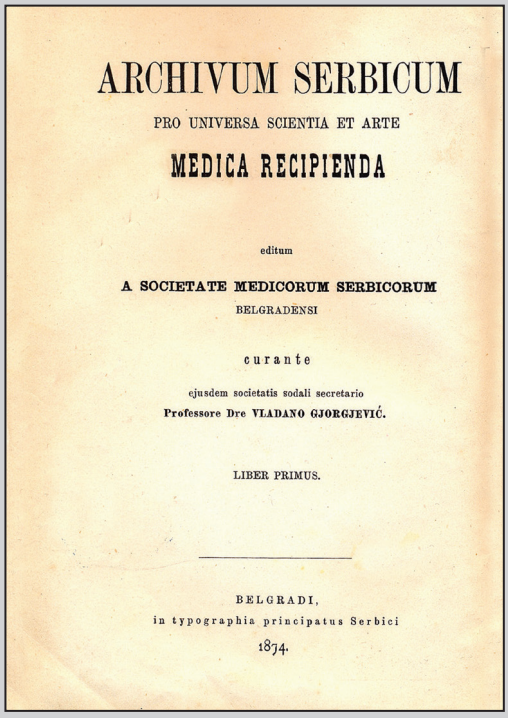

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1874.

The title page of the first journal volume in Latin

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The efficiency of canal cleaning with reciprocating movements instruments – SEM study

Slavoljub Živković, Jelena Nešković, Marijana Popović-Bajić, Milica Medojević-Jovanović

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SUMMARY

Introduction/Objective The application of nickel–titanium (NiTi) instruments in cleaning and shaping of the root canal system is a standard and a precondition for the success of endodontic treatment. The objective of this study was to use scanning electron microscopy (SEM) analysis in order to examine the efficiency of cleaning the apical third of the root canal system using two different NiTi systems with reciprocating movements.

Methods The study included 20 single-rooted teeth (premolars) divided into two groups. In group 1, the canal preparation was realized with a single file UNICONE with reciprocating movements (MEDIN, Inc., Nové Město na Moravě, Czech Republic), and in group 2, with a RECIPROC BLUE instrument with reciprocating movements (VDW GmbH, Munich, Germany). The same quantities of 2% NaOCl solution and 17% EDTA solution were used as irrigation solutions. The samples prepared for SEM analysis of the smear layer in the apical third were evaluated on a scale of 1–5 and at a magnification of 1,000×.

Results SEM analysis pointed to mostly clean canal walls in the apical segment in both tested groups. Slightly cleaner walls were observed after the application of the UNICONE file (78%) compared to the samples of the second group, where the instrumentation was realized by the RECIPROC BLUE file (76%), but without statistically significant differences.

Conclusion Single-file reciprocating systems do not remove the smear layer completely, but provide efficient cleaning of the apical region of the canal.

Keywords: cleaning; smear layer; reciprocating movements; SEM; NiTi instruments

INTRODUCTION

The preparation of the root canal system is one of the most important stages in endodontic therapy, and the application of nickel–titanium (NiTi) instruments in the cleaning and shaping of the canal is a standard and a prerequisite for the success of endodontic treatment [1]. Adequate root canal preparation increases the efficiency of irrigants and medicines and ensures satisfactory geometry and canal dimensions for better quality of the obturation [2].

Problems related to frequent fractures and deformations of NiTi rotating instruments during the preparation influenced the introduction of new systems and concepts of preparation that are based on changing the dynamics of movement and reducing the number of instruments necessary for adequate cleaning and shaping of the canal [2, 3, 4].

A system of preparation based on the application of NiTi instruments with reciprocating movements, and the fact that these systems are most commonly presented with only one instrument, showed new possibilities in the instrumentation [2, 5, 6, 7]. The reciprocating movements of the instrument (based on the technique of balanced forces) imply alternating rotation of the instrument in the direction of counterclockwise movement and much shorter movement in the clockwise direction, which significantly reduces torsional stress and cyclical fatigue, and thus the possibility of breakage of the instrument during preparation [2, 8, 9].

The research indicates that reciprocating movements do not diminish the cutting efficiency of the files and that the quality of the preparation depends primarily on the design of the working part of the instrument, the cross-section, the material from which it was made, and the special treatment of the alloy and the surface of the working part of the file [2, 3, 5, 10]. Cutting efficiency can be reduced only due to prolonged clinical use [11]. The studies also confirmed that the files with reciprocating movements are able to design both straight and curved canals equally well, thanks to the cross-section of the file, the M-Wire alloy, and the reciprocating kinematics [5, 12].

The concept of preparation by a single instrument significantly reduces preparation time, but also allows endodontists to devote more time to irrigation techniques in order to increase the efficiency of cleaning and disinfection of a complex canal system [3, 5, 13]. Endodontic practice confirms that the fundamental cleaning of the canal system is difficult to achieve and that the particular problem is the apical segment of the canal [2, 3, 5].

The data on the effects of files with reciprocating movements on the quality of cleaning and removing the smear layer from the canal walls

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are relatively scarce and mostly indicate similar findings with NiTi full rotation systems [10, 14, 15, 16].

The objective of this study was to use scanning electron microscopy (SEM) analysis in order to examine the efficiency of cleaning the apical third of the root canal system using two different NiTi systems with reciprocating movements.

The null hypothesis of this study is that there will be no significant differences in the amount of the smear layer in different reciprocating systems with a single file.

METHODS

The study included 20 single-rooted teeth (premolars) extracted due to periodontal problems. The study was realized with the permission of the Ethics Committee of the School of Dental Medicine in Belgrade, 36/6, January 10, 2013.

With all the teeth, after the formation of the access cavity on the occlusal surface of the teeth, a certain working length is determined (1 mm shorter than the length at which the top of the instrument appears at the apex). At the top of each root, a pink wax bead is placed in order for the preparation to be carried out under the conditions that are most closely related to the clinical situation. The teeth were then randomly divided into two groups (10 teeth).

In the first group, the canal preparation was realized with a single instrument Unicone with reciprocating movements (MEDIN, Inc., Nové Město na Moravě, Czech Republic), sizes 25/06. After examining the passage through the hand instrument (ISO 15), the canal was filled with 0.5 ml of a 2% NaOCl solution (Chloraxid 2%, PPH Cercamed, Stalowa Wola, Poland) and the instrument was placed at the working length (3–5 times) with gentle pulling movements. After extracting the instrument, the remaining amount of NaOCl solution (0.5 ml) was applied into the canal. Then, a 17% EDTA solution (Calcinase, EDTA solution, Lege Artis, Pharma GmbH, Dettenhausen, Germany) was placed into the canal and with the same movements (3–5 times) was further placed at the working length (1 ml). The final rinsing was done with additional 2 ml of 2% NaOCl solution. The preparation was carried out by Endo A Class endomotor (MEDIN, Inc.).

In the second group, the canal preparation was also realized with a single instrument RECIPROC Blue with reciprocating movements (VDW GmbH, Munich, Germany), sizes 25/08, in the same way as in the first group. The preparation in this group was realized by the VDW Silver endomotor (VDW Gmbh).

SEM analysis

After the completion of instrumentation, the dental crowns were cut in the cementoenamel junction, so that the remaining root length was 10 mm. The roots were then separated into halves (20 in each group) by a diamond disc and a chisel.

Only the apical third of the root (region of 3 mm from the boundary of the preparation) was selected for analysis, so that five standard microphotographs (a total of 200 images) were made for each sample on magnification of 1000× (JSM 6460LV; JEOL Ltd., Tokyo, Japan).

The presence of the smear layer in the apical third of the canal was evaluated according to the criteria of Hülsmann et al. [16]:

score 1 – no smear layer, dentine tubules are open;

score 2 – some smear layer, several tubules are open;

score 3 – a homogeneous smear layer covers the wall, a few tubules are open;

score 4 – the entire wall of the canal is covered with a smear layer, there are no open tubules;

score 5 – non-homogenous smear layer covers the entire wall of the canal.

The analysis and the scoring of the saved microphotographs were done by two independent researchers. In the event of disagreement, the discussion lasted until consensus was reached.

The clean wall of the canal included scores 1 and 2 and the wall with the present smear layer included scores 3, 4, and 5.

The results were processed in IBM SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA) and the descriptive statistics method and the χ^2 test were used in the statistical analysis.

RESULTS

The obtained results are shown in Tables 1, 2, and 3 and in Figures 1 and 2.

Table 1. The average values of the smear layer in the apical third of the canal

Instrument		Score of smear layer					
		n	$\bar{X}$	SD	Med.	Min.	Max.
Group	Unicone	100	1.88	0.89	2	1	4
	RECIPROC Blue	100	2.04	1.04	2	1	5
	Total	200	1.96	0.97	2	1	5

Table 2. Results of the smear layer in the apical third of the canal

Instrument			Score of smear layer					Total
			1	2	3	4	5	
Group	Unicone	n	40	38	16	6	0	100
		%	40	38	16	6	0	100
	RECIPROC Blue	n	34	42	14	6	4	100
		%	34	42	14	6	4	100
Total		n	74	80	30	12	4	200
		%	37	40	15	6	2	100

Table 3. Results of cleaning quality in the apical third of the canal

Instrument			Score of smear layer		Total
			Clean canal	Smeared	
Group	Unicone	N	78	22	100
		%	78	22	100
	RECIPROC Blue	N	76	24	100
		%	76	24	100
Total		N	154	46	200

SEM analysis of the canal walls showed that no reciprocating motion system provided complete cleaning in the apical third. The average values of the smear layer were

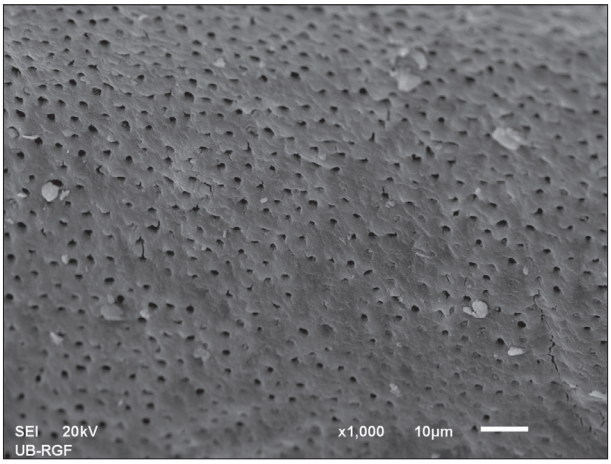

Figure 1. Microphotography of the apical third of the canal after preparation with UNICONE file, score 1, SEM ×1,000

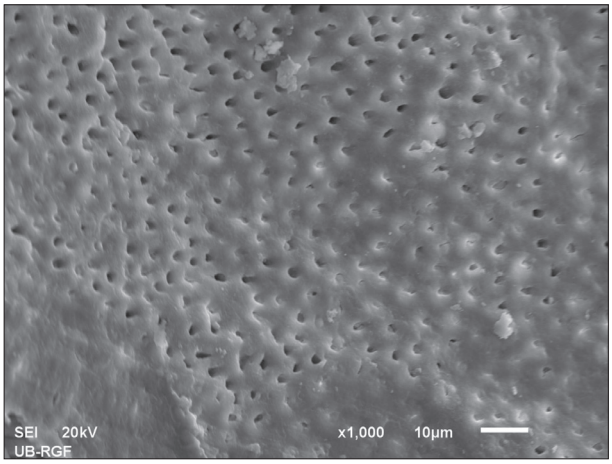

Figure 2. Microphotography of the apical third of the canal after preparation with RECIPROC Blue file, score 2, SEM ×1,000

similar and slightly lower in the first group, where the instrumentation was realized with the Unicone instrument (1.88), compared to the second group, where the RECIPROC Blue instrument (2.04) was used (Table 1).

The obtained results indicated somewhat cleaner walls of the apical part of the canal (scores 1 and 2) in the samples of the first group and after the application of the Unicone instrument (78%), compared to the samples of the second group and the instrumentation with the RECIPROC Blue file (76%) (Tables 2 and 3).

The presence of the smear layer in the first group (Unicone) was most often rated as score 1 (40%) (Figure 1), followed by 2 (38%), 3 (16%), and 4 (6%). No sample was rated as score 5 (Table 2). In the second group, (RECIPROC Blue), the presence of the smear layer was most often rated as score 2 (42%) (Figure 2), followed by 1 (34%), 3 (14%), 4 (6%), and 5 (4%).

DISCUSSION

An effective endodontic treatment involves the complete elimination of microorganisms from the canal system and the prevention of reinfection with adequate mechanical instrumentation, irrigation, and medication [13, 17]. Numerous studies confirm that efficient canal cleaning is difficult to achieve and that the quality of cleaning is reduced starting from the crown and the middle part of the canal according to the apical segment, primarily because of the inaccessibility and inadequate diameter of the apex preparation, i.e. the reduced effect of the irrigation solution [5, 14, 17, 18, 19].

The main objective of this study was to analyze the effects of two different systems with reciprocating movements on the quality of the cleaning of the apical part of the canal and examine the effects of individual files in the removal of the smear layer. The endodontic procedure was performed by a practitioner on simple canal systems, using the same amounts of irrigation solution (NaOCl and EDTA – which are considered to be the gold standards in chemomechanical canal preparation), and instruments with reciprocating movements (Unicone, RECIPROC Blue) were used according to the manufacturer's instructions and standardized irrigation procedure (the same amount and time) for each canal in both experimental groups [5, 14, 20].

The null hypothesis of this study was accepted, because the files with reciprocating movements produced similar amounts of the smear layer in the apical third of the canal in both tested groups.

In the literature, there are few studies that examined the efficiency of the files with reciprocating movements in removing the smear layer [3, 14, 21, 22, 23]. The results indicate that these files have similar cleaning effects to instruments with full rotation and cannot provide complete cleaning of the canal system [2, 3, 21, 23].

It was also confirmed that the efficiency of the cutting of files with reciprocating movements is not influenced by the dynamics of the movement of the instruments in the canal, but primarily by the design of the working part [2, 14, 22].

An attempt to align nearly all the parameters that may be of importance for the formation of the smear layer (the same diameter of the apex preparation – ISO 25, the same time and the amount of irrigants, the same irrigation technique) was made in this study, so that the decisive role in interpreting the obtained results was assigned precisely to the instruments used for the preparation of the canal.

The results of these studies pointed to a rather equable but somewhat lower average value of the smear layer in the apical part of the canal after the application of the UNICONE file. Primarily, this could be explained by the design of the working part of the instrument and by the fact that larger spaces between the blades in the instruments with reciprocating movements allow more efficient elimination of the dentin debris [2, 5, 21]. These findings coincide with the results of the research where the efficiency of cleaning of two systems with a single file, one with full rotation and one with reciprocating movements, was examined [3, 23]. The UNICONE reciprocating file with a specific design of the working part and a different helix angle that allows efficient cutting (triangular cross-section, thermal treatment of the alloy), exceptional flexibility, and improvement of the possibility of eliminating

dentine debris during the canal instrumentation was used in this study [2, 7, 8, 24].

The working part of the RECIPROC Blue file has an 'S' cross-sectional shape and three different horizontal cross-sections along the working part, which, along with an inactive top, increases the efficiency of cutting and debris removal. The instrument tracks the path of least resistance in the canal, so it is usually not necessary to create a canal passage by hand instruments [3, 22, 25, 26].

The study by Bürklein et al. [5] confirmed that continuous rotation instruments produce more smear layers and debris than systems with reciprocating movements, while SEM analysis by Poggio et al. [14] indicates that files with reciprocating movements cause the formation of larger amounts of the smear layer on the canal walls. A *well-packed* smear layer is explained by the dynamics of the instrument's movement in the canal, the reduced effect of the irrigation solution (shorter operating time for systems with one instrument), and the fact that the effect of cutting can be reduced by repeated clinical use [11, 14, 27].

The concept of canal preparation with reciprocating systems with a single instrument pointed to certain advantages (related to speed, safety, reduced fracture) in comparison with full-rotation systems with multiple instruments [2, 3, 5, 11], but also disadvantages that include shortened irrigation time, reduced efficiency of the chemical debridement of the canal system, and slightly more pronounced apical extrusion of dentin debris [3, 11, 26, 28].

Similar values and a little smear layer in the apical segment of the canal in the tested files with reciprocating movements, besides the design of the file, could be attributed to the enhanced activation of the irrigation solution. Actually, the dynamics of the movement of the instrument with reciprocating movements can increase the turbulence of the solution (regardless of the shorter operating time) and reduce the possibility of retaining the smear layer on the canal walls [2, 22, 28].

The studies confirm that reciprocating single-file systems provide fast and effective canal shaping with the preservation of original anatomy, significantly reduce the possibility of torsional fractures and ensure a fairly effective cleaning of the root canal system [2, 15, 27].

CONCLUSION

Within the limits of this study and based on the analyzed parameters, it can be concluded that NiTi single-file and reciprocating movement systems do not remove the smear layer completely, but provide effective cleaning of the apical third of the canal. A small amount of the smear layer in the apical third after the application of both instruments with reciprocating movements indicates their good cleaning possibilities with simple canal systems.

Conflict of interest: None declared.

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Ефикасност чишћења канала инструментима са реципрочним покретима – студија СЕМ

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САЖЕТАК

Увод/Циљ Примена инструмената од легуре никл–титанијум (*NiTi*) у чишћењу и обликовању канала је стандард и предуслов за успех ендодонтског третмана.

Циљ овог рада је био да се анализом скенирајућим електронским микроскопом (СЕМ) провери ефикасност чишћења апикалне трећине канала применом два различита *NiTi* система са реципрочним покретима.

Методе У истраживање је укључено 20 једнокорених зуба (преткутњака) подељених у две групе. Препарација канала у првој групи је реализована једном турпијом са реципрочним покретима *UNICONE* (*MEDIN*, Нове Мјесто у Моравској, Чешка), а у другој инструментом са реципрочним покретима *RECIPROC BLUE* (*VDW Gmbh*, Минхен, Немачка). Као раствори за иригацију коришћене су исте количине 2% раствора *NaOCl* и 17% раствор *EDTA*. Узорци припремљени за анализу СЕМ размазног слоја у апикалној трећини су еволуирани према скали 1–5 и на увеличању од 1000×.

Резултати Анализа СЕМ је указала на углавном чисте зидове канала у апексном сегменту у обе тестиране групе. Нешто чистији зидови уочени су после примене турпије *UNICON* (78%) у односу на узорке друге групе, где је инструментација реализована турпијом *RECIPROC BLUE* (76%), али без статистички значајних разлика.

Закључак Реципрочни системи са једном турпијом не уклањају потпуно размазни слој, али обезбеђују ефикасно чишћење апикалне регије канала.

Кључне речи: чишћење; размазни слој; реципрочни покрети; СЕМ; *NiTi* инструменти

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Evaluation of treatment outcome in patients with acute-on-chronic liver failure using clinical scores

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SUMMARY

Introduction/Objective Due to a very high mortality risk, acute-on-chronic liver failure (ACLF) patients require early identification and intensive treatment. Precise prediction is crucial for determining the urgency degree and therapy appropriateness, considering high mortality and multitude of clinical resources.
The aim of our study was to determine the exact cut-off values of various prognostic scores in the prediction of morality of ACLF.

Methods This prospective study includes chronic liver disease (CLD) patients, admitted due to decompensation, that were subsequently diagnosed with ACLF at the Emergency unit. All patients were evaluated based on various prognostic scores, including Child–Pugh, MELD Na, MELD, SOFA, APACHE II, and CLIF C, which were calculated on admission.

Results Alcoholic liver disease (ALD) was the most common underlying CLD cause (77.9%), followed by viral (8.6%), autoimmune (7.7%), and other causes (5.8%). A total of 37.5% of the patients died at the end of the first month of treatment. Average values of Child–Pugh, MELD Na, MELD, SOFA, APACHE II, and CLIF C scores were significantly higher in patients who died compared to survivors ($p < 0.05$). CLIF C score showed the best performance with a cut-off value of 50.5, with a sensitivity of 94.9% and specificity of 40%.

Conclusion ACLF remains a condition with a high short-term mortality. Of all of the scores examined in our study, CLIF C proved to be the best scoring system for predicting short term and end of treatment mortality in patients with ACLF.

Keywords: liver failure; ACLF; prognosis; mortality; scores

INTRODUCTION

Outcome of cirrhotic patients with acute decompensation (AD) is highly linked to possibility of developing acute-on-chronic liver failure (ACLF) [1, 2]. Introduced in recent years, ACLF is a relatively new term, with several definitions [1, 3, 4]. The joint American Association for the Study of Liver Disease and European Association for the Study of the Liver (AASLD/EASL) identifies ACLF as a syndrome with a high mortality rate, which includes the subgroup of cirrhotic patients who develop organ failure, with/without an identifiable precipitating event, such as variceal bleeding, acute alcoholism or infection [1, 5]. Researchers from the EASL – Chronic Liver Failure Consortium (CLIF) prospectively studied patients with chronic liver disease (CLD) and AD, and found that patients with AD who had organ failure and high 28-day mortality rates, could be diagnosed with ACLF [1, 2, 6, 7]. Due to a very high risk of mortality, ACLF patients require early identification and intensive treatment [7, 8, 9]. Mortality in patients with two organ failures goes up to 32%, and rises to approximately 80% if three or more organ systems fail [10]. In contrast, patients with no organ failure (no ACLF) have a 28-day mortality of approximately 5% [6, 10].

Numerous prognostic scores have been assessed for predicting outcome in ACLF patients [11, 12]. Acute physiology and chronic health evaluation II (APACHE II), Child–Pugh score (CP), model for end-stage liver disease (MELD), model for end-stage liver disease sodium (MELD Na), sequential organ failure assessment (SOFA), and chronic liver failure – sequential organ failure assessment (CLIF-SOFA) are most often used scores in clinical practice [2, 11, 12]. Namely, these scores were developed to assist in clinical decision-making, and should be improved continuously, in order to increase accuracy in outcome prediction of these patients [2, 12, 13]. Precise prediction is crucial for determining adequate therapy because of high mortality and multitude of clinical resources [2, 10]. Outcome prediction in ACLF is not only important for assessing survival in intensive care units, but also for evaluating which patients are in need of curative liver transplant. Furthermore, insufficient number of donor organs make accuracy even more important [2].

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The aim of our study was to determine the exact cut-off values of various prognostic scores in the prediction of morality of ACLF patients, and to define which of the score is the most reliable in determining ACLF patients' prognosis.

METHODS

This prospective study included CLD patients admitted due to AD and subsequently diagnosed with ACLF at the Emergency Unit, Department of Gastroenterology and Hepatology, Clinical Center of Serbia, Belgrade, Serbia, from January 1, 2015 to July 1, 2016. All patients had previously diagnosed CLD or cirrhosis. The diagnosis of CLD or cirrhosis was established either histologically when available, or with a combination of clinical and laboratory findings, and imaging [14]. AD included any of the following: presence of ascites, gastrointestinal (GI) bleeding, hepatic encephalopathy and/or acute bacterial infections [2]. Exclusion criteria were: absence of any CLD, presence of hepatocellular carcinoma, presence of severe chronic extra-hepatic disease, admission due to other chronic illness, human immunodeficiency virus infection, chronic decompensation of end-stage liver disease, less than 28 days of follow-up and incomplete data [15]. All the patients gave their written informed consent for inclusion in the study

This study protocol was done in accordance with the ethical principles of the Declaration of Helsinki and was approved by the institutional Committee on Ethics of the Clinical Center of Serbia (18.11.2014; 1393/9).

Acute-on-chronic liver failure definition and types

ACLF was defined according to the EASL-CLIF Consortium definition in accordance with the CLIF-SOFA organ failure score, as: liver failure: serum bilirubin ≥ 12 mg/dl; renal failure: serum creatinine ≥ 2 mg/dl; cerebral failure: grade III–IV hepatic encephalopathy (West Haven classification); coagulation failure: international normalized ratio ≥ 2.5; circulatory failure: use of vasoconstrictors to treat severe arterial hypotension (use of vasoconstriction for the treatment of type 1 hepatorenal syndrome in patients without severe hypotension not included); respiratory failure: $PaO_2/FiO_2 \geq 200$ or $SpO_2/FiO_2 \geq 214$ [16]. Renal dysfunction was diagnosed when serum creatinine ranged 1.5–1.9 mg/dl; cerebral dysfunction was diagnosed in patients with grade I or grade II hepatic encephalopathy.

Type 1 ACLF was defined by the presence of renal failure alone or by any other type of single system failure, if associated with renal dysfunction and/or cerebral dysfunction. Type II ACLF was defined by the presence of two and type III ACLF was defined by 3–6 organ failures [2, 14].

Patients' clinical and biochemical parameters

Alcoholic liver disease (ALD) was considered as the underlying CLD if there was a positive history of alcohol consumption of at least 50 g per day for the previous five years. Positive hepatitis B surface antigen (HBsAg) or anti-hepatitis C antibodies defined viral etiology. Autoimmune etiology including, autoimmune hepatitis, primary biliary cholangitis, and primary sclerosing cholangitis, was diagnosed using specific antibodies. The remaining study cases had liver cirrhosis of other etiology, including non-alcoholic steatohepatitis, Wilson's disease, α-1 antitrypsin deficiency, hemochromatosis and cryptogenic, and were thus classified as other. The following clinical variables were collected: age, sex, etiology of cirrhosis, blood pressure, mean arterial pressure, heart rate, body temperature, respiratory rate, SpO_2/FiO_2 ratio, neurological status (Glasgow coma scale).

All patients underwent laboratory evaluation at admission, and the following tests were performed: white blood cell count, platelet count, hematocrit, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, serum electrolyte levels, creatinine, international normalized ratio, prothrombin time, albumin, C-reactive protein, venous lactate, and total bilirubin [4].

Prognostic scores and follow-up

The patients were monitored until the end of the hospital treatment at our department, and up to 60 days after hospital discharge. To determine short-term mortality, day 28, or the day of lethal outcome was analyzed and patients were defined as either survivors or non-survivors based on in-hospital death within the follow-up period. Values of prognostic scores at admission were analyzed in correlation to the type of insult (GI bleeding versus non-GI bleeding) [2]. All patients were evaluated based on various prognostic scores, including CP, MELD Na, MELD, SOFA, APACHE II, and CLIF C, which were calculated at the time of admission by previously reported formulas [4, 16].

Statistical analysis

For normal variables, mean and standard deviations were calculated; χ^2 test and independent-sample t-test were used to assess the differences between the groups; p-values less then 0.05 were considered statistically significant. The performance of the MELD Na, MELD, SOFA, APACHE II, CLIF C, and CP score in predicting the 28-day mortality and outcome at the end of treatment was analyzed by calculating the area under the receiver operating characteristics (AUROC) curves. Based on the receiver operating characteristic (ROC) curves, the best cut-offs points were identified. Statistical analyses were performed using SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Clinical characteristics of patients

Demographic and clinical characteristics of the patients are shown in Table 1. A total of 104 patients were included in the study, with 74.1% being male. Mean age of the cohort

was 60.1 ± 9.9 years. ALD was the most common underlying CLD (77.9%), followed by viral (8.6%), autoimmune (7.7%), and other causes (5.8%). The acute insult for ACLF was GI bleeding in 29.8% of the patients. Upper endoscopy was performed in all the patients on admission, 29.8% had variceal bleeding, treated endoscopically and/or with appropriate vasoactive drugs. In patients where hemoglobin levels were below 70 g/l, blood transfusion was administered. Hypovolemic patients were given crystalloid solutions and albumin infusion.

Other non-bleeding insults such as infection, acute drug-induced liver injury, alcoholic hepatitis, reactivation of viral hepatitis, and acute liver vascular disease represented the remaining 70.2% of patients. Infection was identified through laboratory tests, urine analysis, and respective cultures. Third generation cephalosporins or fluoroquinolones were administered empirically, while respective antibiograms were obtained. Vasoactive medications were therapy of choice for 30 %patients. Enteral nutrition was administered in 50% of the patients. Acute drug induced liver injury was treated in 10% of the patients by supportive measures while they were waiting for liver transplant. A total of 37.5% of the patients died at the end of the first month of treatment, while 45% needed mechanical ventilation. By the end of the treatment, the percentage of lethal outcomes rose to 50%.

Prognostic scores

The average values of CP, MELD Na, MELD, SOFA, APACHE II score, and CLIF C used for prediction are shown in Table 1. Average values of CP, MELD Na, MELD, SOFA, APACHE II, and CLIF C scores according to the outcome at the end of the first month are summarized in Table 2. All average values were significantly higher in patients who died compared to survivors ($p < 0.05$). Based on this statistical significance we found the cut-off values of scores for predicting lethal outcome of patients with ACLF at the end of the first month (Tables 3 and 4 and Figure 1).

CLIF C score showed the best performance with a cut-off value of 50.5, which had a sensitivity of 94.9% and specificity of 40%. We also calculated the average values of the scores examined in relation to the outcome of patients with ACLF. As with outcome after one month, there were no statistically significant differences in mean scores investigated ($p > 0.05$). The average value of each individual score was higher in the group of patients who died compared to survivors (Table 5).Based on obtained statistical significance; we investigated the optimal values for predicting death in patients with ACLF. A ROC curve with respective AUROC was created for all scores (Table 6 and Figure 2). For the cut-off value of 49.5 CLIF C score, the sensitivity was 96.2% and specificity of 42.3%, which was the best predictive value relative to all other scores (Table 7). Average values of CP, MELD Na, MELD, SOFA, APACHE II, and CLIF C score depending on acute insults are shown in Table 8. There were no statistically significant differences between the average values of the investigated scores in relation to bleeding *vs.* non-bleeding insult ($p > 0.05$).

Table 1. Demographic and clinical characteristics of the patients (n = 104)

Sex	
Female	27 (25.9%)
Male	77 (74.1%)
Age	60.1 ± 9.9
Etiology of CLD	
ALD	81 (77.9%)
Viral	9 (8.6%)
Autoimmune	8 (7.7%)
Other	6 (5.8%)
Bleeding insult	31 (29.8%)
Non-bleeding insult	73 (70.2%)
Outcome at 28 days	
Lethal	39 (37.5%)
Survivors	65 (62.5%)
Outcome at the end of observation	
Lethal	52 (50%)
Survivors	52 (50%)
Score for Prediction	Mean value
Child–Pugh	11 ± 1.8
MELD Na	23.9 ± 6.5
MELD	21.3 ± 6.8
SOFA	9.5 ± 2.6
APACHE II	14.3 ± 4.2
CLIF C	55.8 ± 8.5

CLD – chronic liver disease; ALD – alcoholic liver disease; MELD Na – model for end-stage liver disease sodium; MELD – model for end stage liver disease; SOFA – sequential organ failure assessment; APACHE II – acute physiology and chronic health evaluation II; CLIF C – chronic liver failure consortium

Table 2. Average values of the investigated scores depending on the outcome at the end of the month

Score	Survived	Died	p-value
Child–Pugh	10.4 ± 1.6	12.1 ± 1.7	< 0.001
MELD Na	21.5 ± 5.5	27.8 ± 6.1	< 0.001
MELD	18.5 ± 5.2	26.1 ± 6.5	< 0.001
SOFA	8.9 ± 2.6	10.4 ± 2.2	0.004
APACHEII	12.9 ± 3.9	16.7 ± 3.4	< 0.001
CLIF C	51.7 ± 5.8	62.6 ± 7.8	< 0.001

MELD Na – model for end stage liver disease sodium; MELD – model for end-stage liver disease; SOFA – sequential organ failure assessment; APACHE II – acute physiology and chronic health evaluation II; CLIF C – chronic liver failure consortium

Table 3. Cut off values of scores in predicting lethal outcome of patients with ACLF at the end of the first month

Score	Cut-off	Sensitivity (%)	Specificity (%)
Child–Pugh	9.5	92.3	26.2
MELD Na	20.5	87.2	47.7
MELD	18.5	87.2	52.3
SOFA	8.5	84.6	44.6
APACHE II	12.5	92.3	46.2
CLIF C	50.5	94.9	40

MELD Na – model for end stage liver disease sodium; MELD – model for end-stage liver disease; SOFA – sequential organ failure assessment; APACHE II – acute physiology and chronic health evaluation II; CLIF C – chronic liver failure consortium

Table 4. Area under the receiver operating curve values for scores of other test scores

Score	AUROC
Child–Pugh	0.760
MELD Na	0.796
MELD	0.843
SOFA	0.714
APACHE II	0.778
CLIF C	0.867

AUROC – area under the receiver operating curve; MELD Na – model for end-stage liver disease sodium; MELD – model for end stage liver disease; SOFA – sequential organ failure assessment; APACHE II – acute physiology and chronic health evaluation II; CLIF C – chronic liver failure consortium

Table 5. The mean value of the scores examined in relation to the final outcome of patients

Score	Survived	Died	p-value
Child–Pugh	10.4 ± 1.6	11.7 ± 1.7	< 0.001
MELD Na	20.7 ± 4.4	27.1 ± 6.7	< 0.001
MELD	17.4 ± 3.5	25.2 ± 7.1	< 0.001
SOFA	8.8 ± 2.7	10.1 ± 2.2	< 0.001
APACHE II	12.7 ± 4.1	16 ± 3.6	< 0.001
CLIF C	50.6 ± 5.6	60.9 ± 7.7	< 0.001

MELD Na – model for end-stage liver disease sodium; MELD – model for end-stage liver disease; SOFA – sequential organ failure assessment; APACHE II – acute physiology and chronic health evaluation II; CLIF C – chronic liver failure consortium

Table 6. Area under the receiver operating curve values for prognosis scores

Score	AUROC
Child–Pugh	0.710
MELD Na	0.785
MELD	0.840
SOFA	0.691
APACHE II	0.744
CLIF C	0.859

AUROC – area under the receiver operating curve; MELD Na – model for end-stage liver disease sodium; MELD – model for end stage liver disease; SOFA – sequential organ failure assessment; APACHE II – acute physiology and chronic health evaluation II; CLIF C – chronic liver failure consortium

Table 7. Cut-off values, sensitivity, and specificity for predicting death in patients with ACLF

Score	Cut off	Sensitivity (%)	Specificity (%)
Child–Pugh	9.5	86.5	25
MELD Na	18.5	88.5	32.7
MELD	15.5	90.4	34.6
SOFA	7.5	86.5	26.9
APACHE II	11.5	92.3	38.5
CLIF C	49.5	96.2	42.3

ACLF – acute-on-chronic liver failure; MELD Na – model for end-stage liver disease sodium; MELD – model for end stage liver disease; SOFA – sequential organ failure assessment; APACHE II – acute physiology and chronic health evaluation II; CLIF C – chronic liver failure consortium

DISCUSSION

Prognosis in ACLF patients is influenced by the extent of acute injury and the degree of hepatic functional reserve. It is important to note that although ACLF represents a curable dynamic syndrome, it has a very unpredictable clinical course, which may improve or worsen in the span of 1–2 days or 2–4 weeks [2, 17].

Table 8. Average values of Child–Pugh, MELD Na, MELD, SOFA, APACHE II score and CLIF C according to bleeding vs. non-bleeding insult (n = 104).

Score	Bleeding insult	Non-bleeding insult	p-value
Child–Pugh	10.7 ± 2	11.2 ± 1.7	0.279
MELD Na	22.2 ± 5.9	24.6 ± 6.6	0.086
MELD	20.2 ± 6.6	21.8 ± 6.9	0.269
SOFA	9.1 ± 2.6	9.6 ± 2.6	0.351
APACHE II	14.0 ± 4	14.5 ± 4.3	0.634
CLIF C	55.9 ± 9.3	55.7 ± 8.2	0.943

MELD Na – model for end stage liver disease sodium; MELD – model for end-stage liver disease; SOFA – sequential organ failure assessment; APACHE II – acute physiology and chronic health evaluation II; CLIF C – chronic liver failure consortium

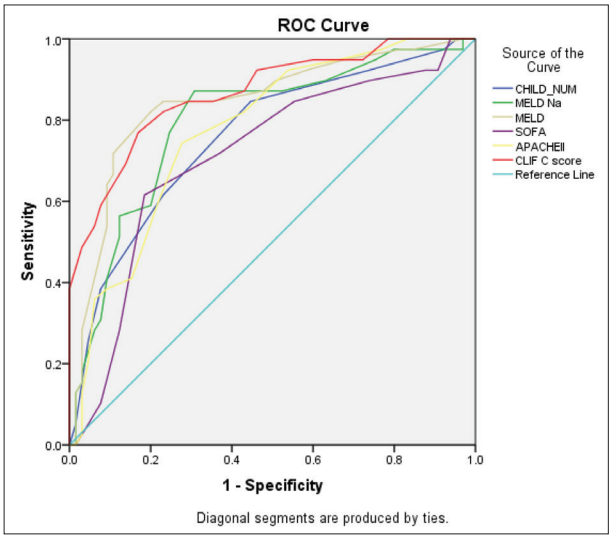

Figure 1. The area under the receiver operating characteristic curve for the following prognostic scores in patients with acute-on-chronic liver failure for prediction of lethal outcome at the end of the month

MELD Na – model for end stage liver disease sodium; MELD – model for end-stage liver disease; SOFA – sequential organ failure assessment; APACHE II – acute physiology and chronic health evaluation II; CLIF C – chronic liver failure consortium

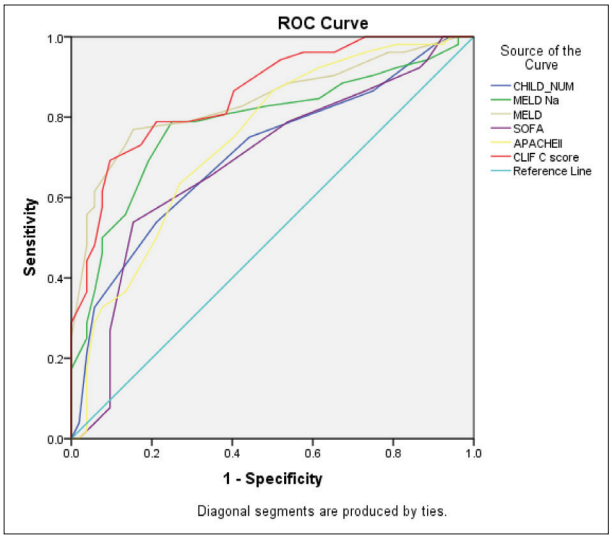

Figure 2. The area under the receiver operating characteristic curve for the following prognostic scores in patients with acute-on-chronic liver failure for prediction of lethal outcome at the end of treatment

MELD Na – model for end stage liver disease sodium; MELD – model for end-stage liver disease; SOFA – sequential organ failure assessment; APACHE II – acute physiology and chronic health evaluation II; CLIF C – chronic liver failure consortium

In our study, approximately a half of the patients had a lethal outcome and the 28-day mortality was 37.5%, indicating that ACLF patients have a very high short-term morality rate. Previous studies have also demonstrated that ACLF is a serious and challenging condition with a very high short-term mortality [2, 6–9]

The mean age in our cohort was 60.1 ± 9.9, which is similar to the previous study of Mikolašević et al. [7], but different from the study of Dhiman et al. [18], where the mean age was 46 ± 13 years. We can explain the differences by the large number of patients with ALD, where the onset of the disease was usually at an older age. Our cohort was predominantly of male sex, which is similar to studies of Dhiman et al. [18] and Amarapurkar et al. [19].

The most common cause of cirrhosis in our cohort was ALD (79.16%), which is consistent with study of Mikolašević et al. [7]. We also found that bleeding was the most common precipitating event, seen in 29.8% of our patients. Dominguez et al. [20] had similar rates of bleeding, while Dupont et al. [21] reported higher bleeding rates (47%). Furthermore, higher occurrence of bleeding as the precipitating event to ACLF was seen in patients with diagnosed hepatorenal syndrome [22].

Previous studies have compared different prognostic scores in order to determine which has the best predictive value [23]. Patients with lethal outcome had significantly higher values of all observed scores on admission compared to other patients. We strove to determine the optimal cut-off value for predicting 28-day mortality of each individual score, and to detect which score is the most reliable one in prediction of short-term mortality.

For the prediction of 28-day mortality, CP score had a cut-off of 9.5 with the sensitivity and specificity of 92.3% and 26.2%, respectively; while the AUROC was 0.760, which is similar to a study conducted by Mikolašević et al. [7], where AUROC for CP in the prediction of short term mortality was 0.707.

In our study, MELD score had a cut-off point of 18.5 with a sensitivity and specificity of 87.2% and 47.7%, respectively; with the AUROC 0.843 which was significantly higher than Mikolašević et al. [7], observed. Namely, AUROC for the MELD score in their study was 0.687. Moreover, a number of studies confirmed that MELD score is discrimination factor similar to SOFA and APACHE II [8].

For MELD-Na, the best cut-off value was 20.5, with a sensitivity and specificity of 87.2% and 47.7%, respectively. In our study, AUROC was 0.796. Mikolašević et al. [7] had an AUROC for MELD-Na of 0.687. MELD-Na score thus also proved to be just as good in predicting short-term mortality and mortality end of treatment.

SOFA score at a cut-off of eight had the sensitivity and specificity of 84.6% and 44.6%, respectively with an AUROC of 0.714. In the studies conducted by Mikolašević et al. [7],the AUROC for the SOFA score was 0.616, which was lower than our results. However, Lee et al. [9] reported AUROC of 0.876, which is higher than our results, in predicting short-term mortality. Moreover, Lee et al. [9] showed that CLIF-SOFA is good in predicting short-term mortality within the first four weeks of an acute episode. Silva et al. [8], showed that the SOFA score was less inferior in predicting 30-day mortality when compared to the MELD and CP score with AUROC values of 0.777, 0.829 and 0.793 respectively, which is similar to our results.

For the APACHE II score, the best cut-off value was 12.5 with a sensitivity and specificity of 92.3% and 46.2% and an AUROC of 0.778. The AUROC for the APACHE II score evidenced by Mikolašević et al. [7], was 0.878, while in studies conducted by Duseja et al. [24, 25], APACHE II score had the highest predictive value with an AUROC of 0.74, as compared to the MELD (AUROC 0.67), CP (AUROC 0.61) and SOFA scores (AUROC 0.65). Cholongitas et al. [26] estimated SOFA, APACHE II, MELD and CP scores and determined the best AUROC using SOFA (0.83), followed by MELD (0.81) and APACHE II (0.78), in the prediction of six week mortality. Better results in predicting mortality using the APACHE II score can be explained by the fact that in the APACHE II score included several physiological variables, thus encompassing more organ dysfunction values when calculated in contrast to other prognostic scores. Some studies imply that the APACHE II is the best predictive scoring system, owing to the fact that in ACLF the prognosis is determined by the degree of multiple organ dysfunction and not solely by the severity of liver failure [4]. Predicting end of treatment mortality with the APACHE II score was best achieved with a cut-off value of 11.5, with a sensitivity and specificity of 92.3% and 38.5% and an AUROC of 0.744.

CLIF-C score proved to be the best predictor of mortality with a cut-off value of 50.5, sensitivity of 94.9% and specificity of 40%, and an AUROC value of 0.867. Based on data from the CANONIC study, a prognostic score for specifically for ACLF evolved and was named the "CLIF CONSORTIUM score for ACLF" (CLIF-C ACLFs) [16]. This score is the result of combining "CLIF-Consortium Organ Failure score (CLIF C-OF) (designed for the diagnosis of ACLF), and two other independent predictors of mortality namely, age and white blood cell count [16]. Thus, the CLIF-C ACLFs score demonstrated a higher predictive accuracy than MELD, MELD-Na, and CP. The best cut-off value for predicting mortality at the end of treatment was of 49.5, with sensitivity of 96.2%, specificity of 42.3%, and an AUROC of 0.859.

Similar to other conducted studies, we did not found a significant difference between the average scores compared to the precipitating insult [11].

CONCLUSION

The results of our study showed that ACLF remains a condition with high short-term mortality. Of all of the scores examined in our study, CLIF-C proved to be the best scoring system for predicting short-term and end of treatment mortality in patients with ACLF.

Conflict of interest: None declared.

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Процена исхода лечења болесника са акутизацијом хроничне инсуфицијенције јетре применом клиничких скорова

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САЖЕТАК

Увод/Циљ Рана идентификација и интензивна терапија су неопходне код болесника са акутизацијом хроничне инсуфицијенције јетре (АХИЈ) због веома високог ризика од смртности. Прецизна предикција је пресудна за одређивање степена хитности и адекватност терапије с обзиром на морталитет и клиничке ресурсе.

Циљ наше студије био је да одредимо тачне граничне вредности различитих прогностичких скорова у предикцији морталитета од АХИЈ.

Методе Ова проспективна студија обухватила је болеснике са хроничном инсуфицијенцијом јетре (ХИЈ) хоспитализоване због декомпензације и касније дијагностиковане АХИЈ у јединици интензивне неге. Сви болесници су процењени према различитим прогностичким скоровима, укључујући *Чајлд–Пју*, *MELD Na*, *MELD*, *SOFA*, *APACHE II* и *CLIF C*, који су израчунати на пријему.

Резултати Алкохолна болест јетре била је најчешћи узрок ХИЈ (77,9%), затим вирусна (8,6%), аутоимуна (7,7%) и друга (5,8%). Укупно 37,5% болесника је умрло на крају првог месеца лечења. Просечне вредности *Child-Pugh*, *MELD Na*, *MELD*, *SOFA*, *APACHE II* и *CLIF C* су биле значајно веће код болесника који су умрли у односу на преживеле ($p < 0,05$). *CLIF C* скор је имао најбољи учинак са граничном вредношћу од 50,5, сензитивношћу 94,9% и специфичношћу од 40%.

Закључци АХИЈ представља стање са високом краткорочном смртношћу. Од свих скорова који су анализирани у нашој студији, *CLIF C* се показао као најбољи скор за предикцију крајњег морталитета болесника са АХИЈ.

Кључне речи: инсуфицијенција јетре; АХИЈ; прогноза; морталитет; скорови

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Comparison among different precursor prostate-specific antigen isoform derivatives on prostate cancer prediction in patients with serum prostate-specific antigen bellow 10 ng/ml

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SUMMARY

Introduction/Objective The precursor prostate-specific antigen (proPSA) especially its isoform p2PSA is useful in the detection of prostate cancer (PCa). However, the prediction value of different p2PSA derivatives remains unclear. The aim of the study was to compare the performance of the p2PSA, percentage of p2PSA to free PSA (%p2PSA), prostate health index (Phi), and one prostate dimension-adjusted index, p2PSA density (p2PSAD), with each other for PCa prediction in patients with serum PSA 10 ng/ml or less.

Methods This prospective study included patients who had undergone ultrasound-guided prostate biopsies and p2PSA testing. The data about patients' clinicopathological characteristics were collected and %p2PSA, p2PSAD and Phi were calculated. Different aspect of predictive performance was assessed using the area under the receiver operating characteristic curve (AUC), the specificities at set sensitivities, and clinical utility using decision curve analyses (DCA).

Results PCa was diagnosed in 23 (32.4%) out of 71 patients. Results of multivariate analysis showed that only the Phi and digital rectal examination were independent predictors of PCa. The AUC of p2PSA, %p2PSA, p2PSAD and Phi were 76.2%, 81.5%, 88.7%, 89.6%, respectively. At pre-specified sensitivity of 90% and 95%, Phi demonstrated a greater specificity than the other p2PSA derivatives. Phi and p2PSAD lead to the higher net benefit in DCA.

Conclusion Compared with other p2PSA derivatives Phi is the most useful parameter for selection of the patients that do not need to be undergone to biopsy and thereby avoiding unnecessary procedures.

Keywords: prostate cancer; p2PSA; prostate health index; early detection of cancer

INTRODUCTION

Prostate cancer (PCa) is the most common cancer among European men and the sixth primary cause of cancer-related mortality in men worldwide [1]. Prostate biopsy is the standard procedure for diagnosing PCa in men with elevated serum prostate-specific antigen (PSA) levels or abnormal findings on digital rectal examination (DRE). Testing men for PSA noticeably increases in the number of those undergoing prostate biopsy in the past decades. However, serum total PSA (tPSA) level itself, in the intermediate range, lacks the specificity, and can needlessly provoke avoidable treatment complications with prostate biopsy.

Continuous efforts are being made to discover novel PCa biomarkers or more complex prediction tools to decrease the number of unnecessary biopsies. Multiple PSA derivatives have been introduced as markers of early detection: age-specific PSA reference ranges, percentage of free PSA (%fPSA), PSA density (PSAD) [2]. Early evidence suggests that measurement of the PSA precursor isoform [-2]proPSA (p2PSA), which is predominantly expressed in malignant prostate tissue, and its derivatives (p2PSA/free PSA [%p2PSA] and prostate health index [Phi]), can offer improvement of PCa detection and management [3]. Phi is calculated by mathematical formula using total PSA, free PSA and [-2]proPSA. Large studies from around the world have consistently demonstrated that p2PSA derivatives both independently and in the models expressed by nomograms, artificial neural networks, or risk calculators adds to specificity and ensures a greater net benefit for PCa diagnostics than total and %fPSA [4–11]. Epstein criteria in predicting insignificant PCa cancer have improved prognostic performance by P2PSA derivatives in men capable for active surveillance [12]. Furthermore, p2PSA and its derivatives may correlate with pathologic cancer features after radical prostatectomy or discriminate whether PCa is clinically significant or indolent [4, 5, 9, 13, 14]. However, some studies did not demonstrate benefit for clinical decision-making and these complex prediction tools are not usually used in daily clinical practice [13]. To overcome this issue, a few other studies have been used prostate dimension-adjusted related indices such as p2PSA density (p2PSAD), %p2PSA density (%p2PSAD) and Phi density (PhiD) [14, 15]. In addition, the

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prediction value of different p2PSA derivatives for detecting PCa when compared to each other remains unclear.

The aim of our study was to compare the performance of the newest p2PSA-based markers including Phi, p2PSA-related indices (p2PSA, %p2PSA) and one prostate dimension-adjusted index (p2PSAD) with each other for PCa prediction in patients with serum PSA level below 10 ng/ml.

METHODS

Patient population

This prospective study was done at the Clinical Centre of Kragujevac, and it involved 71 patients, between May 2017 and December 2017, who had undergone ultrasound-guided prostate biopsies and p2PSA testing. After obtaining institutional Ethical committee approval (01/17/2608), we collected data about clinicopathological characteristics for each patient as follow: age, DRE, tPSA, %fPSA, transrectal ultrasonography (TRUS) findings, prostate volume (PV), PSAD, p2PSA, %p2PSA, p2PSAD, Phi, total number of cores taken, and Gleason score. All patients signed informed consent prior to examination. Exclusion criteria were incomplete data, serum PSA level above 10 ng/ml, and conditions that could alter the p2PSA concentration.

DRE were done on all examined patients. DRE was classified as normal, or suspicious/positive. Ultrasound examination as guidance for biopsy was performed using Toshiba Aplio 300 ultrasound device (United Medical Instruments, Inc., San Jose, CA, USA) with 5–10-MHz probe. After obtaining a median of ten core biopsies, it was assessed by local pathologists. TRUS was used to measure the gland in three dimensions, and the prostate ellipse formula was used to calculate PV. PSAD was calculated by dividing the serum PSA by PV. The primary outcome was the detection of PCa on biopsy.

Specimens and laboratory analysis

At presentation, blood samples were collected before DRE, TRUS or biopsy using standard techniques. Serum samples were obtained from blood and frozen at -70°C within eight hours for future analysis. All serum samples were thawed at the same time and tested for tPSA, free PSA and [-2] proPSA using UniCel DxI 600 Access Immunoassay System, Beckman Coulter, USA. %p2PSA was calculated using following formula: %p2PSA = p2PSA / (fPSA × 1000) × 100; p2PSA density was calculated as ratio of p2PSA level and PV; Phi was calculated using equation (p2PSA/fPSA) × √PSA.

Statistical Analysis

Descriptive statistics was used to characterize patients based on biopsy outcome. In order to identify and quantify potential and independent predictors of PCa, univariate and multivariate logistic regression analysis was performed. The results of regressions were presented in odds ratios (ORs) with 95% confidence interval (CI).

Comparison of different p2PSA-based markers

Cut-off value, area under the receiver operating characteristic curve (AUC) analysis, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, Hosmer–Lemeshow statistic, and the Brier score were calculated for each marker . The comparisons of AUC were performed using the method proposed by DeLong et al. [16]. We also compared the specificities of PHI at 90% and 95% sensitivities [17]. By using decision curve analyses (DCA), clinical usefulness was assessed [18]. Net benefit graph was calculated and made in Excel using the recommended formula [18]. All other calculations were performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $p < 0.05$.

RESULTS

Patients' characteristics

A total of 71 patients were analyzed. The study population included 61 (85.9%) initial biopsies, and 10 (14.1%) repeated biopsies. PCa was detected in 23 (32.4%) patients. Clinicopathological characteristics of patients with/without PCa included in the study are shown in the Table 1. There were no significant differences in TRUS findings between the positive and the negative biopsy groups. However, age, abnormal DRE, tPSA levels, PSAD, p2PSA, %P2PSA, p2PSAD and Phi were significantly higher in patients with PCa, while PV and %fPSA were significantly higher in the group of patients without PCa.

The logistic regression analysis

Univariate analysis revealed that six-reference standard tests/factors displayed significant correlation with PCa (Table 2). Also, at univariate analyses, p2PSA, %p2PSA, p2PSAD and Phi were significant predictors of PCa. During multivariable analysis, DRE and Phi have independent prognostic value of PCa (Table 2).

Performance measure of different p2PSA-based markers

Performance measures of different p2PSA-based markers are summarized in Table 3. AUCs of p2PSA, %p2PSA, p2PSAD and Phi were 76.2%, 81.5%, 88.7%, 89.6%, respectively (Table 3 and Figure 1). P2PSAD and Phi significantly outperformed p2PSA and %p2PSA as judged by AUC. In pairwise comparison of ROC curves, differences between areas Phi and p2PSA and %p2PSA (13.4% and 8.1%, respectively) were significant ($p = 0.003$ and $p = 0.025$). The difference between the AUC of Phi and p2PSAD was not statistically significant ($p = 0.081$). The sensitivity of the test, PPV, NPV was the most optimal using Phi, while the

Table 1. Baseline patients' clinicopathological characteristics

Characteristics	All (n = 71)	BPH (n = 48)	PCa (n = 23)	p-value	LG PCa (n = 13)	HG PCa (n = 10)
Age mean ± SD, years	64.3 ± 5.4	63.4 ± 5.3	66.2 ± 5.3	0.041	64.1 ± 5.7	63.7 ± 5
DRE abnormal n (%)	20 (28.2)	5 (10.4)	15 (65.2)	0.000	8 (61.5)	7 (70)
Total PSA median (IQR) ng/ml	5 (3.7)	4.4 (2.8)	7.1 (3)	0.012	4.8 (3.6)	7.4 (2.5)
%fPSA mean ± SD	19.2 ± 7.6	20.9 ± 7.8	15.7 ± 5.8	0.007	17.1 ± 9.5	12.4 ± 6.4
TRUS findings n (%)	33 (46.5)	19 (39.6)	14 (60.9)	0.128	6 (46.2)	8 (80)
Prostate volume median (IQR), ml	50 (24)	55 (25.2)	45 (19)	0.004	52 (23.2)	39.5 (13.7)
PSAD median (IQR), ng/ml/ml	9.4 (6.5)	8 (4.1)	14.6 (8.4)	< 0.001	8.5 (5.7)	16 (5.6)
p2PSA median (IQR), pg/ml	14.3 (11.7)	12.5 (9)	19.6 (13.5)	< 0.001	13.7 (10.9)	22.6 (16.2)
%p2PSA median (IQR)	14.6 (7)	13.5 (5.5)	23.8 (13.7)	< 0.001	16.9 (16.8)	25.1 (8.2)
p2PSA density median (IQR) pg/ml/ml	0.26 (0.22)	0.23 (0.13)	0.5 (0.37)	< 0.001	0.5 (0.46)	0.49 (0.29)
Phi median (IQR)	37.1 (24.9)	29.1 (13.2)	54.2 (31.2)	< 0.001	49 (26.4)	65.7 (19.8)
Number of biopsy cores median (IQR)	10 (0)	10 (0)	10 (0)	0.006	10 (1)	10 (0.5)

BPH – benign prostatic hyperplasia; DRE – digital rectal examination; HG – high grade Gleason score ≥ 7; IQR – interquartile range; LG – low grade Gleason score ≤ 6; PCa – prostate cancer; Phi – prostate health index; PSA – prostate-specific antigen; PSAD – prostate-specific antigen density; p2PSA – precursor prostate-specific antigen isoform; SD – standard deviation; TRUS – transrectal ultrasound; %fPSA – percentage of free prostate-specific antigen; %p2PSA – percentage of precursor prostate-specific antigen isoform to free prostate-specific antigen

Table 2. The logistic regression analysis of predictors for prostate cancer

Variables	Univariate analysis OR (95% CI)	p-value	Multivariable analysis OR (95% CI)	p-value
Age	1.105 (1.001–1.220)	0.048		
DRE	16.125 (4.562–56.990)	< 0.001	9.432 (1.728–51.492)	0.010
tPSA	1.409 (1.084–1.832)	0.010		
%fPSA	0.895 (0.823–0.974)	0.011		
Prostate volume	0.963 (0.934–0.994)	0.018		
PSAD	1.241 (1.106–1.393)	< 0.001		
p2PSA	1.132 (1.052–1.218)	0.001		
%p2PSA	1.002 (1.001–1.004)	< 0.001		
p2PSAD	1.143 (1.068–1.224)	< 0.001		
Phi	1.130 (1.068–1.195)	< 0.001	1.084 (1.010–1.163)	0.024

DRE – digital rectal examination; CI – confidential interval; OR – odds ratio; Phi – prostate health index; PSAD – prostate-specific antigen density; p2PSA – precursor prostate-specific antigen isoform; p2PSAD – precursor prostate-specific antigen isoform density; tPSA – total prostate-specific antigen; %fPSA – percentage of free prostate-specific antigen; %p2PSA – percentage of precursor prostate-specific antigen isoform to free prostate-specific antigen

predictive accuracy was improved for about 10% (Table 3). The Hosmer–Lemeshow goodness of fit test statistic did not reach statistical significance, thereby demonstrating a good fit. The Brier's scores ranged from a low of 0.112 for the Phi, the best predictive performance, to a high of 0.179 for the p2PSA.

The specificity of serum markers at set sensitivities of 90% and 95% are shown in Table 4. At pre-specified sensitivity of 90% and 95%, Phi demonstrated a greater specificity than the other p2PSA derivatives. For instance, if sensitivity is set at 95%, the specificity of Phi was 66.7% compared to 35.4% for p2PSAD, 31.2 for %p2PSA and 25% for p2PSA. Furthermore, for example, using a Phi cut-off of 31 (95% sensitivity cut-off), 5% of PCa would have been missed and 47% of men with benign disease would not have been undergone to a biopsy. For comparison, 19–26% would have been spared using other markers. Thus, an additional 21–28% of patients could avoid biopsy using Phi, compared to other markers.

Clinical usefulness

Figure 2 shows the results of the DCA. The main assumption of biopsy is that if all patients are undergoing biopsy, it spares them from an unfavorable outcome. DCA suggested that all p2PSA derivatives are likely to be useful for patients whose decision to pursue further intervention is based on a predicted risk above 6–25%. However, Phi (orange line) and p2PSAD (purple line) lead to the higher net benefit compared with p2PSA (blue line), and %p2PSA (green line) in various threshold probabilities above approximately 6% and 10%. For example, if a probability threshold is set at 15%, the use of the Phi and p2PSAD decreases the number of unnecessary biopsies by 26 and nine per 100 patients, respectively, without missing any of PCa. However, their curves are largely overlapping in different threshold probabilities.

DISCUSSION

In the present study, we compared the performance of the newest p2PSA-based markers (p2PSA, %p2PSA, p2PSAD and Phi) to each other for PCa prediction. Our study findings confirmed that Phi is the strongest discriminative parameter between patients with and without PCa at the initial or repeated biopsy, with the PSA value bellow 10 ng/ml. Almost all statistical metrics have demonstrated improved diagnostic performance when Phi was compared with other markers. These findings were further confirmed when we compared the specificities at pre-specified sensitivities and an additional 21–28% of biopsies could be avoided. However, the results of the DCA analysis did not confirm the advantage of the Phi compared with the p2PSAD.

Table 3. Predictive performance of different precursor prostate-specific antigen isoform derivatives

Efficacy measure	p2PSA derivatives			
	p2PSA	%p2PSA	p2PSAD	Phi
Cut-off	> 12.74	> 16.9	> 0.29	> 43.7
AUC (95% CI)	76.2 (64.6–87.8)	81.5 (70.2–92.8)	88.7 (79.6–97.8)	89.6 (81.7–97.4)
Sensitivity (95% CI)	43.5 (23.2–65.5)	52.2 (30.6–73.2)	56.5 (34.5–76.8)	69.6 (47.1–86.8)
Specificity (95% CI)	91.7 (80–97.7)	93.7 (82.8–98.7)	93.7 (82.8–98.7)	93.7 (82.8–98.7)
PPV (95% CI)	71.4 (41.9–91.6)	80 (51.9–95.7)	81.2 (54.4–95.9)	84.2 (60.4–96.6)
NPV (95% CI)	77.2 (64.2–87.3)	80.4 (67.6–89.8)	81.8 (69.1–90.9)	86.5 (74.2–84.4)
Accuracy (95% CI)	76.1 (64.5–85.4)	80.3 (69.1–88.8)	81.7 (70.7–89.9)	85.9 (75.6–93)
HL test, χ^2, p-value	7.313, 0.503	11.945, 0.154	10.127, 0.256	6.503, 0.591
Brier score	0.179	0.143	0.119	0.112

AUC – area under the receiver operating characteristic curve; CI – confidential interval; HL – Hosmer-Lemeshow; NPV – negative predictive value; Phi – prostate health index; PPV – positive predictive value; p2PSA – precursor prostate-specific antigen isoform; p2PSAD – precursor prostate-specific antigen isoform density; %p2PSA – percentage of precursor prostate-specific antigen isoform to free prostate-specific antigen

Table 4. The specificity for precursor prostate-specific antigen isoform and its derivatives at prespecified sensitivity of 90%, and 95%

p2PSA					%p2PSA				p2PSAD				Phi			
Sensitivity (%)	Specificity (95% CI)a	cutoff	Biopsy spread (%)	Missed (%)	Specificity (95% CI)a	cutoff	Biopsy spread (%)	Missed (%)	Specificity (95% CI)a	cutoff	Biopsy spread (%)	Missed (%)	Specificity (95% CI)a	cut-off	Biopsy spread (%)	Missed (%)
90	52.1 (18.7–72.9)	>12.7	38	10	35.4 (16.7–58.3)	>12.7	27	10	47.9 (22.9–93.7)	> 0.22	37	10	66.7 (22.9–81.2)	> 32	48	10
95	25 (8.3–54.2)	> 8.7	19	5	31.2 (14.4–47.9)	>12.5	23	5	35.4 (16.7–87.5)	> 0.16	26	5	66.7 (25–84.4)	> 31.6	47	5

Phi – prostate health index; p2PSA – precursor prostate-specific antigen isoform; p2PSAD – precursor prostate-specific antigen isoform density; %p2PSA – percentage of precursor prostate-specific antigen isoform to free prostate-specific antigen

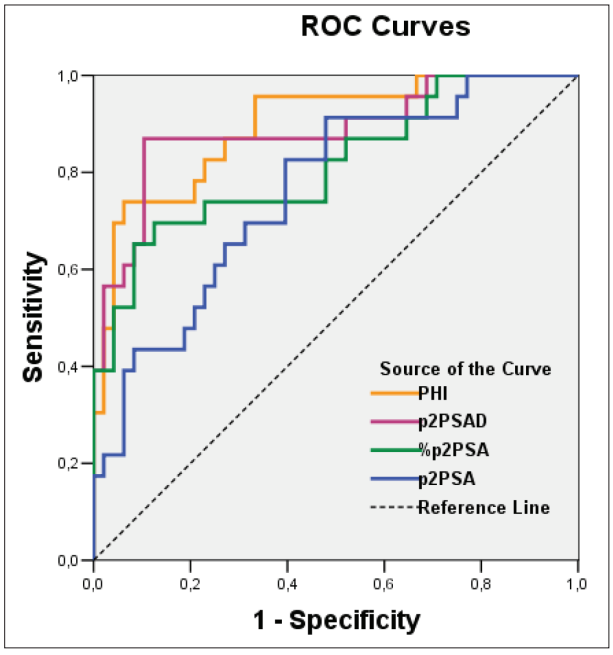

Figure 1. ROC curve analysis

Phi – prostate health index; p2PSA – precursor prostate-specific antigen isoform; p2PSAD – precursor prostate-specific antigen isoform density; %p2PSA – percentage of precursor prostate-specific antigen isoform to free prostate-specific antigen

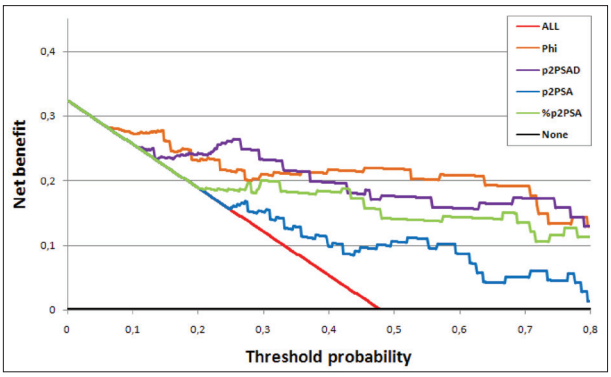

Figure 2. Decision curve analysis of the effect of p2PSA and its derivatives on the detection of prostate cancer

Phi – prostate health index; p2PSA – precursor prostate-specific antigen isoform; p2PSAD – precursor prostate-specific antigen isoform density; %p2PSA – percentage of precursor prostate-specific antigen isoform to free prostate-specific antigen

Previous studies have determined factors related with higher risk of PCa detection in patients with PSA bellow 10 ng/ml. They included age [6, 8, 9, 13], race [4], DRE [8, 9, 11], tPSA [9, 11, 15], %fPSA [4, 9, 14, 15, 19], PV [4, 5, 8, 9, 11, 14], PSAD [14, 15], biopsy history [4, 5, 8, 11], family history [4], p2PSA [4, 15], %p2PSA [9, 15], p2PSAD [15], Phi [4, 5, 8, 9, 15], and PhiD [14]. A broad variety of different combinations of predictive factors has been identified. Like in previous studies, several of those predicting

factors have shown statistical significance in the univariate or multivariate analysis in our study. Nevertheless, some of these parameters did not have value as independent factors. According to the analysis, we found that DRE status and Phi were strong independent predictors of PCa detection. We have included the patients with positive DRE as has been done in other studies [8, 9]. Our prospective study reinforces the evidence that serum isoform p2PSA and its derivatives, particularly Phi, could be useful for discriminating between patients with or without PCa [5, 6, 11, 14].

Unlike other p2PSA derivatives, Phi is considered a three-component marker. The Phi test is a better tool for clinically significant PCa identification, than its individual components [5]. A systematic review by Pecoraro et al. [20] that included 17 studies with 6,912 patients on Phi concluded that Phi increases the specificity for PCa detection [20]. For p2PSA the authors reported AUC ranging from 0.51 to 0.62, highlighting a better performance for %p2PSA (AUC from 0.63 to 0.78) and Phi (AUC from 0.67 to 0.78) [4, 10, 19, 21, 22]. For these biomarkers, we have found a significantly high accuracy for detecting PCa (AUC 76.2%, 81.5%, and 89.7%, respectively) and they are like to be more useful in PCa diagnosis.

For individual risk assessment, the probability of PCa varied considerably depending on Phi values. However, usage of Phi thresholds significantly varied (21.3–29.2) among studies and many studies did not report used the cut-offs, making difficult the generalization of the results [4, 5, 6, 13]. The present study has a higher cut-off value for Phi of 31.6 (the 95% sensitivity cut-off). We estimated that 47% of men with benign disease could have been spared a biopsy and 5% of PCa would have been missed. With similar sensitivity selection others found that avoiding unnecessary biopsy was significantly lower (11–30%) with the same percentage of missed cancer [4, 5, 6, 13, 21].

There are researches that have compared p2PSA and its derivatives with other new biomarkers. Directly compared Phi outperformed PCa antigen 3 performances when added to the Epstein criteria in order to predict the presence of pathologically insignificant PCa [12]. Additionally, in patients who had been undergone to radical prostatectomy, p2PSA-based parameters turned out to be the most accurate predictors for final pathology results [13, 23]. Baseline and longitudinal p2PSA and Phi determinations are reported as significantly related to unfavorable biopsy results in patients that are monitored with active surveillance [9]. Furthermore, if Phi is added to the multivariable risk calculator that increases the predictive accuracy for overall PCa, but differences between risk calculators that include Phi were small [11]. These data suggest that p2PSA-based markers are not only important for PCa diagnosis but also as predictive factors of aggressiveness and possibly of prognosis.

Several studies have demonstrated an inverse relationship between PV and the incidence of PCa. According to the findings of some authors, PV is the most important factor in the interpretation of biomarkers used to detect PCa because PV has an influence in PSA values. Accordingly, bigger AUCs were found for Phi, %p2PSA, %fPSA and tPSA in patients with small prostate volume (≤ 35 ml) then in patients that had large prostate volume (> 50 ml) [24]. It is expected that the use of PV in the structure of p2PSAD shows better diagnostic performance compared to one-component biomarker (p2PSA) as demonstrated in our study. However, a comparison with a three-component biomarker showed slightly lower performance while clinical utility cannot be reliably determined due to overlapping the DCA curve. Unlike other studies that show the same specificity at fixed sensitivity of 95% [25], our results suggest less specificity of p2PSAD compared to the specificity of the Phi (35.4% *vs*. 66.7%).

The study's limitation lies in its relatively small patient cohort. Phi testing was recently set up and that is reason for limited sample size. Furthermore, this analysis is restricted by the bias introduced by false negative biopsies. Latest studies have suggested that systematic biopsies are inferior to extended biopsy schemes and magnetic resonance imaging (MRI)-targeted biopsies for the detection of PCa [26]. However, despite the encouraging results of new markers, the main urologist associations continue to recommend the consideration of DRE status, prostate size, ethnicity, age, comorbidity, family history, previous biopsy results, as well as tPSA values before performing a biopsy, whereas other serum biomarkers require being subject of further investigation to determine their clinical usefulness [27]. However, from a pragmatic viewpoint, all explored p2PSA derivatives are potentially useful in a biopsy decision situation. Cost-effectiveness of PCa detection is improved by using p2PSA derivatives compared to second-line costs caused if PSA-only screening approach is used [28]. Furthermore, in the current MRI era combining p2PSA derivatives and MRI led to even further gains in the detection of PCa that are clinically significant [29]. To our knowledge, this is the first time that comparison among almost all different p2PSA derivatives has been presented. Accordingly, a further study with a large population is needed to evaluate our conclusions. Despite this, the clinical utility of p2PSA derivatives is apparent.

CONCLUSION

This is the first study aimed to determine the diagnostic performance of different p2PSA derivatives in predicting PCa in suspected men. Compared with other markers, Phi was the most useful in selection of patients that do not need to undergo biopsy, thereby avoiding unnecessary procedures.

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Поређење различитих деривата *p2PSA* у предикцији карцинома простате код болесника са серумским нивоом антигена специфичног за простату мањим од 10 *ng/ml*

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САЖЕТАК

Увод/Циљ Изоформе прекурсора антигена специфичног за простату (*PSA*) (*p2PSA*) и његови деривати показали су вредне резултате у детекцији карцинома простате. Међутим, предиктивна вредност различитих деривата *p2PSA* остаје нејасна.

Циљ ове студије је да међусобно упореди перформансе *p2PSA*, процентуални однос *p2PSA* и слободног *PSA* (%*p2PSA*), индекс здравља простате (*Phi*) и један димензији простате прилагођени индекс, густину *p2PSA* (*p2PSAD*), у предвиђању карцинома простате код особа са серумским нивоом *PSA* 10 *ng/ml* или мањим.

Методе Ова проспективна студија укључила је болеснике код којих је учињена ултразвуком вођена биопсија простате и код којих су одређиване серумске вредности *p2PSA*. Прикупљани су подаци о клиничко-патолошким карактеристикама болесника и израчунате вредности %*p2PSA*, *p2PSAD* и *Phi*. Процењени су различити аспекти предиктивних перформанси маркера коришћењем поља испод криве *ROC*, специфичности при предефинисаним оквирима сензитивности, док је клиничка корисност процењена анализом криве одлучивања.

Резултати Карцином простате је утврђен код 32,4% од 71 болесника. У мултиваријантној анализи само су *Phi* и дигиторектални преглед били независни предиктори. Вредности поља испод криве *ROC* за *p2PSA*, %*p2PSA*, *p2PSAD* и *Phi* биле су 76,2%, 81,5%, 88,7% и 89,6%. За предефинисану сензитивност од 90% и 95% *Phi* је показао већу специфичност у односу на друге деривате *p2PSA*. *Phi* и *p2PSAD* доводе до веће нето користи у анализи криве одлучивања.

Закључак У односу на друге деривате *p2PSA*, *Phi* се показао најкориснијим у утврђивању код којих мушкараца не треба учинити биопсију, чиме се избегавају непотребне процедуре.

Кључне речи: карцином простате; *p2PSA*; индекс здравља простате; рана детекција карцинома

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Single center experience in treatment of tibial shaft fractures using the Ilizarov technique

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SUMMARY

Introduction/Objective Since tibial shaft is a common location of opened and closed tibial fractures, it is very important to determine the best method of treating these fractures.
Our objective was to assess whether the Ilizarov technique is appropriate in terms of complications, outcomes, and pain reduction in treatment of patients with tibial shaft fracture.

Methods Retrospective analysis included all consecutive patients with tibial shaft fracture treated with the Ilizarov technique in the period from January 2013 to June 2017 at the Banjica Institute for Orthopaedic Surgery, Belgrade, Serbia. Demographic and clinical data on patients were collected. Pain was assessed using visual analogue scale of pain. Two models of uni- and multi-variate linear regression analysis were performed.

Results The study showed that the overall rate of complications was low, and that hypertension, administration of antibiotics, and reoperation prolonged fixation. Also, severe fractures and longer procedure time delay mobilization. Significant reduction of pain was observed.

Conclusion The Ilizarov technique is a safe and reliable method in the treatment of patients with tibial shaft fractures and is followed by pain reduction, overall improvement of functioning, good outcomes, and is not commonly associated with complications.

Keywords: Ilizarov technique; tibial shaft fracture; disability; functionality; satisfaction; outcome

INTRODUCTION

Tibial shaft is a common location of opened and closed tibial fractures. Opened tibial fractures develop as a consequence of strong force effects, usually seen in traffic accidents [1, 2]. Along with the increased use of motor vehicles and consequential increase of trauma, it is very important to determine the best method and timing of treating tibial shaft fractures [3, 4].

Segmental fractures of tibia are rare, accounting for only 12% of all tibial fractures. It is not unusual for them to be associated with different complications such as malunion and infections. In this case, it is also inconclusive which treatment option to choose and this issue stays unclear and undefined [5, 6].

The generally used treatment method for tibial shaft fractures is still an interlocking nail. This therapeutic approach has its advantages in terms of good mobilization of the patient and prompt return to the usual activities. However, some cohort studies have shown that this approach may be associated with high rate of complications after the insertion of the interlocking nail [7, 8, 9]. The alternative method primarily for opened and complicated fractures is the external Ilizarov fixation. This is considered to be an efficient and safe method [6, 8, 10]. Its unique biomechanical characteristics provide the formation of elastic wires under the tension and maintain stable fixation of bone fragments, while allowing dynamization at the place of fracture. For successful treatment, it is necessary to put the wires under certain tension, which should be maintained during the whole period of treatment [11]. The weakening of tension, loosening of wires or even their breaking add to the instability which further causes deformities and delayed healing of fracture.

External fixation using the Ilizarov fixator is used for treating tibial plateau fractures as well. The majority of the literature data indicate that it is an equally efficient, if not an even more efficient method, in the treatment of tibial plateau fractures, compared to internal fixation [12, 13].

However, treatment of tibial fractures using Ilizarov fixator can be associated with certain complications, especially in cases involving large bone and surrounding soft tissue defects. The most common complications include infection of the surgical region, osteomyelitis, axial deviation, delayed union or malunion [14, 15].

Considering the fact that there is no consensus concerning the best surgical approach for tibial shaft fractures and the lack of studies investigating the long-term prognosis in patients treated with the Ilizarov fixator, the objective of our study was to retrospectively

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analyze patients with tibial shaft fractures in terms of pain, complications, and to determine which characteristics represent significant predictors of the postoperative course.

METHODS

In this retrospective analysis we aimed to review the postoperative course in terms of complications, pain, and to determine which demographic and clinical characteristics of patients represented significant predictors of postoperative course.

The study was conducted at the Banjica Institute for orthopedic surgery, Belgrade, Serbia, in the period from January 2013 to June 2017 and included all consecutive patients with a radiographically confirmed tibial shaft fracture treated with the Ilizarov technique. Classification of tibial fractures was according to the Orthopedic Trauma Association classification system. All fractures were classified as A, B, or C type, in accordance with the radiological finding.

Demographic and clinical characteristics of patients were obtained from their medical records. The following characteristics were analyzed: age, sex, chronic diseases, duration of hospitalization (in days), duration of waiting for the procedure (in days), duration of surgical procedure (in minutes), type of anesthesia, type of fracture, the manner of injury, prophylaxis (antibiotics, nadroparin calcium), complications after the procedure, as well as the duration of fixation. Complications that were analyzed included superficial and deep infection, nonunion, pseudoarthrosis, compartment syndrome, and reoperation.

Intensity of pain at the moment of admission and after a period of recovery, that is, when the Ilizarov fixator was removed, was assessed using visual analog scale (VAS). VAS consisted of a continuous scale that can be horizontal or vertical and is 100 mm in length. It is marked with two perpendicular labels at the end of the 100 mm line that represent the extreme values, i.e. minimal and maximal possible pain in the last 24 hours. VAS is designed to be filled out by the participants themselves. Scoring is performed using the ruler that measures the length from the beginning of the line to the label the participants gave, which represents the intensity of pain from 0 to 100; higher scores indicate higher pain intensity [16].

In order to describe the study sample, measures of descriptive statistics were used: mean values, standard deviation, and relative numbers (percentages). The normality of distribution was assessed using the Kolmogorov–Smirnov test. The differences between groups were evaluated using the Student's t-test. To estimate which characteristics of the participants represent significant predictors of pain, complications, and duration of fixation, we performed two models of linear regression analysis. In the first model, independent variables were clinical and demographic characteristics of patients, while the independent variable was the duration of fixation. In the second model, the independent variables were the same as in the first one, while the dependent variable was mobilization in days. P-values less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS (Statistical Package for Social Sciences), version 22.0 (SPSS Inc, Chicago, IL, USA).

This research was approved by the Council of the Banjica Institute for Orthopedic Surgery and the Ethical Committee of the Faculty of Medicine, University of Belgrade, with the decision that this type of study (retrospective study) does not need any written consent from the patients since it covers the period before the research has been initiated. However, the investigators are under the obligation to keep all personal information on study subjects strictly confidential. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

RESULTS

The average age at admission to the hospital was 47.8 ± 16 years; 63.5% of patients were men and 36.5% were women. Diabetes mellitus was present in 12.2%, hypertension in 28.4%, while coronary artery disease in only 2.7% of patients.

The average duration of hospitalization was 26.5 ± 13.3 days (range 13–85 days), while patients waited for the procedure 7.5 ± 8.1 days, (range 1–61), since the day of admission.

Most of the patients received spinal anesthesia (75.7%), block anesthesia (16.2%), while the least number of them underwent total anesthesia (8.1%).

The average duration of surgical procedure was 68.2 ± 25.8 minutes (range 30–165 minutes). None of the patients received blood transfusion during the procedure.

In the majority of patients (70.3%), A type of fracture occurred. However, a significant proportion of patients (20.3%) had complicated C type of fracture, while B type of fracture occurred in 9.5% of patients. The most frequent manner of injuring were same level falls (67.6%), while falls from height were the rarest manner of injuring (8.1%). Direct force caused trauma in 10.8%, while traffic accidents caused trauma in 13.5% of patients.

An antibiotic was administered in 24.3% of the patients, while nadroparin calcium (Fraxiparine®, Aspen Notre-Dame-de-Bondeville, France) was administered in the majority of patients in the aim of thrombosis prevention (97.3% of cases).

Considering the rate of complications, the overall rate was low; 5.4% of patients underwent the repeated surgical procedure and only 2.7% of patients had pseudo-arthrosis.

The highest value on VAS was observed at the place of fracture, which was expected. This high score remained even after removing the Ilizarov fixator. However, there was a significant difference in pain intensity before and after the procedure, and for each location where pain was assessed (knee, ankle joint, place of fracture). These results are given in Tables 1 and 2.

Table 1. The average pain scores on the visual analogue scale (VAS) on admission and after treatment

Pain (VAS)	On admission		At the end of the treatment	
	Mean	SD	Mean	SD
Knee	64.52	10.15	20.27	8.97
Ankle joint	69.25	13.09	20.27	8.97
Place of fracture	81.64	10.38	23.9	10.52

Table 2. The reduction of pain during the treatment (fixation)

Differences in pain intensity (VAS)	Mean	SD	t-test	p
Knee	44.26	13.79	27.606	< 0.001
Ankle joint	48.11	16.97	24.379	< 0.001
Place of fracture	57.63	16.14	30.723	< 0.001

VAS – visual analogue scale

Table 3 shows the results of uni- and multi-variate linear regression analysis with the duration of fixation, as an independent variable. The average duration of fixation was 6.2 ± 1.9 months. Univariate model showed that significant predictors of the duration of therapy, i.e. the duration of fixation, were the presence of hypertension (p = 0.057), antibiotic prophylaxis (p = 0.029), repeated surgical procedure (p < 0.001), and the presence of pseudo-arthrosis (p = 0.002). These variables entered the model of multivariate linear regression analysis where all variables, except pseudo-arthrosis, remained the significant predictors of the duration of fixation. Patients with hypertension (p = 0.040) were at greater risk of longer therapy duration, as well as those who were on an antibiotic therapy (p = 0.012) and those who underwent the repeated surgical procedure (p = 0.021).

The results of uni- and multi-variate linear regression analysis with the mobilization as a dependent variable are shown in Table 4. The average time of mobilization was 1.3 ± 0.5 days. Univariate linear regression analysis has shown that the duration of procedure, type of fracture, and the manner of injury are significant predictors for

Table 3. Uni- and multi-variate regression analysis with the duration of fixation as an independent variable

Independent variable	Univariate linear regression analysis			Multivariate linear regression analysis		
	β coefficient	IR*	p	β coefficient	IR*	p
Sex	-0.049	-1.115–0.735	0.683			
Age	0.214	-0.002–0.053	0.069			
Diabetes mellitus	0.167	-0.379–2.302	0.157			
Hypertension	0.225	-0.023–1.901	**0.057**	0.220	0.045–1.792	**0.040**
Coronary vascular disease	-0.130	-4.219–1.211	0.273			
Other	0.047	-0.911–1.357	0.696			
Total duration of hospitalization	0.165	-0.010–0.057	0.162			
Waiting for the intervention	-0.036	-0.071–0.041	0.594			
Type of anaesthesia	0.081	-0.472–0.964	0.497			
Duration of procedure	1.023	-0.008–0.026	0.310			
Type of fracture	0.140	-0.220–0.869	0.239			
Manner of injury	0.061	-0.296–0.504	0.606			
Antibiotics	0.255	0.117–2.122	**0.029**	0.261	0.258–2.034	**0.012**
Fraxiparine	-0.137	-4.294–1.132	0.249			
Reoperation	0.451	1.190–5.496	**< 0.001**	0.345	0.447–5.237	**0.021**
Pseudo-arthrosis	0.359	1.595–6.707	**0.002**	0.79	-2.030–4.721	0.429

Table 4. Uni- and multi-variate linear regression analysis with mobilization as independent variable

Independent variable	Univariate linear regression analysis			Multivariate linear regression analysis		
	β coefficient	IR*	p	β coefficient	IR*	p
Sex	0.171	-0.060–0.395	0.146			
Age	-0.180	-0.112–0.001	0.124			
Diabetes mellitus	-0.178	-0.593–0.076	0.128			
Hypertension	-0.069	-0.319–0.173	0.557			
Coronary vascular disease	-0.119	-1.028–0.333	0.312			
Other	0.020	-0.260–0.307	0.868			
Total duration of hospitalization	0.956	-0.004–0.012	0.324			
Waiting for the intervention	0.158	-0.004–0.023	0.178			
Type of anaesthesia	-0.098	-0.254–0.104	0.408			
Duration of procedure	0.445	0.004–0.012	**< 0.001**	0.385	0.004–0.011	**< 0.001**
Type of fracture	0.512	0.181–0.417	**< 0.001**	4.113	0.004–0.011	**< 0.001**
Manner of injury	0.242	0.006–0.200	**0.037**	0.061	-0.082–0.087	0.952
Antibiotics	0.061	-0.191–0.326	0.605			
Fraxiparine®	0.119	-0.333–1.028	0.312			
Reoperation	-0.044	-0.548–0.398	0.707			
Pseudo-arthrosis	-0.119	-1.028–0.333	0.312			

mobilization time ($p < 0.001$, $p < 0.001$, and $p = 0.037$, respectively). These three variables entered the model of multivariate linear regression analysis, which showed that the duration of procedure and the type of fracture were independent predictors of mobilization, while the manner of injury has not remained significant ($p = 0.952$). Patients with more severe fractures ($p < 0.001$) and those who underwent longer procedures could stand on their feet later, compared to those with shorter procedure times ($p < 0.001$).

DISCUSSION

We observed that the overall rate of complications in our study was low, with only 5% of patients undergoing repeated surgical procedure and about 2% with pseudo-arthrosis. There were no other complications observed. In the study by Lan et al. [17], which investigated the outcomes after the lengthening procedure, and compared the Ilizarov technique with nailing, one of the outcome measures after the Ilizarov technique was also the complications rate. They followed the rate of pin-site infections and deep infections. The rate of pin-site or superficial infections was about 2%, and there were no deep infections observed. Our results are in concordance with this study. Pin sites may become colonized with bacteria and much shorter time needed for external fixation may be the possible explanation for low rate of infections in this group of patients. In other studies, the rates of infections were 1.7–21%, but bony union rates were high only when the nail was inserted after the initial external fixation, for high energy and opened tibial fractures [18, 19, 20]. However, in the study by Lan et al. [17], all tibiae were well vascularized, which could also be an explanation to the low rate of infection. In our sample, the majority of cases were non-complicated fractures, which is a possible explanation for the findings. However, we had a significant percentage of complicated fractures without any infection as a complication and this goes in favor of the Ilizarov technique, in terms of safety and good outcomes.

Some authors found that nonunion represented a relatively frequent complication. Surgery to treat pseudoarthrosis and nonunion is difficult and can be a serious problem, followed by severe complications [21, 22]. In their study, Gulabi et al. [23] stated that nonunion was the result of closed fractures in two patients and opened fractures in three patients. Our results are in accordance with these studies, considering the fact that in our sample nonunion was a result of complicated fracture.

In the study by Sen et al. [24], the rate of complications was 2.08% per patient. Other studies reported the rate of 2.2 complications per patient and 2.5 complications per patient, respectively [25, 26]. The study by Gulabi et al. [23] reported the rate of 2.6 complications per patient, but most of them were minor and could be resolved without any additional surgical procedure. Only one patient had deep chronic bone infection, so he had to be re-operated. Although in our study the rate of complications was not calculated per patient, the overall rate was presented so the results could not be adequately compared. We can conclude that in all the studies using the Ilizarov external fixation, the rate of complications was low and our results are in accordance to theirs. This further implicates that the Ilizarov fixation method is safe and provides good results. In addition, the results of Meleppuram and Ibrahim [27] showed the similar rate of complications per patient, which was 1.6.

Tibial fractures range from low-energy injures, like in women with osteoporosis, to high-energy injures with severe soft tissue damage, along with bone trauma. The most common clinical finding associated with tibial fracture is soft tissue damage. This injury is particularly serious when there is metaphysial-diaphyseal dissociation. The treatment of such injures with external fixation dramatically improved results. The advantage of the Ilizarov fixator over closed fixation is that it allows closed reduction, minimal soft tissue damage, early mobilization, and a minor procedure of removal of the Ilizarov fixator [28]. Our results are in concordance with these particular findings. We have also shown that complicated fractures and longer duration of the procedure postponed mobilization time. However, we could not compare our results with previously mentioned study since the authors have not investigated the predictors of faster mobilization.

Early removal of external fixation reduces the risk of complications, i.e. the risk of infections, and allows earlier rehabilitation [23]. One of the objectives of our study was to show how certain demographic and clinical characteristics of patients influence the length of fixator carrying – the time of fixator removal. We have shown that patients with hypertension, those who received antibiotic therapy, and those who underwent repeated surgical procedures were at greater risk of later removal of fixation. In other words, we may say that complications (repeated procedure in our sample) delay the removal of fixation and that further leads to other complications, such as infections.

The Ilizarov technique offers an effective and safe manner of treating some of the most challenging conditions in orthopedics, such as complicated fractures, infected fractures, or nonunions of tibia. In our study, the average duration of fixation was about six months, which is slightly shorter than in the study by Meleppuram and Ibrahim [27], who showed that the average duration of fixation was 8–10 months. Some studies have shown that smoking had negative effects on fixation, in terms of lengthening the time of fixation, as well as on bone lengthening index [29]. We have not investigated the influence of smoking status on the duration of fixation, but, as it was already mentioned, we have shown that hypertension, repeated procedure, and the use of antibiotics were independent predictors of fixation duration.

We have investigated the functionality after the procedure – the pain and the reduction of pain after fixation. We have observed that the reduction of pain was significant, even in those with complicated fractures. The other authors also measured functionality after procedure. Meleppuram and Ibrahim [27] showed good bone results in 60% of patients, but functional results were worse than

bone results. It shows that excellent bone results do not guarantee good functionality. Functional results, as well as the pain reduction, are affected by damage of soft tissue and neurovascular structures. There are many published papers investigating long bone defect managing and describing complications, but pain, long treatment process, and prolonged external fixation are the main shortcomings. This could be a significant physical and mental burden for the patient. The study by Wang et al. [30], which investigated the overall wellbeing and pain after the Ilizarov fixation, showed that treatment deteriorated physical and emotional wellbeing and patients experienced severe pain for a long time. At the end of the follow-up, although with severe pain, the overall functioning was significantly improved. Our results differ significantly from these findings. This study included only patients with infected tibial nonunions, unlike our sample, which involved patients with less complicated fractures as well, which could be the possible explanation for the different results.

Our study has some limitations. Firstly, although we analyzed the representative sample of all consecutive patients with tibial shaft fractures in a given period, sample size is still small, which can disable the generalization of the results. In addition, we used only VAS without analyzing the overall physical and mental condition of the patients, so we cannot have the comprehensive view on the situation. Furthermore, this is a retrospective analysis that relied on medical records of the patients, and not a prospective one, where we could further follow the outpatients. Clinical data were taken from the medical history of a patient that had been obtained partly by the anesthesiologist and partly by the orthopedic surgeon, which could affect the consistency of data and further results. Also, the small number of studies investigating this medical problem in the manner we did make the comparison difficult.

CONCLUSION

In conclusion, we may say that the Ilizarov technique in the treatment of tibial shaft fractures is a safe and reliable method, not commonly associated with complications, and is characterized by pain reduction, overall improvement of functioning, and good outcomes. It is important for surgeons to consider the factors influencing the outcome, such as the duration of fixation, pain, and mobilization time, so that they could better cope with the problem of their patient at an individual level.

Conflict of interest: None declared.

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Искуство једног центра у третману прелома дијафизе тибије Илизаровљевом методом

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САЖЕТАК

Увод/Циљ Дијафиза тибије је често место настанка отворених и затворених прелома тибије, те је веома важно изабрати најбољи метод за третман ових повреда.
Циља рада је био да се процени да ли је Илизаровљева техника прикладна у смислу компликација, исхода лечења и редукције бола у третману болесника са преломом дијафизе тибије.

Методе Ретроспективна анализа је укључила све болеснике са преломом дијафизе тибије који су третирани Илизаровљевом методом у периоду од јануара 2013. до јуна 2017. године на Институту за ортопедско-хируршке болести „Бањица“ у Београду. Анализирани су демографски и клинички подаци о болесницима. Бол је процењен коришћењем визуелно-аналогне скале за бол. Коришћена су два модела униваријантне и мултиваријантне линеарне регресије за анализу података.

Резултати Резултати студије су показали мали број укупних компликација. Показано је да високе вредности крвног притиска, употреба антибиотика и реинтервенције продужавају фиксацију, као и тежина прелома и дужина интервенције. Такође је показано значајно смањење нивоа бола после интервенције.

Закључак Илизаровљева метода је сигурна и поуздана у третману болесника са преломом дијафизе тибије, праћена је смањењем јачине бола, свеукупним побољшањем функционалности и добрим исходом, и нема честе компликације.

Кључне речи: Илизаровљева метода; прелом дијафизе тибије; исход лечења; задовољство болесника лечењем

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Comparison of efficacy and safety of preemptive infusion protocols of ephedrine and phenylephrine – prevention of hypotension and effects on hemodynamic parameters during spinal anesthesia for caesarean section

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SUMMARY
Introduction/Objective Spinal anesthesia (SA) for cesarean section may lead to significant changes in hemodynamic parameters, especially hypotension.
The aim of this study was to determine and compare the efficacy and safety of preemptive infusion protocols of the two most commonly used vasopressors, ephedrine (Group E, n = 29) and phenylephrine (Group P, n = 31) not only on prevention of hypotension but also to determine their effect on hemodynamic parameters, such as stroke volume (SV) and cardiac output (CO) using a continuous non-invasive hemodynamic monitor.
Methods The infusion of ephedrine was administered at the rate of 5 mg/min. immediately after SA. Phenylephrine was administered at an infusion rate of 25 μg/min for two minutes prior to SA.
Results In Group E, mean systolic blood pressure (SBP) and heart rate (HR) were similar to baseline. CO was higher ($p < 0.001$), while systemic vascular resistance (SVR) was lower than baseline ($p < 0.001$). In Group P, mean SBP and diastolic blood pressure (DBP) were lower than baseline, respectively ($p = 0.006$, $p < 0.001$). SBP, DBP, CO, SV, SVR, and HR were significantly different between the E and P groups ($p < 0.001$).
Conclusion E and P vasopressors are both effective in the prevention of hypotension during SA.
Keywords: cesarean section; spinal anesthesia; ephedrine; phenylephrine; hypotension; hemodynamic parameters

INTRODUCTION

Due to the significantly higher percentage of morbidity and mortality under general anesthesia, spinal anesthesia (SA) is now the method of choice [1]. Cesarean section under SA leads to significant changes in hemodynamic parameters, such as preload, stroke volume (SV), cardiac output (CO), heart rate (HR) and systemic vascular resistance (SVR) [2]. Hypotension occurs within approximately 70–80% of cases as a consequence of sympathetic blockade in the affected areas of anesthesia, which might cause organ and placental hypoperfusion. Acute hypotension reduces cerebral perfusion, which leads to transient ischemia and activates the vomiting center [3–6]. Fall in CO reduces oxygen delivery to organs and tissues, results in buildup of oxygen debt, causing complications after SA [7, 8, 9]. Other side effects of hypotension during SA are nausea, vomiting, dizziness, respiratory problems, and fetal acidosis [10, 11].

The most commonly used drugs for hemodynamic optimization during cesarean section are ephedrine and phenylephrine [6, 11, 12]. Ephedrine leads to greater venoconstriction than arteriolar constriction, increases BP, HR, improves venous return (preload), increases CO, and restores uterine perfusion [13]. Ephedrine may cause tachyphylaxis, and is associated with increased risk of fetal acidosis [11, 13]. Phenylephrine increases venoconstriction and arterial constriction, which increases BP, results in venous tone increase and favors venous return (preload) and increases SVR [13–16].

The main goal of this study was to determine and compare the efficacy and safety of preemptive infusion protocols of ephedrine and

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phenylephrine not only on hypotension prevention but also the associated hemodynamic changes during SA for cesarean section. Our hypothesis was that the protocol of application of these drugs is of importance for hemodynamic stability and that application of the given doses of ephedrine and phenylephrine infusion prevents hypotension during the planned caesarean section in SA.

METHODS

This study was designed as prospective and randomized and was approved by the ethics committee of Dr Dragiša Mišović – Dedinje University Hospital Center, Belgrade, Serbia, with the protocol no. 01-5293/23, on April 28, 2017. This study included 60 patients (from June 25, 2017 to April 25, 2018) divided randomly into Group P and Group E. The patients gave written informed consent to participate in the study. Inclusion criteria were as follows: patients aged between 18 and 40, American Society of Anesthesiologists (ASA) 1 or 2 physical status, and single fetus. Exclusion criteria were as follows: less than 36 weeks gestation, presence of cardiac, vascular, or neural diseases, body weight under 50 kg or greater than 100 kg, height under 150 cm, and presence of contraindications for SA.

Protocol P Group

Two minutes before the administration of SA, Group P received 25 μg/min. of phenylephrine infusion and this was continued at 25 μg/min. for the next three minutes. If SBP was unchanged or reduced, the infusion resumed at the same rate. If SBP was greater than 20% below baseline, patients received a rescue bolus of 50 μg phenylephrine intravenously (iv). If bradycardia occurred together with SBP less than 20% below baseline, the infusion of phenylephrine was continued at 25 μg/min., and 0.5 mg atropine was administered iv. If bradycardia occurred with SBP equal to or higher than baseline, phenylephrine infusion was discontinued. If SBP exceeded 20% of the baseline, the infusion of phenylephrine was discontinued.

Protocol E group

Group E patients received ephedrine immediately after SA injection at a dose of 5 mg/min. for the first three minutes. The same dose was continued where SBP was unchanged or lower than the baseline. If SBP decreased more than 20% of baseline, a rescue bolus dose of 5 mg ephedrine was given iv. Where SBP was greater than 20% of the baseline, ephedrine infusion was discontinued. Both infusions were administered via infusion pump Argus 600S (Argus Medical, Heimberg, Switzerland).

Bradycardia was defined as a HR under 60 beats per minute while hypotension was defined as a reduction in SBP greater than 20% of baseline. Hypertension represents increase of SBP greater than 20% above the baseline.

All the patients received 50 mg of ranitidine iv and were preloaded with 500 ml of Hartmann's solution. During the cesarean section the infusion of Hartmann's solution was resumed. BP, HR, electrocardiogram, and oxygen saturation were recorded using the DASH® 4000 monitor (GE Medical Systems Information Technologies Inc., Chicago, IL, USA). BP was measured automatically at three-minute intervals. Pre-induction values of BP, HR, CO, SV, and SVR were recorded with continuous non-invasive hemodynamic monitoring LiDCO Rapid^{V2}CNAP (LiDCO Ltd, London, UK) and the parameters were measured continuously up to the end of the surgical procedure. LIDCO Rapid^{V2}CNAP contains a module for non-invasive continuous monitoring of arterial pressure using a double finger cuff. The Pulse CO^{R} is a pulse pressure algorithm that calculates SV values by the detection of variations in arterial pulse, from the BP waveform using pulse power analysis pressure. It provides a nominal value for SV, CO and SVR using patient demographic data of height, weight, and age. SA was given in the sitting position using a "pencil point" spinal needle of 25G (Pencan® B.Braun, Melsungen, Germany). The patients received bupivacaine-spinal 0.5% 2–2.2 ml in the L3–L4 intervertebral space. The patients were then returned to the previous supine position with the operating table tilted to the left side by 15°.

Umbilical vein blood gas analyses were performed for acidity (pH), partial oxygen pressure (PO_2), partial carbon dioxide pressure (PCO_2), and base excess (BE). Apgar score at the first and fifth minute was recorded for each newborn. The times from spinal injection to baby delivery and from SA until the end of surgery were also recorded.

For statistical analysis we used Kolmogorov–Smirnov test to examine distribution, then parametric Student's t-test for two independent groups' comparison, the Wilcoxon signed-rank test for paired groups, and χ^2 test for frequency distribution analysis, using SPSS Version 19.0 (IBM Corp., Armonk, NY, USA). Before the beginning of the study, we performed the statistical power of study analysis, and our sample size was sufficient at power of 80%; $p < 0.05$ was considered significant.

RESULTS

Demographic characteristics and medical history of the patients are presented in Table 1. The body weight and height were higher in Group E ($p = 0.002$, $p = 0.086$, respectively, Student's t-test, Table 1). Changes in the mean values of hemodynamic parameters between the groups were analyzed before (baseline), and at the time of vasopressor infusion (Table 2).

Mean baseline values of the hemodynamic parameters were not significantly different between the two groups (baseline values, p^{EP}, Table 2). The mean values SBP, DBP, CO, SV, SVR, and HR changed significantly during the ephedrine and phenylephrine infusions ($p^{EP} < 0.001$, Student's t-test, Table 2).

During the infusion, the mean values of SBP, DBP, CO, SV, and HR were significantly higher in the Group E compared to Group P, while the mean SVR was significantly lower in the Group E compared to the Group P (Table 2).

Table 1. Patient characteristics

Charactheristic	Group E (n = 29)	Group P (n = 31)	p
Age (year)	32 (4)	31 (4)	0.335
Weight (kg)	83 (10)	75 (9)	0.002*
Height (cm)	170 (6)	167 (6)	0.086
Gestational age (weeks)	39 (38–40)	39 (38–40)	0.942
Number of previous deliveries	2 (1–3)	2 (1–3)	–
ASA physical status I	18 (62%)	18 (58%)	0.752
ASA physical status II	11 (38%)	13 (42%)	

E – ephedrine; P – phenylephrine; ASA – American Society of Anesthesiologists physical status
*significant p < 0.05, mean (sd), median (min–max. range), n (%), Student's t-test, χ^2 test

During the ephedrine infusion, the mean DBP was significantly lower compared to the baseline values ($p^{12} = 0.005$, Wilcoxon's test, Table 2). Mean values of CO and SV were significantly higher during ephedrine infusion compared to the baseline values ($p^{12} < 0.001$, Wilcoxon's test, Table 2); while SVR was significantly lower ($p^{12} < 0.001$, Wilcoxon's test, Table 2).

In Group P, mean values of CO and SVR were not significantly changed during the infusion compared to the baseline values. During phenylephrine infusion, the mean SBP was lower than baseline ($p^{12} = 0.006$, Wilcoxon's test, Table 2), as was DBP ($p^{12} < 0.001$, Wilcoxon's test, Table 2). SV was significantly increased compared with baseline ($p^{12} = 0.001$, Wilcoxon's test, Table 2). HR was significantly higher in Group E compared to Group P ($p^{EP} < 0.001$, Student's t-test, Table 2), and significantly lower than the baseline in Group P ($p^{12} < 0.001$, Wilcoxon's test, Table 2).

During the first 5–6 minutes following SA and vasopressor administration until skin incision, mean SBP values were similar in Groups E and P. During the delivery, and at the fifth and 10th minute after delivery, and up to the end of the procedure, significantly higher mean SBP values were recorded in Group E compared to Group P (p < 0.001, Figure 1A).

Table 2. Hemodynamic changes between/within the two groups: ephedrine (E) and phenylephrine (P) vasopressors

Parameter	Baseline values		p^{EP}	During vasopressor infusion		p^{EP}	E	P
	Group E (n = 29)	Group P (n = 31)		Group E (n = 29)	Group P (n = 31)		p^{12}	p^{12}
SBP (mmHg)	122 (12)	121 (12)	0.828	122 (21)	114 (15)	< 0.001*	0.938	0.006*
DBP (mmHg)	74 (9)	73 (11)	0.685	66 (15)	61 (15)	< 0.001*	0.005*	< 0.001*
CO (L/min)	8 (2)	8 (2.2)	0.645	11 (3.7)	9 (3.5)	< 0.001*	< 0.001*	0.424
SV (mL)	91 (26)	88 (22)	0.668	111 (36)	110 (38)	< 0.001*	0.002*	0.001*
SVR (dyn s/cm^5)	878 (204)	852 (233)	0.643	671 (291)	777 (366)	< 0.001*	< 0.001*	0.253
HR (bpm)	93 (23)	97 (18)	0.444	97 (21)	83 (16)	< 0.001*	0.333	< 0.001*

SBP – systolic blood pressure; DBP – diastolic blood pressure; CO – cardiac output; SV – stroke volume; SVR – systemic vascular resistance; HR – heart rate; p^{12} – baseline values compared with values during vasopressor infusion in the respective group
*significant p < 0.05, mean (sd) Student's t test

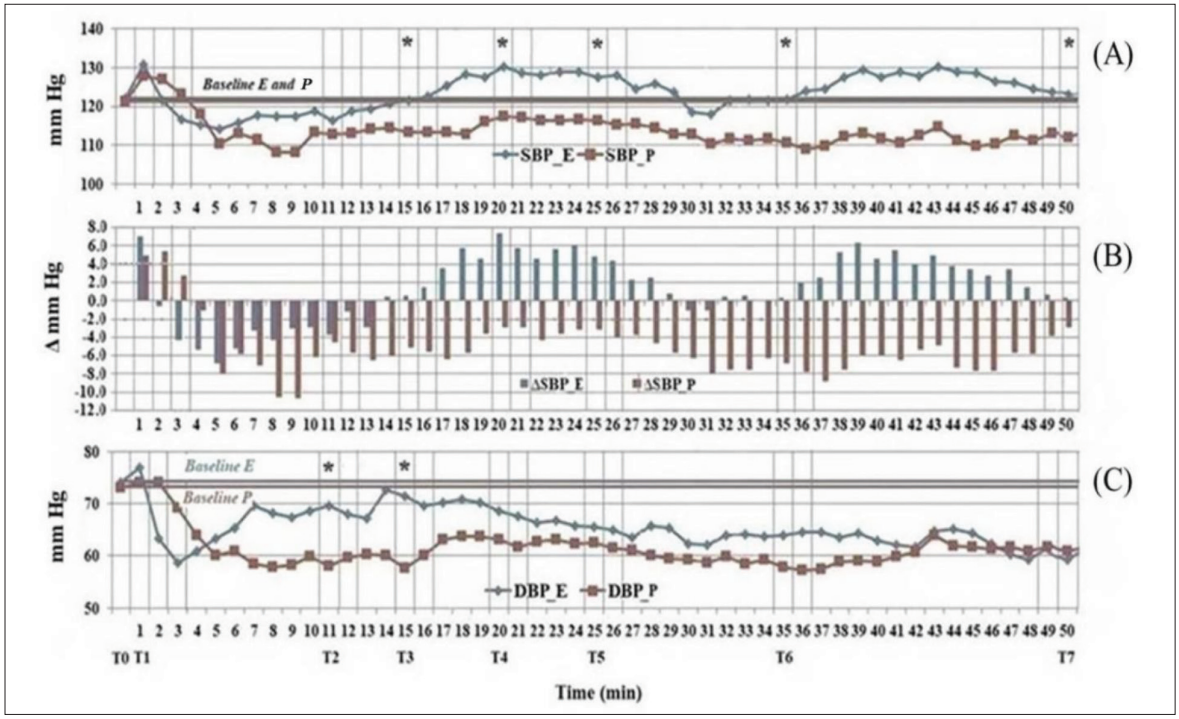

Figure 1. Changes in hemodynamic parameters; comparison to baseline values, and values after spinal anesthesia/after administration of vasopressors; SBP-1A – systolic blood pressure; ΔSBP-1B – average changes of systolic blood pressure; DBP-1C – diastolic blood pressure; T0 – start of infusion P; T1 – spinal anesthesia (both groups) and start of ephedrine infusion; T2 – skin incision (both groups); T3 – delivery (both groups); T4 – five minutes after delivery; T5 – 10 minutes after delivery; T6 – 20 minutes after delivery; T7 – end of surgery
*significant p-values

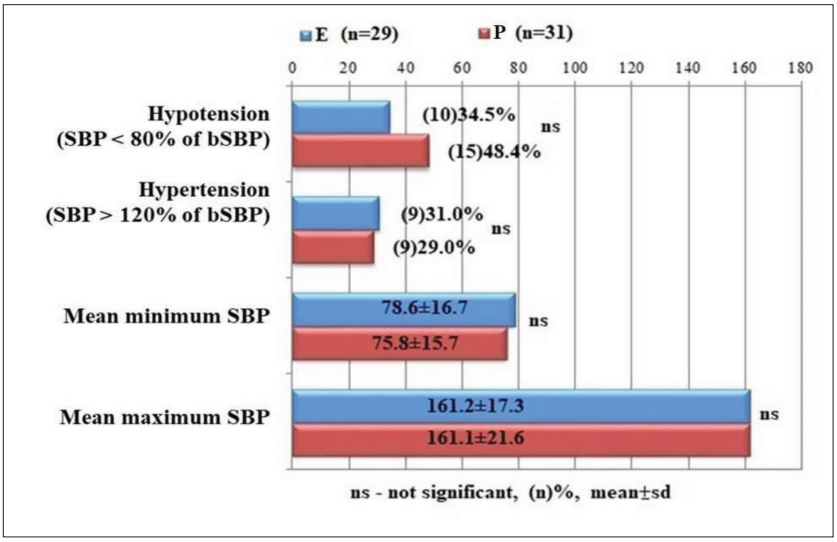

Figure 2. Incidence of hypotension/hypertension and mean minimum/maximum systolic arterial pressure; SBP – systolic blood pressure; bSBP – baseline systolic blood pressure

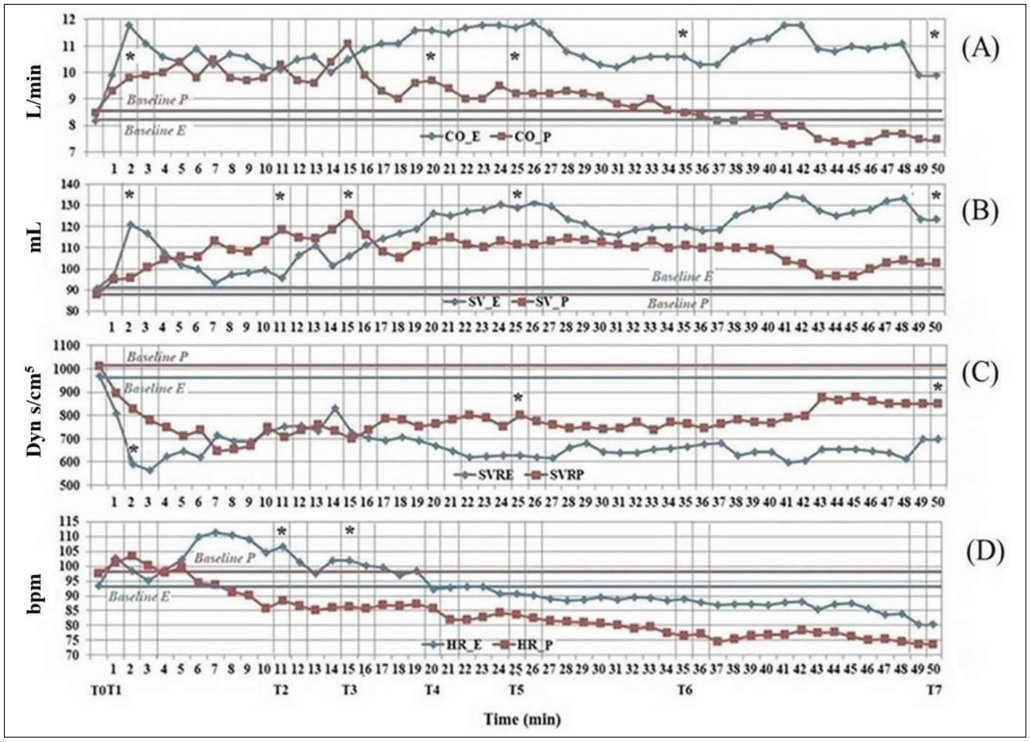

Figure 3. Changes in hemodynamic parameters, before (baseline) and after spinal anesthesia/after administration of vasopressors; CO-3A – cardiac output; SV-3B – stroke volume; SVR-3C – systemic vascular resistance; HR-3D – heart rate; T0 – start of phenylephrine infusion; T1 – spinal anesthesia (both groups) and start of ephedrine infusion; T2 – skin incision (both groups); T3 – delivery (both groups); T4 – five minutes after delivery; T5 – 10 minutes after delivery; T6 – 20 minutes after delivery; T7 – end of operation;

*significant p-values

In Group P, except in the first few minutes, SBP was lower than baseline. The largest decrease in SBP was seen after 8–9 minutes of phenylephrine infusion, but average values were only about 10 mmHg lower than baseline (Figure 1B). There was a decrease of SBP in Group E between 3–13 minute up to 6.5 mmHg (Figure 1B). Mean values of DBP in both groups were lower than basal levels (Figure 1C).

No significant differences in the incidence of hypotensive and hypertensive episodes were detected, and the average minimum and maximum SBP were similar between groups (Figure 2).

Mean CO values in Group E were consistently higher than baseline after SA and up to the end of the surgical procedure. In Group P, mean CO values were higher than baseline only up to the 36th minute (Figure 3A). After SA, during the second minute, mean values of CO in Group E increased significantly compared to the baseline, and compared to Group P ($p < 0.001$, Figure 3A).

After delivery and up to the end of the procedure, lower mean Group P CO values were recorded compared to those in Group E (Figure 3A). However, mean Group P CO values were similar to baseline values. After the 36th minute, (20 minutes away from delivery), and up to the end

Table 3. Intraoperative characteristics, umbilical vein gases and Apgar scores

Variables	Group E (n = 29)	Group P (n = 31)	p
Intraoperative characteristics			
Time from spinal anesthesia to the end (min)	49 (8)	51 (9)	0.291
Duration of I–D (min)	15 (3)	15 (3)	0.982
Vasopressor infusion duration (min)	23 (6)	50 (15)	< 0.001**
Sensor block level before skin incision	T5 (T4–T6)	T5 (T4–T6)	-
Modified Bromage score for motor block	3 (3–4)	3 (3–4)	-
Number of patients received rescue bolus doses (%)	7 (24%) dose 5–15 mg	7 (23%) dose 0.05–0.15 μg	0.887
Number of rescue bolus doses	13	11	-
Number of rescue bolus doses before delivery	10	7	-
Number of rescue bolus doses after delivery	3	4	-
Mean doses of vasopressors (mg)	49.3 (9.3)	1.3 (0.4)	-
Total fluid-crystalloid (ml)	1551 (244)	1419 (291)	0.061
Incidence of nausea			
Yes	7 (24%)	3 (10%)	0.133
No	22 (76%)	28 (90%)	
Incidence of vomiting			
Yes	1 (3%)	0 (0%)	0.297
No	28 (97%)	31 (100%)	
Medicaments			
Atropine			
Yes	1 (3%)	7 (23%)	0.029*
No	28 (97%)	24 (77%)	
Metoclopramide			
Yes	7 (24%)	3 (10%)	0.133
No	22 (76%)	28 (90%)	
Umbilical vein gases and Apgar scores			
pH	7.36 (7.14, 7.49)	7.36 (7.29, 7.45)	0.668
PO2 (mmHg)	27.3 (24.4, 30.5)	27.7 (26.1, 28.8)	0.657
PCO2 (mmHg)	37.3 (32.7, 41.8)	38.2 (32.6, 42.6)	0.706
BE (mEq/L)	-3.8 (-4.7, -1.6)	-2.6 (-4.0, -1.1)	0.122
Apgar first minute	8.97 (0.19)	8.90 (0.30)	0.342
Apgar fifth minute	9.93 (0.26)	9.87 (0.34)	0.447

I–D – time from the induction of spinal anesthesia to delivery of the baby; pH – acidity; PO_2 – partial oxygen pressure; PCO_2 – partial carbon dioxide pressure; BE – base excess
*significant $p < 0.05$, mean (sd), n (%), Students t-test, χ^2 test, pH, PO_2, PCO_2, BE median (min., max. range), Apgar mean (sd); Modified Bromage score: 1 – able to raise legs above the table; 2 – able to flex knees; 3 – able to move feet only; 4 – no movement in legs or feet

of the procedure, mean values of Group P CO decreased compared with baseline. In Group E, CO was significantly higher up to the end of the procedure compared with baseline and Group P, ($p < 0.001$, Figure3A).

Both vasopressors increased SV. In Group E, SV increased significantly after SA, with the addition of ephedrine infusion in the second minute ($p < 0.001$, Figure 3B). During skin incision and delivery, SV was significantly higher in Group P compared to Group E ($p < 0.001$, Figure 3B). From that point on, Group E had higher SV than Group P until the end of the procedure.

SVR values were lower than baseline in both groups (Figure 3C).

After five minutes of infusion, Group E's HR was increased above baseline up to 20th minute. After 24th min, mean HR of Group E was below baseline, but it was consistently higher than mean HR of Group P (Figure 3D). HR was higher at skin incision and delivery in Group E ($p < 0.001$, Figure 3D). Mean Group P HR was below baseline from the fifth minute up to the end of the procedure.

Mean values of vasopressors infusion duration were significantly longer ($p < 0.001$, Student's t-test, Table 3) in Group P compared with Group E.

Incidence of nausea and vomiting were similar in both groups. The administration of atropine was significantly higher in Group P ($p = 0.029$, χ^2 test, Table 3).

Umbilical venous pH was lower than 7.2 in one case in Group E. However, the mean pH values in both groups were identical (7.36). No newborn had Apgar score lower than 8 in the first minute, and mean values of Apgar score were similar between groups. Gas analysis of umbilical vein showed no significant differences between Groups E and P.

DISCUSSION

In our study, after SA, CO values increased along with concomitant increase of HR and SV. Liu et al. [17] detected a significant decrease in SVR and an increase in CO after SA both before administration of phenylephrine and before hypotension occurred.

In our study, phenylephrine infusion was introduced two minutes prior to administration of SA. This was followed by an increase in CO following SA, as noted above, but these changes were significantly lower than in Group E where ephedrine infusion was given immediately after SA. With both phenylephrine and ephedrine, the drop in SBP did not exceed 20% and the changes were relatively minor.

We have shown that patients from Group E had significantly higher SBP, DBP, CO, SV, and HR, but lower SVR than in Group P. Similarly, Gunda et al. [18] in their study showed that HR was also significantly higher using ephedrine *vs.* phenylephrine. However, they used a single bolus dose of 5 mg of ephedrine or 100 μg of phenylephrine, but both were administered only after the occurrence of hypotension. Furthermore, the same authors did not detect significant differences in SBP (although slightly higher in Group P than in Group E) [18].

Allen et al. [19] investigated four groups of patients who received different doses of prophylactic fixed-rate phenylephrine infusions. In the groups that received 25 μg/min. and 50 μg/min. P, SBP was higher than 80% of baseline and

in the groups that received 75 μg/min. and 100 μg/min. the incidence of hypertension was increased [19]. Also, our study showed that patients who received phenylephrine at a dose of 25 μg/min., SBP remained greater than 80% of baseline.

Langesæter et al. [20] examined the effects of two different intrathecal doses of bupivacaine, with or without iv phenylephrine infusion on hemodynamic parameters. This study showed that a low dose of prophylactic phenylephrine infusion (0.25 μg/kg per min.) provided the best hemodynamic stability [20]. In our study, patients in Group P received 10 mg spinal bupivacaine and prophylactic phenylephrine infusion (25 μg/min.) two minutes prior to SA and were also quite effective.

Mon et al. [21] in their study examined the effects of ephedrine and phenylephrine infusion on hemodynamics. In Group P, CO was significantly lower than the baseline in the 10th and 15th min. after application of SA, in contrast with Group E, in which CO values were not significantly changed [21]. Ephedrine administration was associated with significantly more cases of fetal acidosis despite good SBP control and increased CO [21]. Our study showed that after the initial increase in CO in both groups, there was a reduction in CO in the Group P, but values below the baseline were detected only from 36th minute up to the end of the surgery, which might be important for fetal outcome. It should be recalled that the dose of phenylephrine in our study was four times lower than in the previously described and continued for a longer time. Unlike in the previously mentioned study, where no significant changes in SV were detected, in our study SV was increased in both groups [21]. Numerous studies have shown associations between HR and CO, as was the case here [14].

The lowest average HR values in Group P were recorded in the 37th minute of phenylephrine infusion, which coincides with the time when CO in the same group drops below baseline values. A total of seven patients in Group P had HR < 60 and received atropine (five of them before delivery), which may be associated with the administration of seven phenylephrine rescue doses before the birth of a baby. Also, other authors have found higher incidence of bradycardia in patients receiving phenylephrine than those receiving ephedrine [22].

Ngan et al. [23] showed that prophylactic phenylephrine infusion of 100 μg/min. decreased the incidence of hypotension during SA for cesarean delivery compared with control group, who received bolus phenylephrine at 100 μg after each event of SBP < 80% of baseline. Results in our study, using four times smaller dose of phenylephrine infusion, show that reactive hypertension was recorded in 29% of the patients.

Although other studies reported higher incidences of nausea and vomiting in groups on ephedrine, we did not detect significant differences between Group E and Group P [10, 19]. We are of the opinion that not only type of vasopressor, but the protocol of administration and dosage significantly influences the incidence of side effects.

CONCLUSION

In this study, SBP remained in the normal range during infusion in both groups, which indicated that ephedrine and phenylephrine are both effective. Both vasopressors had similar effects on newborns. Continuous monitoring of hemodynamic parameters, with a well-defined administration protocol and dosing regimen are considered important for a favorable outcome, as shown in this study.

Conflict of interest: None declared.

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Поређење ефикасности и безбедности преемптивних протокола инфузије ефедрина и фенилефрина – превенција хипотензије и утицај на хемодинамске параметре током спиналне анестезије за царски рез

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САЖЕТАК

Увод/Циљ Током спиналне анестезије (СА) за царски рез долази до значајних хемодинамских промена, као и до хипотензије.

Циљ ове студије био је да се утврде и упореде ефикасност и безбедност преемптивних инфузионих протокола два најчешће коришћена вазопресора, ефедрина (група Е, $n = 29$) и фенилефрина (група П, $n = 31$), не само у циљу превенције хипотензије већ ради утврђивања њиховог утицаја на хемодинамске параметаре, као што су ударни волумен и минутни волумен, коришћењем континуираног неинвазивног хемодинамског монитора.

Методе Инфузија ефедрина је укључена у дози од 5 *mg/min.*, одмах после СА. Инфузија фенилефрина је укључена у дози од 25 *μg/min.*, на два минута пре СА.

Резултати У групи Е средње вредности систолног крвног притиска и срчана фреквенција нису се значајно промениле у односу на базалне вредности. Минутни волумен је био значајно виши ($p < 0,001$), док је системски васкуларни отпор био значајно нижи у односу на базалне вредности ($p < 0,001$). У групи П средње вредности систолног крвног притиска и дијастолног крвног притиска биле су значајно ниже у односу на базалне вредности ($p = 0,006$, односно $p < 0,001$). Средње вредности систолног крвног притиска, дијастолног крвног притиска, минутног волумена, ударног волумена, системског васкуларног отпора и срчане фреквенције су се статистички значајно разликовале између група Е и П ($p < 0,001$). Гасне анализе венске умбиликалне крви и оцена Апгар су биле сличне у обе групе.

Закључак Вазопресори ефедрин и фенилефрин су веома делотворни у превенцији хипотензије током СА.

Кључне речи: царски рез; спинална анестезија; ефедрин; фенилефрин; хипотензија; хемодинамски параметри

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Long-term outcomes of catheterizable continent urinary diversion in children

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SUMMARY

Introduction/Objective The use of bladder augmentation and/or continent urinary diversion has gained wide acceptance, particularly in children with small, abnormally developed bladder or high-pressure bladder that poses great risk for renal deterioration and incontinence. We discuss indications, results, and complications with various types of continent vesicostomy (CV) in children.

Methods Sixty-eight patients with CV are retrospectively reviewed (51 boys and 17 girls) 1987–2008. The median follow-up time was 17.8 years (3–22 years). CV included appendicovesicostomy in 31 (41.3%), vesicostomy with distal ureter in 27 (36.0%), and preputial CV in 10 (13.3%) patients. CV in patients with augmented bladder was in 18 (26.47%) children.

The indications for performing CV were various types of neurogenic and myogenic dysfunctions of urine bladder with incontinence due to the following pathoanatomical substrates: anomalies of the brain–spine segment development (27), bladder exstrophy (10), posterior urethral valve (15), expansive processes (4), and other anatomical defects in 12 patients.

Results Continence was achieved in 94.64% of the cases, without statistically significant difference between particular types of the stoma (p = 0.065). Early complications included stoma necrosis, stoma bleeding, peristomal infection in 5/68 (7.35%) patients, and late complications included calculosis, in 20/68 (29.4%), stomal stenosis, in 8/68 (11.5%), and difficulties of catheterization, in 3/68 patients (4.08%). Calculosis was predominant in appendicovesicostomy (p = 0.012).

Conclusion CV is a safe procedure applied with the main purposes of achieving continence, preservation of renal function, and improvement of the quality of life, along with an acceptably low rate of complications.

Keywords: children; continent vesicotomy; postoperative complications

INTRODUCTION

Resolving urinary incontinence and preserving renal function in children with neurogenic bladder dysfunction has been a serious challenge for pediatric surgeons and urologists for years. Until the mid-1970s, urinary diversion by means of the intestinal conduit was practically the only solution to the problem when Lapides et al. [1] introduced the technique of clean intermittent catheterization (CIC) through the native urethra, which specifically addressed these issues. However, in a relatively high percentage of cases, the method appeared to be inefficient due to the difficulty in performing self-catheterisation (pain in males, orthopaedic problems) or continuous urinary leakage between catheterizations.

In 1976, in order to overcome this obstacle, Mitrofanoff [2] proposed the alternative forms of continent urinary diversion – appendicovesicostomy or ureterovesicostomy – with bladder neck closure (in most cases). These methods have been most widely applied after the reports of Duckett and Snyder [3], and along with the introduction of other possibilities (prepuce, a segment of small intestine, bladder wall, oviduct...) they justifiably named it the Mitrofanoff principle [4, 5, 6].

Considering any reservoir and any alternative urinary diversion, continent diversion is associated with a low-pressure reservoir [3, 4]. In most cases, the goal is native bladder preservation, with or without augmentation.). The most common complications following continent vesicostomy (CV) are stoma stenosis and urinary leakage [7, 8]. Solving these problems requires further urodynamic volume–pressure testing and further revision or reimplantation if needed [9].

The objective of the study is to present our experiences with indications for performing specific types of CV and complications following the procedure.

METHODS

We performed a retrospective study in children (aged 3–8 years) who underwent a continent urinary diversion at the University Children's Hospital in Belgrade, Serbia, in the 1987–2008

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period. Patients were divided into the following three groups: appendicovesicostomy, vesicostomy with distal ureter, and preputial vesicostomy.

The indications for performing CV were various types of neurogenic and myogenic dysfunctions of the urinary bladder with incontinence due to the following pathoanatomical substrates: anomalies of the brain–spine segment development (27), bladder exstrophy (10), posterior urethral valve (15), expansive processes (4), and other anatomic defects (12).

The patients with other ways of bladder emptying (Credé's maneuver) or those emptying through the native urethra were excluded.

Operating technique

The principle consists of the interposition of the appendix vermiformis or other tubular structure between the bladder and the skin, with an anti-reflux technique, which facilitates self-catheterisation and establishes a continent mechanism. When necessary, urinary bladder augmentation was performed during the same operation.

Ureteral reimplantation was done using the extravesical approach (Lich–Gregoire technique) or detrusor submucosal tunnelling anti-reflux technique. Stoma should be located as close as possible to the bladder reservoir to provide a short and straight pathway. It is usually the right lower abdominal quadrant when using the appendix, and the distal right ureter, or the left lower abdominal quadrant when using the left ureter. Some authors suggest the placement of the stoma in the umbilicus because of the lower incidence of stenosis and less visibility [10].

The patient carries a suprapubic catheter in the Mitrofanoff canal for 21 days, and then commences CIC.

CIC was used in the standard manner together with the oxybutynin and prophylaxis of urinary infections (first- and second-generation cephalosporins or co-trimoxazole) [1]. By urodynamic testing or measuring bladder capacity, the need for oxybutynin was eliminated occasionally

Bladder augmentation was performed in children with low-capacity bladder and/or poor detrusor compliance.

The data collected refers to the basic illness, age of the child when performing a stoma placement, the spot of the stoma placement, continence, complication rate, indications for surgical revision, type of surgical revision, and results. We divided the complications into two groups – early (up to 12 months after the stoma placement) and late.

We classified the stoma-revision procedures into suprafascial (skin level revision) and infrafascial (deep complications that require additional laparotomy). Suprafascial revisions were applied in cases of stoma stenosis, stoma prolapse, or granulation tissue around the stoma, and were categorized as stoma revisions. Subfascial revisions was performed due to difficulties in performing catheterization. The most common reasons were channel angulation and diverticulum. The incontinence due to insufficient anti-reflux mechanism was solved by a subfascial revision or endoscopically (STING procedure)

The study was approved by the Research Ethics Committee of the institution where it was conducted.

Statistical analysis was performed using the G*Power software (Heinrich Heine University Düsseldorf, Düsseldorf, Germany). The results are expressed in numbers and percentages along with the mean value ± standard deviation. Comparisons were done by Student's t-test and descriptive statistics by Fisher's exact test and χ^2 test.

RESULTS

The analysis included 68 patients, 17 (25%) of which were girls and 51 (75%) were boys. CV included appendicovesicostomy in 31 (41.3%), vesicostomy with distal ureter in 27 (36%), and preputial vesicostomy in 10 (13.7%) patients.

Additional surgery was performed in 18 (26.47%) patients in the form of augmentation of the urinary bladder, and bladder neck reconstruction (exstrophy-epispadias complex) was performed in 10 patients. Bladder neck closure was not performed. The median follow-up time was 17.8 years (3–22 years). Clinical details of our patients are presented in Table 1.

Continence achieved in 63 patients (94.64%) without significant statistical difference between the types of stomas ($p = 0.063$).

Early complications (infection, dehiscence, gastrointestinal problems, febrile conditions) occurred in 5/68 patients (7.35%) – in three patients with appendicovesicostomy, and in one each for distal ureteral stoma and preputial tube. No statistically significant difference between the types of CV (χ^2 test, $p = 0.233$).

Stomal stenosis occurred in 9/68 (11.7%) patients who underwent CV – six patients with appendicovesicostomy, two (7.1%) patients with preputial vesicostomy, and one patient (3.5%) with CV created by the distal ureter. Statistically, stenosis was significantly more frequent in patients with appendicovesicostomy ($p = 0.010$).

Table 1. Clinical details of patients

Variables		Appendico Vesicostomy n (%)	Distal ureteral vesicostomy n (%)	Preputial vesicostomy n (%)	Total n (%)
Anomalies of the brain–spine segment development		17 (25)	5 (7.4)	5 (7.4)	27 (39.8)
Exstrophy-epispadias complex		8 (11.7)	2 (2.9)	0 (0)	10 (14.6)
Posterior urethral valve		3 (4.41)	11 (16.22)	1 (1.47)	15 (22.1)
Expansive processes		2 (2.9)	0 (0)	2 (2.9%)	4 (5.9)
Other diseases		1 (1.47)	9 (13.23)	2 (2.9%)	12 (17.6)
Total		31 (45.6)	27 (39.7)	10 (14.7)	68 (100)
Median follow-up time (years)		19.7	14.9	10.9	17.8
Stoma location	umbilical non-umbilical	7 24	0 27	3 7	10 58
Augmented urinary bladder		10	6	3	18
Sex	Male Female	20 11	21 06	10 0	51 17

Table 2. Most common complications

Variable	Appendicoves-icostomy n (%)	Distal ureteral vesicostomy n (%)	Preputial Vesicostomy n (%)	Total n (%)	p
Early complications	3 (9.3)	1 (3.7)	1 (3.7)	5/68 (7.35)	0.233
Stomal stenosis	5 (13.2)	1 (3.5)	2 (7.1)	8/68 (11.7)	0.012*
Calculosis	8 (38)	2 (7.1)	2 (7.1)	12/68 (17.64)	0.018*
Channel angulation	3 (9.3)	3 (9.3)	0 (0)	6/68 (8.8)	0.028
Continency	29 (90.3)	25 (89.7)	9 (92.8)	63/68 (94.64)	0.063

*statistically significant

In only 1/68 (1.47%) of the patients, a stomal prolapse (distal urethral stoma) developed, which was resolved by revision surgery and fixation.

Calculosis was predominant in patients with appendicovesicostomy (eight patients or 38%), especially if it was associated with bladder augmentation (substitution) using an intestinal segment. One patient in each of the remaining groups was also detected. Even if only patients without augmentation were analyzed, calculosis appeared significantly more frequently in patients with appendicovesicostomy ($p = 0.0015$).

There was no statistically significant difference between the specific types of CV regarding difficulty in catheter angulation ($p = 0.028$, test: binary logistic regression).

Incontinence occurred in 5/68 (7.36%), mostly in patients with distal ureter stoma (3/27; 14.6%). No statistically significant difference between the types of CV ($p = 0.065$).

Most complications occurred in patients with exstrophy-epispadias complex – 6/10 (60%) – and in patients with anomalous brain–spine segment development.

Six patients with appendicovesicostomy were subjected to stoma revision due to stomal stenosis, with a median follow-up time of 2.3 years (min. 1.8 years, max. 9.8 years). In three patients, stoma-specific complications required a surgical revision, and in another three patients the problem was resolved after the dilatation of the stenosis.

In one patient with a distal ureteral stoma, the stoma revision (prolapse) was performed 2.6 years after the major surgery.

Two patients with preputial vesicostomy were subjected to stoma revision 2 years and 4.5 years, respectively, after the performance due to stenosis.

Most additional interventions, due to incontinence, were performed in patients with CV with distal ureter. STING procedure was employed in four patients. A secondary revision was performed in two patients (one was subjected to the procedure twice and the other one three times) due to incontinence (eight months and 2.6 years after the primary revision). In one patient, stenotic ureteral orifice was balloon dilated.

A subfascial revision of appendicovesicostomy was performed in three patients (channel angulation). There were no revisions to the secondary surgery. Statistically, there was a significant difference related to this type of revision in favor of patients with CV formed from the distal ureter.

DISCUSSION

The role of CIC is to protect the upper urinary tract and to achieve continence [4]. CV enables easier execution of CIC [5]. CV should be performed in carefully selected patients when CIC through the native urethra is not possible. Difficulties due to the sensitivity of the urethra, especially in boys, are the main reason [7, 8, 9]. Accessing the urethra in children with orthopaedic deformities or paraplegia appeared to be considerably difficult, which is why CV is clearly indicated in this group of patients. Patients with exstrophy-epispadias complex or posterior urethral valve have difficulty while performing self-catheterisation due to anatomical reasons.

CV is associated with a number of early and late complications [9, 10]. Children and their parents should be properly informed about numerous benefits, but also potential risks and complications [10, 11, 12].

The appendix vermiformis, rather than other parts of the gastrointestinal system, is preferable for CV since intestinal anastomosis is not required, it has adequate lumen and sufficient vascularisation, suggesting significantly lower predisposition for ischemia. Damage due to frequent catheterisation or channel diverticular pouches is most often the consequence of a slightly longer intravesical part of the channel [12].

Prior to CV, it is necessary to estimate compliance, urinary bladder capacity, and detrusor overactivity by urodynamic testing. CV is best performed on low-pressure bladder [13]. Regardless of our channels being implanted using an anti-reflux method, provided that the intravesical pressure was not low, the likelihood of urinary leakage was considerably higher [14].

Despite the increasing number of catheterizations and high-dose anticholinergic therapy, the incontinence problems were more frequent in patients with CV created using the distal ureter (14.6%). In these patients, CV was created from a very wide ureteral reflux, and the length of the submucosal tunnel (anti-reflux mechanism) had to be longer than in the other two types. The good side of these CVs is that incontinence can be endoscopically resolved (STING procedure) [13].

Stoma stenosis is the most common complication in our group of patients – 8/68 – with a somewhat more frequent occurrence in patients with appendicovesicostomy (Table 2). Even at 82% of our patients, it was reported between the second and third year after CV. Similar results were reported by Leslie et al. [15] in their slightly larger group of patients, with the highest incidence in stoma from gastrointestinal segments. Stenosis usually occurs on mucocutaneous junction due to poor vascular support [14]. Later, this is largely due to micro trauma resulted from frequent catheterizations. The stoma location has no effect on the incidence of stenosis [15].

It is known that vesicostomy and urinary bladder augmentation are associated with an increased risk for

calculosis [16]. In our group of patients, those with appendicovesicostomy were more often diagnosed with calculosis. Chronic bacteriuria, mucous production, and urine pathways are the causes of calculosis. All three risk factors can be present in appendicovesicostomy. In our institution, we suggest that parents use saline solution for more aggressive bladder irrigation once a day for preventing urolithiasis.

The pathological condition with the largest number of complications was exstrophy-epispadias complex, which is different if compared to numerous other studies, where it was more common in patients with the posterior urethral valve or central nervous system anomalies [17, 18].

Our median follow-up time was not long enough to enable a discussion on complications such as malignancy or nutritional deficiencies, which was not the subject of this research. Nevertheless, it was long enough so that we could infer that CV significantly improved the quality of life of our patients.

CONCLUSION

Continent urinary diversions are safe procedures whose main purposes are continence, preserving renal function, and improving the quality of life, with an acceptably low rate of complications. Stenosis, calculosis, and incontinentia are the three most common complications inherent in certain methods of CV.

Conflict of interest: None declared.

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Удаљени резултати континентних везикостомија код деце

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САЖЕТАК

Увод/Циљ Аугментација мокраћне бешике са континетном везикостомијом или без ње је широко примењена код деце са малом, абнормално развијеном мокраћном бешиком, која води оштећењу бубрега и иконтиненцији. Описане су индикације, резултати и компликације болесника с разним типовима континентних уринарних диверзија код деце. Циљ рада је био да се прикажу дугорочни резултати и компликације појединих континентних уринарних деривација код деце, као и начин њиховог решавања.

Методе Ретроспективно је приказано 68 болесника (51 дечак и 17 девојчица) са континентним уринарним дериватима у периоду 1987–2008. Средње време праћења је 17,8 година (3–22 г.). Континентне везикостоме укључују апендиковезикостому код 31 (41,3%), везикостому дисталним уретером код 27 (36%), препуцијумску везикостому код 10 (13,3%) болесника. Индикације за извођење континентне везикостоме су разни облици неурогених и мишићних дисфункцаја мокраћне бешике са инконтиненцијом различитих патоанатомских супстрата: аномалије развоја кичмено-можданог сегмента (27), екстрофија мокраћне бешике (10), валвула задње уретре (15), експанзивни процеси (4), остале аномалије код 12 болесника. Аугментација мокраћне бешике урађена је код 18 болесника (24,3%).

Резултати Континентност је постигнута код 94,64% случајева, без значајне разлике између појединих типова стома ($p = 0,065$). Компликације укључују ране (стомална некроза, стомално крварење, парастомална инфекција) код 8/68 (11,5%) болесника и касне: калкулоза 20/68 (29,4%), стеноза стоме 8/68 (11,5%) тешкоће изводљивости катетеризације 3/68 (4,08%). Калкулоза је предоминантна код апендиковезикостоме ($p = 0,012$).

Закључак Континентне везикостомије дају висок степен континентности, мали број компликација и позитивно утичу на бубрежну функцију.

Кључне речи: дете; континентне везикостомије; постоперативне компликације

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Frequency and correlates of depression at the primary health care level in Belgrade

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SUMMARY

Introduction/Objective The prevalence of depression in primary care is relatively high. The aim of the study was to assess the frequency of depression among patients in Zvezdara Primary Health Care Center in Belgrade. We also examined the relationship between depression and individual risk factors (socio-demographics, lifestyle characteristics, and health-related factors).

Methods A cross-sectional study, which included 422 adult patients, under 65 years of age, was conducted at the Zvezdara Primary Health Care Centre in Belgrade, Serbia, during January of 2018. The instrument used was Patient Health Questionnaire-9 (cut-off score ≥10). Multivariate logistic regression analysis was applied.

Results Depression, at least of moderate intensity, was found in 36%of the respondents. Around 1.4% of the participants confirmed suicidal thoughts almost every day during the previous two weeks. The logistic regression model showed the association with depression and being married (OR: 0.24, 95% CI: 0.13–0.44), single (OR: 0.43, 95% CI: 0,22–0.83), unemployment (OR: 3.83, 95% CI: 1.51–9.76), lifetime contact with mental health services (OR: 3.79, 95% CI: 2.19–6.57), and regular treatment for chronic illnesses (OR: 3.22, 95% CI: 1.94–5.34).

Conclusion This study found a relatively high prevalence of depression among patients in the primary health care center. We found an association between depression and marital status, employment, previous contact with mental health services, and regular treatment for chronic illnesses. The Patient Health Questionnaire-9 instrument could be implemented in primary health care settings in Serbia.

Keywords: depression; prevalence; primary health care

INTRODUCTION

Major depressive disorder (depression) is a common mental health condition in which the absence of positive affect is associated with mental health problems. The World Health Organization estimates that depression will be the second leading cause of the global burden of disease by 2020 [1]. Mood changes cover a spectrum from transitory "normal" low mood to clinically significant affective disorder (such as major depression), which may be life-threatening. Nevertheless, the higher the degree of affective disorder, the higher is the mortality rate and the prevalence of adverse outcomes [2].

There is evidence that almost half of patients with depression in Europe have been unrecognized or inadequately treated. This gap results from the reluctance of patients to seek help, as well as from misdiagnosis at the primary care level [3]. In health care systems, general practitioners are the first line contact with patients with mental health problems. It could be stated that timely diagnosis and the efficiency of treatment are affected by the general practitioners' knowledge and training on the proper communication with this group of patients [4, 5]. The prevalence of depression varies among patients in primary health care and it ranges from 2.3% up to 48.5% [6–9]. The most recent meta-analysis from 2018 (n = 1,112,573 adults) showed no difference between the rural and urban settlements (13% *vs.* 17.7%, respectively) [10].

Different social factors could affect the development of depression, such as female sex, lower education, economic inactivity, and being divorced or widowed, or lifestyle characteristics and habits: diet, exercise, sleep [11–16]. If inadequately treated, depression can lead to many complications; in particular, it significantly increases the risk for suicide. Primary health care plays an important role in suicide prevention as more than one half of suicide victims contact their general practitioner one to four weeks prior to death, which creates the window of opportunity for the healthcare system to provide preventive measures [17, 18].

Screening instruments for depression are numerous and include Beck Depression Inventory, Zung Self-Rated Depression Scale, Kessler Psychological Distress Scale [19]. Patient Health Questionnaire (PHQ-9), and its versions (PHQ-8 and PHQ-2), is widely proposed to be used in these settings, as it has been shown to have higher specificity and sensitivity compared to primary health care physicians' diagnoses [3, 20]. Routine

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use of the nine-item Patient Health Questionnaire (PHQ-9) may be useful at primary care level and it may identify individuals at risk for depression who would not otherwise have been identified [21]. Routine use of PHQ-9 is still not a frequent practice in Serbia. According to the education and practice in Serbia, general practitioners should be able to recognize the depression and refer the patient to the psychiatry treatment. The study among general practitioners in five Southeastern Europe countries showed that the majority of our general practitioners consider recognizing the depression as their responsibility [22].

Serbia National Health Survey conducted in 2013 (n = 19,079) found a 4.1% prevalence of depression (PHQ-8 total score 10–24) in the general population [23]. The prevalence of depression in primary health care centers in Serbia was examined by the Lisulov and Nedić [24], with PHQ-9 and MINI test.

The objective of the study was to assess the frequency of depression among patients at the Zvezdara Primary Health Care Center in Belgrade. We also examined the relationship between depression and individual risk factors (socio-demographics, lifestyle characteristics, and health-related factors).

METHODS

Patients and setting

A cross-sectional study was conducted at the Zvezdara Primary Health Care Center in Belgrade, Serbia. The study included patients aged 18–65 who visited three general practitioners in January 2018. The exclusion criteria were the following: age under 18 jears, age over 65 years, pregnancy and postpartum, mental retardation or intellectual disability. Patients over 65 were excluded since it was shown that screening methods available are less robust for this age group and symptoms of depression often coexist with medical comorbidities [25, 26]. We excluded 14 patients who had an appointment with a psychiatrist before the study began and four who had not filled out the questionnaire. The final sample consisted of 422 patients. The required sample size (two-tailed) was calculated for a significance level of 5% and the power was set at 95%, whereas the proportion of depression was estimated to 25% by the Lisulov and Nedić [24] study. Our final sample of 422 exceeded the required minimum sample size of 72 patients. All the patients were informed on the study objective and the data collection. The patients gave their written consent to participate in the study.

The study was approved by the Ethics Committee of the Zvezdara Primary Health Care Center (No 1641/3) and the Faculty of Medicine, University of Belgrade (No 29/VI-15).

Data collection

The study instrument was a questionnaire, which consisted of four sections: socio-demographic and socio-economic characteristics, lifestyle characteristics, physical health, and mental health. Socio-demographic data were obtained by a questionnaire which included the following information: sex; age (for further analysis it was stratified into three clusters – 19–34 years, 35–54 years, and above 55 years), marital status (single, married, widowed, and divorced), educational level (elementary school, high school, college, and university), employment status (employed, unemployed, other – retired or student), monthly income per person in the household in Serbian dinars (RSD) – one euro is approximately 120 RSD (< 10,000 RSD, 10,000–25,000 RSD, 25,000–50,000 RSD, > 50,000 RSD), housing space per person (less than 10 m^2, 11–30 m^2, and above 31 m^2), number of family members in the household (one, two, three to four, five or more members). The lifestyle characteristics were 'Tobacco' (yes/no) and 'Alcohol' (no/regularly/occasionally) consumption. The third part of the questionnaire included questions on health-related factors: whether they regularly took any prescribed medication at the time of this evaluation (yes/no) and whether they had contact with mental health services during their lifetime (yes/no).

Mental health was assessed by the PHQ-9 questionnaire, which has been widely used in primary care to quickly assess symptoms of depression and is considered a screening gold standard [18]. It has nine items scoring nine common symptoms of depression in the previous two weeks. It has a four-point rating scale from 0 – 'not at all' to 3 – 'always'. Score 5–9 indicates mild depression, 10–14 moderate depression, 15–19 is considered moderate-severe depression, and 20 and above severe depression. The validated cut-off score of ≥ 10 (sensitivity of 0.85, specificity of 0.89) has been recommended as an indicator for moderate to severe depression symptoms [19]. The ninth question of PHQ-9 measures suicidality (questioning if there were any "thoughts that you would be better off dead or of hurting yourself in some way" could be scored "not at all," "several days," "more than half the days," or "nearly every day"). The cut-off score of ≥ 1 was used as an indicator of suicidality (endorsement of "several days" or more to the item).

Data analysis

Descriptive statistics was used to show socio-demographic, socio-economic, and lifestyle characteristics, as well as health-related factors of the respondents (age, sex, education, employment status, marital status, number of family members in the household, monthly income per person, housing space per person, whether participant is taking any prescribed medication on a daily basis, and previous contact with mental health service). The difference in proportions was tested by the χ^2 test. Multivariate logistic regression analysis was performed to obtain significant factors (independent variables) associated with depression (dependent variable) and presented by odds ratio (OR), 95% confidence interval (CI), and p-value. All the data were analyzed using the IBM SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Based on PHQ-9 scores, the patients were divided into two groups: in the first one there were patients with the scores ≤ 9, who had none, minimal, or mild depression (n = 270; 64%), and in the second one there were patients with scores ≥ 10, who had moderate, moderately severe, or severe depression (n = 152; 36%). Cronbach alpha for PHQ-9 was 0.90 and ICC was ≥ 0.90.

About two-thirds (68%) of the patients were female. The majority of these were married (50.2%), 66.8% were employed, and the majority had a high school education (46.7%). The monthly income per person ranged 10,000–25,000 RSD (85–210 euros) for most of the patients. About 64% of the patients had no lifetime contact with mental health services. More than half were non-smokers (57.8%) and most of them reported no alcohol consumption (69.9%). About 59% of the patients were not using any medication on a daily basis (Table 1).

Table 1. Socio-demographic and other characteristics and percentages of positive screens for depression (PHQ-9 ≥ 10)

Characteristics	Total		PHQ-9 ≤ 9		PHQ-9 ≥ 10		p
	n	%	n	%	n	%	
Total	422	100	270	64	152	36	
Sex							
Female	287	68	188	65.5	99	34.5	0.342
Male	135	32	82	60.7	53	39.3	
Age (years)							
19–34	122	29	82	67.2	40	32.8	0.061
35–54	219	52	145	66.2	74	33.8	
> 55	80	19	42	52.5	38	47.5	
Marital status							
Married	212	50.2	159	75	53	25	< 0.001
Single	125	29.6	76	60.8	49	39.2	
Widowed, divorced	85	20.1	35	41.2	50	58.8	
Education							
< High school	28	6.6	14	50	14	50	0.216
High school	197	46.7	124	62.9	73	37.1	
College	74	17.5	46	62.2	28	37.8	
University	123	29.1	86	69.9	37	30.1	
Employment status							
Employed	282	66.8	201	71.3	81	28.7	< 0.001
Unemployed	77	18.2	33	42.9	44	57.1	
Other (student, retired)	63	14.9	36	57.1	27	42.9	
Monthly income per person (RSD)							
< 10,000	47	11.2	27	57.4	20	42.6	0.092
10,000–25,000	223	55.3	136	61	87	39	
25,000–50,000	117	28	86	73.5	31	26.5	
> 50,000	31	7.4	19	61.3	12	38.7	
Housing space per person (m^2)							
0–10	26	6.2	15	57.7	11	42.3	0.701
11–30	304	72.9	195	64.1	109	35.9	
> 30	87	20.9	58	66.7	29	33.3	
Number of family members in the household							
1	37	8.8	19	51.4	18	48.6	0.420
2	72	17.1	47	65.3	25	34.7	
3–4	248	58.8	162	65.3	86	34.7	
5 and more	65	15.4	42	64.6	23	35.4	
Any lifetime contact with mental health service							
No	270	64	239	72	93	28	< 0.001
Yes	152	36	31	34.4	59	65.6	
Smoking							
No	244	57.8	164	67.2	80	32.8	0.105
Yes	178	42.2	106	59.6	72	40.4	
Alcohol consumption							
No	295	69.9	195	66.1	100	33.9	0.167
Yes	127	30.1	75	59.1	52	40.9	
Regular treatment for chronic illnesses							
No	249	59	185	74.3	64	25.7	< 0.001
Yes	173	41	85	49.1	88	50.9	

RSD – the Serbian dinar currency;
p-value < 0.05 was considered statistically significant

The frequency of the patients who answered positively on the last question on the PHQ-9 (considering suicidality) was 10.8%. Around 1.4% of all the patients answered that they had suicidal thoughts or thoughts about hurting themselves almost every day.

The logistic regression model showed the association with depressive symptoms and being married (OR: 0.24, 95% CI: 0.13–0.44), single (OR: 0.43, 95% CI: 0.22–0.83), unemployment (OR: 3.83, 95% CI: 1.51–9.76), lifetime contact with mental health services (OR: 3.79, 95% CI: 2.19–6.57), and regular treatment for chronic illnesses (OR: 3.22, 95% CI: 1.94–5.34) (Table 2).

Table 2. Factors associated with depression

Parameters	Positive screening for depression	
	p	OR (95% CI)
Marital status		
married	< 0.001	0.24 (0.13–0.44)
single	0.013	0.43 (0.22–0.83)
widowed, divorced	/	Ref. category
Employment status		
employed	0.955	1.02 (0.44–2.36)
unemployed	0.005	3.83 (1.51–9.76)
other (student, retired)		Ref. category
Any lifetime contact with mental health service	< 0.001	3.79 (2.19–6.57)
Regular treatment for chronic illnesses	< 0.001	3.22 (1.94–5.34)

OR – odds ratio, CI – confidence interval;
p-value < 0.05 was considered statistically significant

DISCUSSION

This cross-sectional study of depression prevalence in an urban population of Serbia indicated that more than one-third of adults attending the Primary Health Care Center had depressive symptoms of moderate, moderately severe, or severe intensity. Another study of the primary health care center population in Vojvodina, by Lisulov and Nedić [24], registered a prevalence of 24.5%. Studies in primary health care in the world reported prevalence in a wide range, 2.3–48.5% [6, 7, 9]. Overall, the prevalence in the primary health care center population is much higher than prevalence of depression reported in the general population. For example, prevalence reported by the National Health Survey conducted in the general population of Serbia in 2013 was only 4.1% [23].

In our study, 1.4% of participants answered that they had suicidal thoughts almost every day during the previous two weeks. In the literature, the prevalence of suicidal thoughts was estimated at around 10%, which meant that suicidal thoughts were present "more than half the days" or "nearly every day" in 1/10 adults who were visiting their general practitioner [27]. By showing that cumulative probability of both nonfatal and fatal suicidal attempts (according to response to item 9 of the PHQ-9) was ranging from approximately 0.4% (1/250) for those responding "not at all", to approximately 4% (1/25) for those responding "nearly every day," Simon et al. [27] emphasized the need for sustained and organized follow-up care to address an ongoing risk of suicide. We fully support this statement, in particular related to primary health care center screening practices [27].

Several individual-level factors: lower education, female sex, economic inactivity and being divorced or widowed, were associated with increased odds of depression in a large, multilevel cross-national study of prevalence of depression, which was conducted in 68 countries [4]. It was shown previously that women are twice more likely to experience depression during their lifetime in most studies [7, 15]. The results of the National Health Survey of the Republic of Serbia 2013 demonstrated that symptoms of depression were present in a significantly higher percentage in women (5.3%) than in men (2.9%) [23]. In our study, sex was not associated with the likelihood of depression. This could be explained by numerous social factors in Serbia, which could have led to an increase in prevalence of mental health problems. Previous studies have shown that women are more likely to be depressed in countries in which they have lower income, and lower socio-economic status. Women are more likely to be prescribed with more antidepressants than men are, which may be associated with higher prevalence reported [22].

Our study showed that married patients are less likely to have depression compared to single/widowed/divorced patients, which is in agreement with previous studies [28]. Kessler et al. found that being separated/divorced was associated with an increased risk of depressive disorders in 12 countries (OR from < 4 to > 8) [13]. Married participants have strong social support from their partners, which can serve as a protective factor for depression [16].

Unemployed participants had almost four times higher likelihood for developing depression disorders. The reason could be that increase in household spending could stress unemployed participants more and create suitable environment for depression [29]. In our study, the likelihood for developing depression in unemployed patients was threefold higher than that for the employed.

Another independent factor associated with depression in our study was previous contact with a mental health service (almost four times higher likelihood). Having in mind that our participants were attending their general practitioners for general medical care and that those who already had an appointment with a psychiatrist were excluded, the correlation of actual depression and any lifetime contact with mental health services is to be considered further. The implications of these findings are many, but still beyond the scope of this paper.

In our study, regular treatment for chronic illnesses was also associated with higher likelihood (more than three times higher likelihood). Previous research confirmed that chronic diseases were predictors for depression. However, recent studies have demonstrated the inverse causality, i.e. depression precedes chronic illness [28]. Comorbidities associated with depressive disorders are highly prevalent in primary health care practice and a causal link between comorbid physical disorder and depression is yet to be studied [30].

LIMITATIONS

Our study revealed a relatively high proportion of depression in adults visiting their general practitioner in the primary health care center. These results should be treated with some caution. PHQ-9 scores do rate some of the patients as depressive despite the fact that psychiatric clinical examination may often reject this diagnosis (false positive findings). In the opposite direction, our question regarding alcohol habits was answered with 'no' in 70.2% of the cases. There is a slight chance that it was a false negative finding, because the latest reports of alcohol consumption in Serbian population aged 15 years and more, both sexes, showed higher prevalence of alcohol consumption in the population (48.4%) in comparison to our findings. Aforementioned limitations are usual in the evaluations based on self-report instruments and the truth is that only physician-administered interview tools with clinical accuracy will lead to a sufficient diagnostic evaluation for those at risk. Nevertheless, on a day-to-day basis, the use of the self-report PHQ-9, with evaluation of both alcohol/drug consumption and anxiety by screening questions, remains the briefest, simplest, most accurate way to diagnose depressive and other frequent psychiatric symptoms in the adult population. Patients can complete and score the questionnaires themselves in the waiting room prior to seeing their doctor. Consistent use of this approach in primary health care centers could improve our national general medical practices, helping to choose the most appropriate interventions and to monitor the outcomes.

CONCLUSION

Depression is highly prevalent in many settings. Early screening for depression in primary health care using the PHQ-9 instrument is essential for early recognition and management of the disorder. Depression and depressive disorders are often associated with numerous socio-demographic factors. In our study, we found the association between depression and marital status, employment, previous contact with mental health services, and regular treatment for chronic illnesses. We found relatively high prevalence of depression in our sample, which supports the need for training of primary health center doctors to implement screening instruments for depression.

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Учесталост и корелати депресије на примарном нивоу здравствене заштите у Београду

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САЖЕТАК

Увод/Циљ Учесталост депресије међу испитаницима на нивоу примарне здравствене заштите је релативно висока. Циљ ове студије био је да се испита учесталост депресије међу болесницима који су посетили свог изабраног лекара у Дому здравља „Звездара" у Београду, као и да се испита повезаност депресије са индивидуалним факторима ризика (социјално-демографским, карактеристикама животног стила и факторима повезаним са здрављем).

Метод Студија пресека, која је обухватила 422 одрасла учесника млађа од 65 година, спроведена је у Дому здравља „Звездара" у Београду, Србија, током јануара 2018. године. Инструмент истраживања био је Упитник о здрављу пацијената (*Patient Health Questionnaire 9, PHQ-9*). Гранична вредност је износила ≥10. Примењена је мултиваријантна логистичка регресиона анализа.

Резултати Код 36% особа утврђена је депресивност (умерени, умерено тешки или тешки степен израженості), док је 1,4% свих испитаника имало суицидне мисли скоро сваки дан током последње две недеље. Мултиваријантна логистичка регресија је показала повезаност депресивности и брачног статуса – у браку (*OR:* 0,24; 95% *CI:* 0,13–0,44), неожењен/неудата (*OR:* 0,43; 95% *CI:* 0,22–0,83), као и незапослености (*OR:* 3,83; 95% *CI:* 1,51–9,76), претходног контакта са службама за ментално здравље (*OR:* 3,79; 95% *CI:* 2,19–6,57) и регуларне терапије за хроничне болести (*OR:* 3,22; 95% *CI:* 1,94–5,34).

Закључак Ова студија је показала релативно високу учесталост депресивности међу испитаницима у дому здравља. Пронашли смо повезаност између депресије и брачног стања, запослења, претходних контаката са службом менталног здравља и редовне терапије хроничних болести. Инструмент *PHQ-9* може се примењивати у примарној здравственој заштити у Србији.

Кључне речи: депресија; учесталост; примарна здравствена заштита

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Factors that are associated with the development of acute respiratory infections in the kindergarten – an analysis of the population of 1,528 children

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SUMMARY

Introduction/Objective Acute respiratory infections (ARI) are very common amongst children attending preschool institutions (PIs) and they represent a significant public health problem. Strict compliance with the legal regulations should reduce the incidence of respiratory infections in children.
The aim of this research was to determine predictors for the appearance of ARI in kindergarten.

Methods The research was conducted as a cross-sectional study at the six PIs located on the territory of Voždovac municipality during a six-month period (from January to June 2016). The target population consisted of 1,528 children who were between three and seven years old. Data analysis was done using descriptive statistics (χ^2 test and Spearman's rank correlation coefficient).

Results The increase in the number of children for 30% above the norm was a predictive factor for the increase of respiratory infections ($p < 0.001$). Sex differences were not a predictive factor for the emergence of respiratory infections in any age group. In all organizational units, March was the period when respiratory infections were on the increase (37.2%, $p < 0.001$), and it was significantly higher ($p < 0.001$) in cases, when the diseased child had a brother and/or a sister in the same kindergarten. During the observed period, the number of teachers corresponded to the range of one educator per 12 to 19 children. A constant number of educators could not reduce the incidence of respiratory infections, especially during the February–March period ($p < 0.001$).

Conclusion In addition to active treatment and strict compliance with the legal regulations in the organization of PIs, the reduction in incidence of respiratory infections in preschool children could be achieved if the number of enrolled children complied with the standard, as well as by preventive procedures in children's groups: avoiding overheated and dry air, regular ventilation, fresh air, well-planned diet, physical activity, personal hygiene and cleanliness.

Keywords: kindergarten; respiratory infections; predictors

INTRODUCTION

Preschool upbringing and education of children in Serbia is regulated by the Law on the Education System Foundations, as well as the Law on Preschool Education within the framework of a unified system of education that is in accordance with the Constitution and ratified international conventions, such as Convention on the Rights of the Child, which emphasizes the rights of preschool children to develop, as well as meeting their educational, cultural, health, and social needs [1, 2]. In addition to educational work with children as defined by the law, preschool institutions (PIs), which comprise of nurseries and kindergartens that provide daycare accommodation, meet the needs of preventive health care, nutrition and social protection of children as well. These functions are performed by nursery and kindergarten teachers. At the level of PIs, there are pedagogues, psychologists, speech therapists, social workers, dietitians, and nurses, who work on prevention and, if necessary, other associates.

The preschool age of a child carries specific risks of increased incidence of acute respiratory infections (ARI). Primarily, there is decreased ability to produce antibodies for certain groups of bacteria, given the dynamics of the production of Immunoglobulin G antibody subtypes. Immunoglobulin G2 antibodies work on bacteria with a polysaccharide capsular membrane, and their maximum level of action is expressed at the age of 12 [3]. Staying in children's nurseries, where children are in contact with peers of the same age, leads to a greater exposure to potential sources of infection. In children's groups with the mixed age structure, this danger is lower, and thus is the incidence of respiratory tract infections. In addition, older children have already adopted hygienic habits, and there is less chance of spreading the infection with dirty hands, by the secretion from the nose and mouth.

Children who are not in the system of daycare accommodation institutions like kindergarten, mostly suffer from viral infections. On the other side, children who are in daycare accommodation suffer equally from viral, bacterial, and mixed infections. The risk of respiratory infections is three times higher in the age group between two and five, compared to children of the same age who are at home, and it is more frequent in the

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urban population group. The early enrollment to nursery is associated with frequent childhood illnesses (averagely up to three times a month). For the first time, those children are in contact with a larger number of children, whereas some of whom have a cold, some are in the infectious disease incubation, and some are healthy [4, 5]. Only after the third year of stay in the collective, the incidence of illness falls to the incidence rate of children who do not attend kindergarten. After a three-year-old child goes to the kindergarten for the first time, he is exposed to one or two episodes of airway inflammation annually, and after two years, the frequency decreases to the level of children who do not attend the kindergarten. Children who have an older brother or sister, who attends kindergarten as well, meet these diseases at home, and when they go to the kindergarten, they are less likely to be sick.

The aim of this research was to determine factors that are associated with the appearance of ARI among children who are attending daycare accommodation in the kindergarten.

METHODS

The research was conducted as a cross-sectional study at the Čika Jova Zmaj PI in Belgrade, which organizes and delivers daycare in 29 kindergartens in the area of Voždovac municipality. This study was conducted from January to June 2016, in six kindergartens that belong to the Čika Jova Zmaj PI (Čika Jova Zmaj, Plavi Čuperak, Sestre Bukumirović, Mala Sirena, Vivak, and 1001 Radost), which presents 20% of all kindergartens (6/29). A total of 1,528 children were enrolled there (739 girls and 789 boys, 48.4% *vs.* 51.6%), and they were organized in sixty educational groups. This number of children accounts for 35% of all enrolled children within this PI, and 40% of all educational groups. Internal questionnaires were used for the purposes of this research, as well as records and workbooks for each educational group. Socio-demographic data on the age, sex, and siblings in the family were retrieved from these records. In addition, we used data on the number of children enrolled in educational groups in relation to age; the total number of children enrolled at each kindergarten (all groups); the prescribed legal norm of the maximal number of children that could be enrolled in one particular kindergarten (it is determined by the capacity of the kindergarten) and the number of absent children from the group due to the ARI (ill episode). Data on the number of engaged educators per group were also recorded on a daily basis at each kindergarten.

Data analysis was performed using descriptive statistical methods. Results were presented in absolute numbers and as percentages. χ^2 test was used to assess statistically significant differences between groups. Spearman's correlation analysis was used to assess significant correlations. The line graph was used to present the most important findings. All data were analyzed using SPSS 20.0 (IBM corp., Armonk, NY, USA) statistical package and MS Excel 2007 (Microsoft Corp., Redmond, WA, USA). Statistical analysis was based on the probability level of 95%.

All procedures performed in the study were in accordance with the institutional Committee on Ethics.

RESULTS

The number of children enrolled in six kindergartens within the Čika Jova Zmaj PI during the observed period from January to June 2016, compared to the prescribed norm for the ages of three, four, five and six is shown in Table 1, while the number of registered ARI in kindergartens in this period are shown in Table 2.

In every kindergarten, the number of registered children was higher than the number that is recommended by the norm in all age groups. Mala Sirena kindergarten was leading when it came to the number of children enrolled. There were 441 children in total, or 32% children more than the envisioned norm in the period from January to June 2016. During this period, there were 678 cases of registered ARI, and on average, it is 47% more infections compared to other kindergartens (Figure 1). Correlation analysis found that there was a statistically significant association between the number of children above the norm and the occurrence of

Table 1. The number of enrolled children, the norm, and percentage of difference (%)

Age	The number of enrolled children / the norm (% difference)						Total
	Čika Jova Zmaj	Plavi čuperak	Sestre Bukumirović	Mala sirena	Vivak	1001 Radost	
3	63/52 +21%	31/20 +55%	69/40 +73%	146/100 +46%	33/20 +65%	84/60 +40%	426/292 +46%
4	36/24 +50%	68/48 +42%	66/48 +38%	92/72 +28%	35/24 +46%	64/48 +33%	361/264 +37%
5	36/24 +50%	56/48 +17%	87/68 +28%	112/72 +56%	11/12 -8%	85/72 +18%	387/296 +31%
6	33/24 +38%	72/48 +50%	75/48 +56%	91/72 +26%	24/24 0%	59/48 +23%	354/264 +34%
Total	168/124 +35%	227/164 +38%	297/204 +46%	441/316 +40%	103/80 +29%	292/228 +28%	1,528/1,116 +37%

Table 2. The number of acute respiratory infections (ARI) episodes in six kindergartens in the period from January to June 2016

Number of ARI episodes	Čika Jova Zmaj	Plavi čuperak	Sestre Bukumirović	Mala sirena	Vivak	1001 Radost	Total
Total in the observed period	186	275	249	678	127	347	1,862
Average number per month	31	45.8	41.5	113	21.2	57.8	310.3

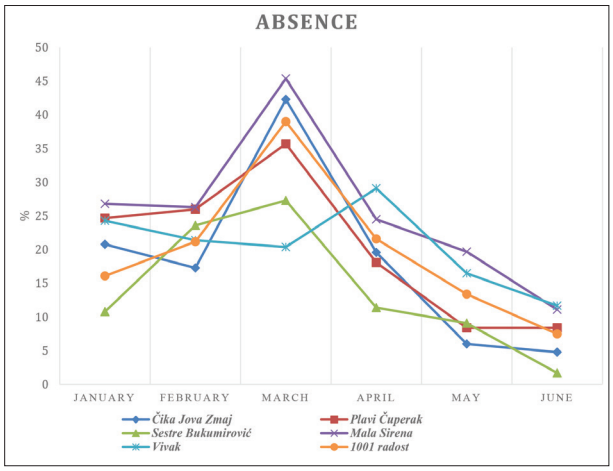

Figure 1. The absence of ill children in the observed period for each kindergarten

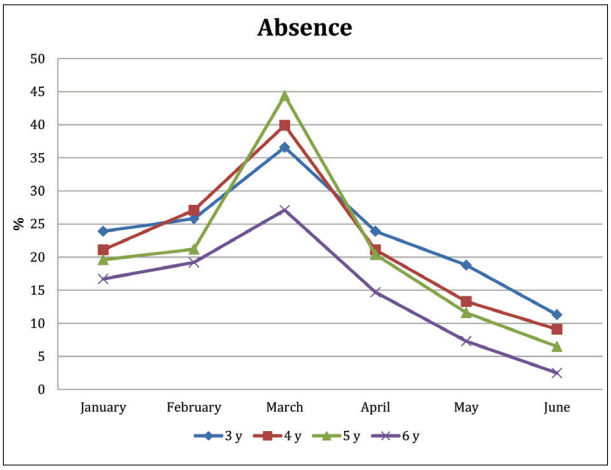

Figure 2. The absence of children in the observed period for all kindergartens

ARI ($\rho = 0.475$; $p < 0.001$). Figure 2 presents the illustration of the total absence of children between three and six years due to ARI during the observed period for six kindergartens covered by the observed sample in Čika Jova Zmaj PI. In the group of preschool children, the highest number of respiratory infections was registered in the period from January to April, with a peak in March.

Number of registered absences due to the ARI ill episodes in all kindergartens during the six-month period, and characteristics of children, in terms of their sex and whether they have siblings in the same kindergarten, is presented in Table 3. There was no statistically significant difference in relation to the sex of children, while statistically higher incidence of ARI was registered among children who had a brother and/or sister in the same kindergarten, in comparison to those who did not ($\chi^2 = 29.864$; $p < 0.001$).

As shown in Figure 3, the incidence of ARI in all six kindergartens was highest in March, 37.2% ($\chi^2 = 172.069$; $p < 0.001$), with the tendency to decline toward June, when just 7.5% were absent due to the ARI ($p < 0.001$). During the observed period, a constant number of teachers was present in six kindergartens at the Čika Jova Zmaj PI level, despite the fact that there were 25–45% less children than usual, just due to the ARI ($\rho = 0.492$; $p < 0.001$).

Table 3. Number of registered absences due to acute respiratory infections (ARI) episodes in all kindergartens during the six-month period and characteristics of children

Children characteristics	Number of children registered as absent due to ARI						Total number of registered absences
	January	February	March	April	May	June	
Sex							
Female	152	192	275	152	98	52	921
Male	161	166	293	157	101	63	941
Siblings in the kindergarten							
No	117	138	225	118	75	51	724
Yes	196	220	343	191	124	64	1138
Total	313	358	568	309	199	115	1862

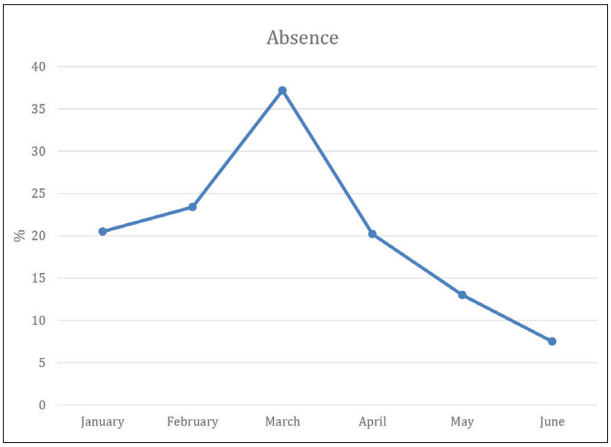

Figure 3. The absence of children in all kindergartens, all ages, during the observed period

DISCUSSION

The results of our study indicate that an increase in the number of children above the norm by 30% is a statistically significant preemptive factor for the increase in respiratory infections ($p < 0.001$). The sex of a child in any age group was not a predictive factor for the emergence of respiratory infection.

In all organizational units, March was the period when there was a statistically significant ($p < 0.001$) increase in respiratory infections and it was significantly higher ($p < 0.001$) in cases when the ill child had a sibling in the same kindergarten. During the observed period, the number of educators corresponded to the range of one educator per 12–19 children. A constant number of educators due to the number of children, which was above the norm, could not reduce the incidence of respiratory infections, especially in the period from February to March ($p < 0.001$). In the observed period from January to June 2016, the maximum incidence of respiratory infections was in March at 37.2%, with the tendency to decline to 7.5% in June ($p < 0.001$).

The consequences of respiratory infections of children attending PIs represent a significant public health problem. The ill child has to remain at home and be cared for until it recovered. It implies that one parent has to take days off work and stay at home as well, which causes work absenteeism, if there are no available family members who would look after the ill child (usually grandparents). This type of infections requires medical checkup and often prescription of antibiotic therapy, which implies certain costs. By changing the norm (by increasing the size of the space in which children stay and creating age-mixed groups), the rate of respiratory infections could be reduced. In Denmark, children enter the national kindergartens at the age of 18 months [6]. In the age group of 18–24 months, there are six children in the group, who are looked after by two people, while children 24–36 months are in groups of 12 with two educators assigned.

The risk degree for airway inflammation is three times higher in the group of children, who are two and a half years old in kindergartens, compared to the group of children of the same age, who are at home [7–10]. A Swedish group of authors state that children attending classical kindergartens are twice as likely to be absent due to illness, compared to children in small groups of three to six children [11]. Similar results are also found by an American Prospective Study, where the frequency of respiratory infections was monitored in three groups of children; the first group consists of children who do not reside in the collective daycare; the second group of children who were accommodated in family groups of two to six children; and the third group consisted of children who were attending classic kindergartens with more than seven children in the group [12]. In the third group of children aged two, there was significantly higher incidence of respiratory infections. This difference disappears after three years of residence in the collective. Danish authors did not find the connection between the amount of time children spend outdoors and in the kindergarten. However, they did find that the higher level of hygiene standards has an effect on reducing the incidence of respiratory tract infections [6]. This study shows that about 30% fewer sick days are present with children under parental care, who do not attend classic kindergartens. Chinese authors found higher risk of ARI in the group of children with allergies [13].

Within one year, out of 100 children suffering from ARI, 58 children were in kindergarten, while additional 12 were ill because of an older sibling who has been sick in the nursery. Only 30 of them had no contact with the group and they were infected by another source (inside the household 83%, neighborhood 11% and unknown 6%). Bacterial inflammation of the airways is dominant in kindergarteners, whose treatment requires the use of antibiotics, which increases the cost of treatment [12, 14].

Therefore, special significance has to be given to preventive procedures in children's collectives: avoiding overheated and dry air, regular ventilation, fresh air, well-planned nutrition, physical activity, personal hygiene, and cleanliness. Hygienic habits are an important factor in the emergence of infectious diseases in children's collectives. Thus, washing hands (a child or an educator) after wiping the nose, changing diapers, before feeding, or preparing a meal is to a large extent statistically associated with an increased incidence of respiratory illnesses [15, 16]. The equal degree of association exists regarding the claim that there are greater chances of ARI when using a commonly used towel (instead of paper towels), as well as when washing blankets less than once a week [17]. Pan et al. [18] find that increasing age of children and higher paternal education are associated with lower risk of bacterial carriage.

By changing the norm (by increasing the size of the space in which children stay and creating mixed age groups), the amount of infectious diseases could be reduced. It is also necessary to consider the socioeconomic justification of the early departure of children into nurseries (already at the age of six months), especially having in mind the spatial and staff inadequacy of the institutions for the care of infants. It should be emphasized that the cost of nursing care for a sick child is considerably higher than that of a child who stays at home because they often have someone to take care of them during the absence of their parents (unemployed parent, grandmother, etc.). In our conditions, the direct costs, the cost of sick leave (without indirect costs) and the kindergarten costs, triple the cost of treatment.

CONCLUSION

Respiratory infections at preschool age present a specific sociomedical and public health problem that could be prevented to some extent. The population of children of preschool age during the first phase of staying in a collective goes through the process of adaptation and separation from parents. The probability of early prevention is significantly reduced if we consider staying in a group during the incubation period of respiratory infections. Frequent lack of understanding of employers for the absence of parents, who need to take care of their children, causes the reduction of time used for treatment. Thus, insufficiently recovered children are often sent back to kindergarten, which causes the increased risk of repeated infection. It further induces a longer absence of parents from work in order to take care of an ill child. Therefore, strict adherence to the regulated norms of the organization of PIs and preventive measures in order to keep down respiratory infections during the winter time could be the basis for the reduction of the number of children, who suffer from respiratory infections.

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Фактори удружени са појавом акутне респираторне инфекције у предшколским установама – анализа популације од 1528 деце

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САЖЕТАК

Увод/Циљ Последице акутних респираторних инфекција деце која похађају предшколске установе представљају значајан јавноздравствени проблем. Стриктно поштовање прописаних норматива и превентивних поступака у дечјим колективима довело би до смањења стопе респираторних инфекција код деце.

Циљ нашег истраживања био је одређивање предиктора за настанак акутних респираторних инфекција у вртићу.

Методе Истраживање је спроведено као студија пресека у предшколској установи на подручју општине Вождовац током периода јануар–јун 2016. године. Циљану популацију чинило је 1528 деце узраста од три до седам година уписаних у шест вртића. Анализа података вршена је методом дескриптивне статистике (χ^2 и Спирманов тест).

Резултати Повећање броја деце изнад норматива за 30% предикторни је фактор за пораст респираторних инфекција ($p < 0,001$). Пол детета ни у једној узрасној групи није предикторни фактор за настанак респираторне инфекције. У свим организационим јединицама март је период када долази до пораста респираторних инфекција (37,2%, $p < 0,001$), значајно више ($p < 0,001$) у случајевима када оболело дете има брата и/или сестру у истом вртићу. Током периода праћења број васпитача је одговарао опсегу једног васпитача на 12 до 19 деце. Сталан број васпитача није могао да смањи учесталост респираторних инфекција, посебно у периоду фебруар–март ($p < 0,001$).

Закључак Поред активног лечења и стриктног поштовања законских прописа у организацији предшколских установа, смањење учесталости респираторних инфекција у предшколском узрасту се постиже превентивним поступцима у дечјим колективима: избегавање прегрејаног и сувог ваздуха, редовно проветравање, боравак на свежем ваздуху, добро планирана исхрана, физичке активности, лична хигијена и хигијена простора.

Кључне речи: предшколске установе; респираторне инфекције; предиктори

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Inhibitory effect of propafenone derivatives on *Pseudomonas aeruginosa* biofilm and pyocyanin production

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SUMMARY

Introduction/Objective Biofilm and pyocyanin production are essential components of *Pseudomonas aeruginosa* virulence and antibiotic resistance.

Our objective was to examine inhibitory effect of synthetized propafenone derivatives 3-(2-Fluorophenyl)-1-(2- (2-hydroxy-3-propylamino-propoxy)-phenyl)-propan-1-one hydrochloride (5OF) and3-(2-Trifluoromethyl-phenyl)-1-(2-(2-hydroxy-3-propylamino-propoxy)-phenyl)-propan-1-one hydrochloride (5CF3) on biofilm and pyocyanin in *Pseudomonas aeruginosa* clinical strains.

Methods Effects were tested on nine clinical isolates and one control laboratory strain of *P. aeruginosa*. *In vitro* analysis of biofilm growing was performed by incubating bacteria (0.5 McFarland) with 5OF and 5CF3 (500–31.2 μg/ml) and measuring optical density (OD) at 570 nm. Bacteria in medium without compounds were positive control. Blank medium (an uninoculated medium without test compounds) was used as negative control. Pyocyanin production was estimated by OD at 520 nm, after bacteria incubated with 5CF3 and 5OF (250 and 500 μg/ml), treated with chloroform, and chloroform layer mixed with HCl.

Results A total of 500 μg/ml of 5OF and 5CF3 completely inhibited biofilm formation in 10/10 and 4/10 strains, respectively. A total of 250 μg/ml of 5OF and 5CF3 strongly inhibited biofilm formation in 7/10 strains, while inhibition with 125 μg/ml of 5OF and 5CF3 was moderate. Lower concentrations had almost no effect on biofilm production. Pyocyanin production was reduced to less than 40% of the control value in 6/9, and less than 50% of the control in 7/9 strains with 500 μg/ml of 5OF and 5CF3, respectively. At 250 μg/ml 5OF and 5CF3, most strains had pyocyanin production above 50% of the control value.

Conclusion Synthetized propafenone derivatives, 5OF and 5CF3, inhibited biofilms and pyocyanin production of *Pseudomonas aeruginosa* clinical strains. Presented results suggest that propafenone derivatives are potential lead-compounds for synthesis of novel antipseudomonal drugs.

Keywords: propafenone derivatives; *Pseudomonas aeruginosa;* biofilm; pyocyanin

INTRODUCTION

As an opportunistic human pathogen, *Pseudomonas aeruginosa* has evolved a number of immunoevasive strategies to impair host defense, including growing in biofilm [1, 2].

Biofilms are bacterial clusters encased in self-produced polymeric matrix attached to the epithelial surfaces or surface of medical implants. They are characterized by lower metabolic activity, increased synthesis of protective molecules, prolonged doubling time, and genetic diversity of bacterial cells, all together improving bacterial tolerance to antibiotics and survival in harsh conditions [3, 4]. Biofilm production in P*seudomonas aeruginosa* is a well-known causative agent of antibiotic resistant infections in humans, such as pneumonia, and infections in patients with bronchiectasis and cystic fibrosis [5, 6]. Due to resistance to phagocytosis and pronounced antibody response, those infections lead to chronic inflammation, often with a severe fatal outcome [7, 8]. Thus, there is an urgent need to develop new drugs for the treatment of *Pseudomonas aeruginosa* biofilm-associated infections. In addition, *Pseudomonas aeruginosa* pathogenicity is intimately linked to its ability to produce large variety of virulence factors, including phenazine, and most abundant pyocyanin [9, 10]. Pyocyanin is highly diffusible blue pigment, which can interact with molecular oxygen and stimulate generation of oxygen radicals**,** leading to redox disbalance, injury and death of host cells [11]. As virulence factor in chronic lung infection pyocyanin disrupts redox control, inhibits respiration in human cells, accelerates neutrophil apoptosis, therefore impairing host defense and favoring bacterial persistence [12, 13, 14].

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Considering that ion channels are integral part of each living cell, which play a key role in cell division, proliferation, excitation, and apoptosis, modulators of ion channel activity have become important target molecules in medical chemistry [15]. Propiophenone is relatively simple compound commercially obtained from benzoic and propionic acid, it has channel-modulatory effect and serve as a precursor of numerous drugs (e.g. ephedrine, arylalkene) [16, 17, 18]. Propiophenone derivatives called propafenone are primarily known on their antiarrhythmic action, but they are also involved in treatment of many different diseases including lupus erythematosus, epilepsy, Alzheimer's disease, malaria, ebola, cancer [19–25]. In addition, recent studies have shown that analogs of propafenone exhibit antifungal activity [26]. Therefore, the molecule of propafenone has become a model of compounds used in multidrug-resistant studies [27].

Since data on antibacterial activity of propafenone derivatives are scarce, we decided to test potential antibacterial activity of 3-(2-Fluoro-phenyl)-1-(2- (2-hydroxy-3-propylamino-propoxy)-phenyl)-propan-1-one hydrochloride (5OF) and 3-(2-Trifluoromethyl-phenyl)-1-(2-(2-hydroxy-3-propylamino-propoxy)-phenyl)-propan-1-one hydrochloride (5CF3). Even more, because the influence of propafenone derivatives on the *Pseudomonas aeruginosa* biofilm and pyocyanin production has not yet been tested, we expanded our examinations on the influence of propafenone derivatives on expression of *Pseudomonas aeruginosa* virulence factors.

In the present study, we aimed to evaluate the inhibitory effects of ortho-fluorinated propafenone derivatives, which were synthetized in our laboratory, on biofilm and pyocyanin production in *Pseudomonas aeruginosa* clinical strains.

METHODS

Effect of ortho-fluorinated propafenone derivatives on the *Pseudomonas aeruginosa* biofilms

Test compounds

Ortho-fluorinated propafenone derivatives were synthesized at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Serbia: 5CF3: 3- (2-Trifluoromethyl-phenyl) -1- (2- (2-hydroxy-3-propylamino-propoxy) -phenyl) -propan-1-one hydrochloride and 5OF: 3- (2-Fluoro-phenyl) -1- (2- (2-hydroxy-3-propylamino-propoxy) -phenyl) -propan-1-one hydrochloride [28]. The structure of synthesized derivatives was spectrophotometrically analyzed at FT-IR spectrophotometer Nicolet iS10 (Thermo Fisher Scientific Inc., Waltham, MA, USA) [29].

The stock solutions of 5CF3 or 5OF (1 mg/ml) were prepared in 5% dimethyl sulfoxide (DMSO). The working solutions were prepared in trypticase soybean broth (TSB) with the addition of 1% glucose (Lab M Limited, Lancashire, UK) according to Knobloch et al. [30]. The concentrations of working solutions of 5CF3 or 5OF were 31.2, 62.5, 125, 250, and 500 μg/ml. In previous studies, we already investigated antimicrobial effect of tested compounds in the concentration range from 500 μg/ml to 62.5 μg/ml, and the best activity was observed for 250 μg/ml and 500 μg/ml of 5OF and 5CF3 [31].

Pseudomonas aeruginosa *clinical isolates*

The effects of tested compounds were investigated on nine clinical isolates obtained from urine (strains 1, 2, 5, 8, 9), ear swab (strains 3, 6, 7) or sputum (strain 4) and one laboratory control strain (ATCC 27853). Bacteria were stored at -70°C in Brain Heart Infusion Broth (Lab M Limited) until needed.

Culture medium

TSB and trypticase soybean agar (Lab M Limited) were used.

Analysis

Biofilm production and quantification were performed according to protocols described by Stepanović et al. [32]. Briefly, bacteria were resuspended in saline to the density of a 0.5 McFarland standard (~10^8 CFU/ml). In 96 microtiter plates, 180 μl of test compounds and 20 μl of bacterial suspension were added in triplicate. Bacteria incubated in medium without test compounds were used as positive control, while blank medium (uninoculated medium without test compounds) represented negative control. After incubation, which lasted 24h at 35°C, plates were washed in phosphate buffer (PBS, pH 7.2), dried, fixed with methanol, and stained with 2% crystal violet (Himedia, Mumbai, India). After washing, the color was extracted from bacteria with 96% ethanol. The OD was measured spectrophotometrically at 570 nm (ICN Flow Titertek Multiscan Plus, ICN, USA). Each experiment was repeated three times. To calculate the category of biofilm production, the optical density cut-off (ODc) was determined as three standard deviations above the mean OD of the negative control. According to the calculated results, all tested strains were categorized into four groups: $OD \leq ODc$ - category 0 (no biofilm production); $ODc < OD \leq 2 \times ODc$ - category 1 (weak biofilm production); $2 \times ODc < OD \leq 4 \times ODc$ - category 2 (moderate biofilm production), and $4 \times ODc < OD$ - category 3 (strong biofilm production).

Effect of ortho-fluorinated propafenone derivatives on the *Pseudomonas aeruginosa* pyocyanin production

Test compounds

Ortho-fluorinated propafenone derivatives, 5CF3 and 5OF, were synthesized at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Serbia.

Working dilutions of 250 and 500 μg/ml 5CF3 and 5OF in 5% DMSO were prepared from the stock solution of 1 mg/ml in 5% DMSO. Working concentrations were chosen based on our results about 5CF3 and 5OF effect on biofilm formation, where concentrations of 250 μg/ml and 500 μg/ml appeared to have the strongest inhibitory effect.

Pseudomonas aeruginosa *clinical strains*

The effects of tested compounds were investigated on nine *Pseudomonas aeruginosa* clinical isolates. The sources of bacteria and storage conditions were the same as previously described in section Methods.

Culture medium

Mueller–Hinton broth for bacteria (Torlak, Belgrade, Serbia) was used (Figure 1).

Pyocyanin determination

Pyocyanin was determined as previously described by Glamočlija et al. [33]. Five milliliters of bacterial cultures in exponential phase of growth were incubated with test compounds for 24h at 37°C and then treated with 3 ml of chloroform. Separated chloroform layer was mixed with 1 ml of 0.2 M HCl. OD was measured at 520 nm [34]. Positive controls for each isolate were cultivated at the same conditions in medium without tested compounds. Values were expressed as a ratio $(OD_{520}/OD_{600}) \times 100$. Two experiments, each in triplicate, were performed. Results were calculated as the percent of the pyocyanin production compared to the positive control (expressed as 100% ± SD).

Statistical analysis

Obtained data were analyzed using statistical analysis software package – SPSS Statistics Version 18.0 for Windows (SPSS Inc., Chicago, USA) and Student's t-test [35].

RESULTS

Effect of ortho-fluorinated propafenone derivatives on the *Pseudomonas aeruginosa* biofilm formation

Ortho-fluorinated propafenone derivatives, 5OF and 5CF3, inhibited production of *Pseudomonas aeruginosa* biofilms. The intensity of inhibitory effects changed in concentration dependent manner, thus, higher drug concentrations lead to stronger inhibition. The highest inhibition occurred at concentration of 500 μg/ml of both compounds. When the 5OF and 5CF3 concentrations decreased to 250 μg/ml, 125 μg/ml, 62.5 μg/ml, or 31.2 μg/ml, the inhibitory effect was also decreased. In addition, there was a variance in different isolates sensitivity to particular drug concentration. Biofilm formation was completely inhibited by 500 μg/ml of 5OF and 5CF3 in 10/10 and 4/10 strains, respectively. In 7/10 strains, biofilm formation was strongly inhibited by 250 μg/ml 5OF and 5CF3, while inhibition with 125 μg/ml 5OF and 5CF3 was moderate. In the presence of lower 5OF and 5CF3 concentrations, 62.5 μg/ml and 31.2, 8/10 tested strains exerted strong biofilm production. Categories of biofilm production in different isolates and in the presence of various concentrations of tested compounds are presented in Table 1 and 2.

Table 1. *In vitro* effect of 5OF on the biofilm production of *Pseudomonas aeruginosa*

Parameters	5OF μg/ml					
	500	250	125	62.5	31.2	Positive control
Pseudomonas aeruginosa Strain number	Category of biofilm production					
1	0	1	2	2	2	2
2	0	1	1	3	3	3
3	0	0	1	2	2	2
4	0	1	1	3	3	3
5	0	1	2	3	3	3
6	0	1	2	3	3	3
7	0	2	2	3	3	3
8	0	1	2	3	3	3
9	0	1	1	2	3	3
ATCC 27853	0	0	2	3	3	3

5OF – 3- (2-fluoro-phenyl) -1- (2- (2-hydroxy-3-propylamino-propoxy) -phenyl) -propan-1-one hydrochloride; Positive control – bacterial growth in medium without tested compound; 0 – no biofilm production; 1 – weak biofilm production; 2 – moderate biofilm production; 3 – strong biofilm production

Table 2. *In vitro* effect of 5CF3 on the biofilm production of *Pseudomonas aeruginosa*

Parameters	5CF3 μg/ml					
	500	250	125	62.5	31.2	Positive control
Pseudomonas aeruginosa Strain number	Category of biofilm production					
1	0	1	2	2	2	2
2	1	1	2	3	3	3
3	0	1	2	2	2	2
4	1	2	2	3	3	3
5	1	1	2	3	3	3
6	0	1	2	3	3	3
7	1	2	2	3	3	3
8	1	1	3	3	3	3
9	1	2	2	3	3	3
ATCC 27853	0	1	0	3	3	3

5CF3 – 3- (2-trifluoromethyl-phenyl) -1- (2- (2-hydroxy-3-propylamino-propoxy) -phenyl) -propan-1-one hydrochloride; Positive control – biofilm production in medium without tested compound; 0 – no biofilm production; 1 – weak biofilm production; 2 – moderate biofilm production; 3 – strong biofilm production

Effect of ortho-fluorinated propafenone derivatives on the *Pseudomonas aeruginosa* pyocyanin production

Ortho-fluorinated propafenone derivatives, 5CF3 and 5OF, inhibited production of pyocyanin in *Pseudomonas aeruginosa*. In the presence of 500 μg/ml 5OF or 5CF3

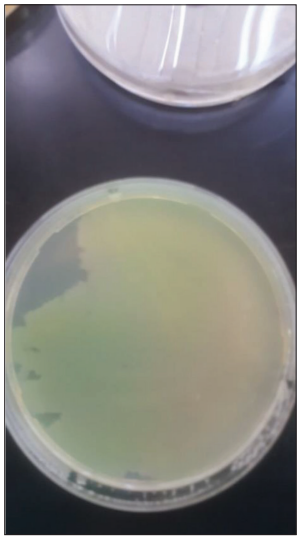

Figure 1. *Pseudomonas aeruginosa* growth on Mueller–Hinton agar

production of pyocyanin was reduced to less than 40% of the control value in 6/9 strains, and less than 50% of the control in 7/9 strains, respectively. In the presence of 250 μg/ml 5OF or 5CF3, most strains had pyocyanin production above 50% of the control value. The difference in the sensitivity to the tested compounds among various strains was also detected. Results of inhibitory action of 5OF and 5CF3 on the pyocyanin production in *Pseudomonas aeruginosa* are expressed as the percentage of the absorbance of positive controls (presented as 100% ± SD) (Table 3).

Table 3. *In vitro* effect of ortho-fluorinated propafenone derivatives 5OF and 5CF3 on the production of pyocyanin in *Pseudomonas aeruginosa* strains

Parameters	5OF μg/ml		5CF3 μg/ml	
	500	250	500	250
Pseudomonas aeruginosa Strain number	Pyocyanin production as% of positive control			
1	48.6	70.5	74	79.8
2	27.1	42	36.6	79.8
3	39.3	51.8	68.5	99.4
4	33.7	31.9	34.6	54.2
5	39.4	61.1	48.3	104.9
6	34.3	49.5	35.2	46.6
7	42.7	53.4	51	56.3
8	29.8	54.2	36.9	52.4
ATCC 27853	43.6	57.6	47.9	64

5OF – 3- (2-fluoro-phenyl) -1- (2- (2-hydroxy-3-propylamino-propoxy) -phenyl) -propan-1-one hydrochloride; 5CF3 – 3- (2-trifluoromethyl-phenyl) -1- (2- (2-hydroxy-3-propylamino-propoxy) -phenyl) -propan-1-one hydrochloride; Positive control – pyocyanin production of each isolate in the absence of the tested compounds (100%).

DISCUSSION

Antibiotic compounds that inhibit different virulence factor, such as enterotoxins, hemolysins, biofilm, or pigments, became the focus of the present research [36]. The resistance of *Pseudomonas aeruginosa* isolates to antimicrobial drugs is largely attributed to its ability to form a biofilm and produce bacterial pigment pyocyanin [37]. In this study, we used synthesized propafenone derivatives, 5CF3, and 5OF, to test inhibitory effect on *Pseudomonas aeruginosa* biofilm and pigment production.

Antimicrobials are generally dedicated to kill bacteria (bactericidal) or to inhibited bacterial growth (bacteriostatic). However, mostly due to frequent chromosomal mutations, *Pseudomonas aeruginosa* appeared to be extremely adaptive and acquired resistance to many antibiotics such as carbapenems, penicillins and cephalosporins. Recent efforts to develop novel class of anti-pseudomonas agents moved their focus to *Pseudomonas aeruginosa* physiology and collective behavior of bacterial population [38]. Therefore, biofilm formation and its modulation became a subject of our research interest. Our results have shown that propafenone derivative 5OF and 5CF3 significantly reduced biofilm production in all tested isolates of *Pseudomonas aeruginosa.* Previous study on propafenone compounds reported antimicrobial effect due to inhibition of ubiquitous bacterial multidrug efflux pumps [39]. Thus, by channel-blocking propafenone may decrease drug resistance and positively influence clinical outcome of *Pseudomonas aeruginosa* infections [40]. On the other hand, to the best of our knowledge, this study revealed identification of ortho-fluorinated propafenone derivatives as efficient agents that inhibit *Pseudomonas aeruginosa* biofilm formation for the first time. The inhibitory effects of both 5OF and 5CF3 were found. Numerous external factors affect biofilm formation by *Pseudomonas aeruginosa*. In addition, the type of tissue has strong impact on biofilm formation, and researchers commonly test biofilm formation of *Pseudomonas aeruginosa* from a variety of clinical sources [41]. In our study, various clinical strains showed differences in sensitivity to tested compounds, but those variations were not connected to specific bacterial source (urine, ear swab, sputum). However, we tested only nine clinical isolates (5 – urine, 3 – ear swab, 1 – sputum) and for such a small number of samples statistical data processing is not relevant.

The highest tested dose of both compounds (500 μg/ml) was the most efficient, reducing bacterial growth to the highest extent. However, when the concentration of test agents decreased bacterial growth recovered. In the present study, 5OF was more effective in reducing bacterial growth compared to 5CF3. This could be explained by higher binding affinity to bacterial transport porin in a case of monofluorinated propafenone derivatives (such as 5OF), compared to trifluoromethyl derivative (5CF3), as found in our docking studies (data not shown) [31]. Namely, biofilm formation depends on the presence of an extracellular matrix, which is a mixture of polysaccharides, proteins, and nucleic acids [extracellular DNA (eDNA)]. Matrix polysaccharides (alginate and lipopolysaccharides), which are synthetized in bacterial cytoplasm**,** bind to membrane transporters to be extruded out of the cell [42]. It was found that both fluorinated derivatives tested in this study briefly occupied key substrate-specific sites in the bacterial porin (Arg124). This discovery might be associated with interruption of the transport of carbohydrate compounds involved in synthesis of biofilm [43].

The blue pigment pyocyanin, chemical derivative of phenazine, is one of the most important virulence factors in *Pseudomonas aeruginosa* [44]. Pyocyanin is toxic for respiratory epithelium, it acts on the cell structure and function, disrupts normal expression of genes involved in efflux pumps, redox homeostasis and iron acquisition in human cells [45, 46, 47]. Thus, control of pyocyanin production may be a mechanism to reduce bacterial pathogenicity. Results of our study have shown that both 5OF and 5CF3 inhibited production of pyocyanin in all tested *Pseudomonas aeruginosa* isolates. The inhibitory effect was concentration dependent, higher concentrations caused stronger inhibition, while inhibitory effect decreased with lower drugs concentration. Literature survey on other drugs suggests that ortho-fluorinated propafenone derivative 5OF had significantly stronger inhibitory effect on the production of pyocyanin in *Pseudomonas aeruginosa* strains than commercial antibiotics ampicillin or streptomycin. Namely, we have shown that lower concentrations of 5OF, 500μg/ml, and 250 μg/ml, exerted same or even enhanced inhibitory effect compared to commercial antibiotics when applied in 2–4 times higher concentration (1 mg/ml) [33, 48]. Similarly, the concentrations of 5CF3, which led to pyocyanin reduction, were within the same range as concentration of standard drugs. The observed propafenone-induced pyocyanin inhibition could be discussed in a view of recent results on pyocyanin impact on eDNA and biofilm formation [11]. It was found that pyocyanin decreases eDNA content within the *Pseudomonas aeruginosa* biofilm. Since eDNA promotes bacterial adhesion and cellular aggregation, depletion of eDNA can reduce biofilm strength and disturb protection of bacterial cells against antibiotics. Based on the aforementioned, it was assumed that reduction of pyocyanin production as detected in our study could be a model of propafenone derivatives action against *Pseudomonas aeruginosa* pathogenicity and infection.

CONCLUSION

The results of the study suggest that synthetized ortho-fluorinated propafenone derivatives inhibit biofilm and pyocyanin production in *Pseudomonas aeruginosa* clinical strains. Presented results suggest that propafenone derivatives could be considered as potential lead-compounds for synthesis of novel antipseudomonal drugs.

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This study was done in accordance with the institutional standards of the Committee on Ethics.

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Conflict of interest: None declared.

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Инхибиторни ефекат пропафенонских деривата на продукцију биофилма и пиоцијанина код бактерије *Pseudomonas aeruginosa*

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САЖЕТАК

Увод/Циљ Производња биофилма и пиоцијанина је важан фактор вируленције и антибиотске резистенције бактерије *Pseudomonas aeruginosa.*

Циљ рада је био да се испита инхибиторни ефекат синтетисаних пропафенонских деривата, 3-(2-флуоро-фенил)-1-[2-(2-хидрокси-3-пропиламино-пропокси)-фенил]-пропан-1-он-хидрохлорид) (5*OF*) и 3-(2-трифлуорометилфенил)-1-[2-(2-хидрокси-3-пропиламино-пропокси)-фенил]-пропан-1-он-хидрохлорид) (5*CF*3), на продукцију биофилма и пиоцијанина код клиничких изолата бактерије *Pseudomonas aeruginosa.*

Методе Ефекат пропафенонских деривата испитан је на девет клиничких изолата и једном стандардном сојe бактерије *P. aeruginosa.* Утицај на продукцију биофилма испитан је *in vitro,* инкубацијом бактерија (0,5 по Макфарланду) са 5*OF* и 5*CF*3 (500–31,2 *μg/ml*), и мерењем оптичке густине на 570 *nm*. Бактерије у медијуму без испитиваних једињења су биле позитивна контрола, а сам медијум негативна контрола. Производени пиоцијанин, који је одређиван мерењем оптичке густине на 520 *nm*, на коинкубације бактерија са 5*CF*3 или 5*OF* (250 и 500 *μg/ml*), третиран је хлороформом и мешањем хлороформског слоја са *HCl*.

Резултати При концентрацији од 500 *μg/ml* 5*OF* је довео до потпуне инхибиције продукције биофилма код свих испитиваних сојева (10/10). Инхибиција биофилма са 500 *μg/ml* 5*CF*3 била је потпуна код 4/10 сојева. При концентрацији 5*OF* и 5*CF*3 од 250 *μg/ml* продукција биофилма код већине испитаних изолата била је слаба, док је при концентрацији 125 *μg/ml* 5*OF* односно 5*CF*3 продукција била умерена. Ниже концентрације 5*OF* и 5*CF*3 нису имале инхибиторни ефекат на формирање биофилма. У присуству 500 *μg/ml* 5*OF* у 6/10 испитиваних сојева продукција пиоцијанина пала је на мање од 40% у односу на контролну вредност. Иста концентрација (500 *μg/ml*) 5*CF*3 снизила је продукцију пиоцијанина на мање од 50% од контроле у 7/9 сојева. При концентрацији 250 *μg/ml* 5*OF* или 5*CF*3 већина сојева продуковала је пиоцијанин изнад 50% у односу на позитивну контролу.

Закључак Синтетисани пропафенонски деривати, 5*OF* и 5*CF*3, инхибирају продукцију биофилма и пиоцијанина код клиничких сојева бактерије *Pseudomonas aeruginosa.* Добијени резултати указују на то да пропафенонски деривати представљају могућа полазна једињења за синтезу нових антипсеудомонасних агенаса.

Кључне речи: пропафенонски деривати; *Pseudomonas aeruginosa;* биофилм; пиоцијанин

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Novel *PANK2* mutation identified in a patient with pantothenate kinase-associated neurodegeneration

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SUMMARY

Introduction Pantothenate kinase-associated neurodegeneration (PKAN) is a rare, recessively inherited disorder caused by mutations in the pantothenate kinase 2 (*PANK2*) gene on chromosome 20p13. The objective of this report is to present a patient with atypical PKAN with the novel heterozygous *PANK2* mutation.

Case outline We present a 32-year-old female who had disease onset at the age 20 (depression, speech, chewing problems and backward falls) with progressive course. Neurological examination revealed hypomimia, *risus sardonicus,* dysphagia, tachylalia and severe dystonic dysarthria, moderate arms, legs, and jaw-opening dystonia, postural instability, urge incontinence, and decreased visual acuity. Brain magnetic resonance imaging revealed iron accumulation in the bilateral globus pallidus and putamen ("eye-of-the-tiger"), a radiological finding pathognomonic for PKAN. Genetic analysis revealed known mutation p.T528M (c.1583C>T) in exon 6, and novel p.Y405D (c.1213T>G) in exon 3 of the *PANK2* gene. *In silico* analyses strongly suggested this mutation to be pathogenic.

Conclusion We report a patient with PKAN, and novel substitution p.Y405D (c.1213T>G) in *PANK2* that has not been previously described in PKAN patients.

Keywords: neurodegeneration with brain iron accumulation; pantothenate kinase-associated neurodegeneration; *PANK2*

INTRODUCTION

Pantothenate kinase-associated neurodegeneration (PKAN) is a recessively inherited disorder caused by bi-allelic mutations in the pantothenate kinase 2 (*PANK2*) gene on chromosome 20p13 [1]. Two most frequent mutations (c.1231G > A, c.1253C > T) account for about one-third of all cases; however, to date, 155 different mutations have been reported [2].

Typical PKAN presents in early childhood with gait difficulty (spastic/dystonic gait), in almost 90% of the patients, followed by generalized pyramidal and extrapyramidal features (mainly dystonia), neuropsychiatric involvement, and pigmentary retinopathy. Clinical course is progressive and affected children generally become wheelchair-bound within a few years [3, 4].

Atypical PKAN presents later with less pronounced motor involvement, but cognitive decline and psychiatric features may be prominent [5]. Disease progresses over the first five years, followed by a long-lasting, rather stable period of slower progression [6].

In this report, we present a patient with atypical PKAN with the novel heterozygous *PANK2* mutation.

CASE REPORT

A 32-year-old female, born from a non-consanguineous marriage, had unremarkable family history (her brother was diagnosed with spondylitis ankylopoietica). Delivery and developmental milestones were normal. At the age of 20, she was treated by a psychiatrist due to depression. At that time, she noticed speech and chewing problems, and frequent backward falls. Three years later, urge incontinence appeared and gradually worsened. The patient sought medical care at the Clinical Centres of Zagreb and Clinical Hospital in Osijek (Croatia) and Belgrade (Serbia). In the course of years, she experienced slow progression of symptoms and gradual but slight worsening of gait, speech, and postural stability.

Laboratory findings examined in the course of her illness (since the onset of the symptoms until present) included normal serum ferritin, ceruloplasmin, albumin, liver tests, copper (workup for Wilson's disease), and lipoprotein levels. The blood smear was negative for acanthocytes. After several years of the disease, brain magnetic resonance imaging (MRI) revealed iron accumulation in the bilateral globus pallidus and putamen ("eye-of-the-tiger") (Figure 1). We examined her after obtaining brain MRI, and due to the typical "eye-of-the-tiger"

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finding, we diagnosed her with neurodegeneration with brain iron accumulation (NBIA) – PKAN.

Personal neurological examination (neurological reports were also used for the follow-up of the patient's status) 12 years after disease onset, revealed hypomimia, *risus sardonicus*, dysphagia, tachylalia and severe dystonic dysarthria, moderate arms, legs, and jaw-opening dystonia, postural instability, and decreased visual acuity. Tendon reflexes were brisk, Babinski sign negative. Her gait was unstable. Cerebellar signs and Romberg test were negative. The Mini Mental State Examination score was 30/30. Twelve years after the symptoms onset she was still able to walk unassisted and to take care of herself.

At the moment of examination, her psychological status was within the normal range, without symptoms of depression.

DNA was extracted using a commercial kit. After PCR amplification of the *PANK2* exons 5 and 6 and surrounding regions, direct Sanger sequencing was performed using BigDye Terminator v.3.1 Cycle Sequencing kit (Thermo Fisher Scientific – Life TEchnology, Waltham, MA, USA) on ABS 3500 Genetic Analyzer (ABS Global, Inc., DeForest, WI, USA). For data analysis, Sequencher software (Gene Codes Corporation, Ann Arbor, MI, USA) was used. After detection of only one heterozygous *PANK2* mutation in exon 6, the analysis was continued by next generation sequencing (NGS) of DNA. We used TruSight One Panel (Illumina, Inc., San Diego, CA, USA) and MiSeq NGS platform (Illumina, Inc.). Data analysis was performed by the Variant Studio provided for Illumina users. *In silico* characterization of the detected gene variants was performed by PolyPhen, Shift, MetaLR, REVEL, and MutationTaster software. Confirmation of NGS-detected *PANK2* mutation was done by Sanger sequencing after PCR amplification of the target region, as described above.

Initial targeted sequencing of selected *PANK2* gene exons reveled known mutation p. T528M (c.1583C>T) in exon 6, in heterozygous state. In addition, NGS analysis detected substitution p.Y405D (c.1213T>G) in exon 3, also as a heterozygous change.

This change was confirmed in our patient by another targeted Sanger sequencing. Substitution c.1213T>G at transcript NM_153638.2 is the missense mutation leading to replacement of tyrosine to aspartic acid at amino acid position 405. This change was not detected previously in population databases ExAC and 1000G and it is also absent from disease-related bases ClinVar, LVOD, and HGMD. The variant is located in exon 3 of the *PANK2* gene that corresponds to catalytic domain of the protein, and this nucleotide and amino acid position is evolutionary highly conserved. According to the *in silico* prediction, p.Y405D (c.1213T>G) is ranged as deleterious (by Sift), probably damaging (by PolyPhen), or damaging (by MetaLR), likely disease-causing (by REVEL), and disease-causing (by MutationTaster). Aforementioned features are sufficient to classify this variant as (likely) pathogenic [7].

DNA analysis revealed that the proband's neurologically healthy father and brother were heterozygous carriers of the known p.T528M (c.1583C>T), while healthy mother was a heterozygous carrier of the newly described p.Y405D (c.1213T>G) mutation.

DISCUSSION

Clinical presentation of our patient was consistent with atypical PKAN based on time of the disease onset, neurological features, the presence of behavioral and psychiatric abnormalities, and the mode of disease progression. In addition to characteristic MRI scans, mutational analysis confirmed the diagnosis.

Initial complaints were psychiatric, in accordance with the previous findings that psychiatric symptoms (depression, anxiety, emotional lability, tics, obsessive-compulsive disorder, and psychosis) were common in the atypical PKAN, often preceding motor features [6, 8, 9].

DNA analysis showed one known mutation and one newly described variant in the *PANK2* gene. Substitution c.1583C>T (p.T528M) is one of the most common mutations in European NBIA patients, and confirmed founder mutation in the Serbian population [10]. This variant affects catalytic domain of the enzyme; frequently it is associated with an atypical form of PKAN, supporting biochemical data of residual enzyme activity.

Substitution p.Y405D (c.1213T>G) has not been previously described in NBIA patients. Also, this variant was not found in 1000 Genomes and ExAC population databases nor in disease-related databases such as ClnVar, LVOD, and HGMD. Several *in silico* predictions indicate that this variant is damaging. In addition, segregation analysis confirmed p.Y405D (c.1213T>G) is in trans with an already known disease-related mutation p.T528M, which all support its own pathogenicity.

Previous reports have demonstrated that in patients with two loss-of function alleles, symptoms were always presented at an early stage of life, while those in atypical patients often resulted in amino acid changes. This indicated that many of the patients with an atypical form of the disease may have residual *PANK2* activities. It is believed that in the presence of missense mutations, residual activity of the *PANK2* determines the age of onset, without playing a role in the progression of the disorder [11]. Although variable expressivity of alleles, as well as the combination and the concentration of the mutant proteins, were the features that mainly affected the PKAN phenotype, there were also other genetic and non-genetic modifiers that might alter *PANK2* catalytic activity [12–15].

Although we were unable to determine the enzymatic activity of *PANK2* in our case, these compound heterozygous mutations may have been responsible for the adult onset and delayed progressive nature of the disease. Our novel *PANK2* mutation may probably add to understanding the clinic–genetic correlations in atypical PKAN.

NOTE

Ethical compliance statement: We confirm that we have read the journal's position on issues involving ethical publication and affirm that this work is consistent with those guidelines.

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

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Нова мутација у гену *PANK2* код болесника са неуродегенерацијом удруженом са пантотенат-киназом

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САЖЕТАК

Увод Неуродегенерација удружена са пантотенат-киназом (*PKAN*) ретко је, аутозомно рецесивно обољење узроковано мутацијама у гену за пантотенат-киназу 2 (*PANK2*) на хромозому *20p13*.

Циљ овог рада је приказивање болесника са атипичним обликом *PKAN* који је носилац новооткривене хетерозиготне мутације.

Приказ болесника Приказујемо жену стару 32 године чија је болест почела у двадесетој години (депресија, проблем са говором и гутањем и падови уназад) и има прогресиван ток. Неуролошким прегледом уочени су хипомимија, *risus sardonicus*, дисфагија, тахилалија и тешка дистоничка дизартрија, умерена дистонија руку, ногу и дистонија отварања вилице, постурална нестабилност, ургенција микције и смањена оштрина вида. Преглед мозга магнетном резонанцом указао је на таложење гвожђа у глобусу палидусу и путамену обострано (знак тигровог ока), а радиолошки налаз је био патогномоничан за *PKAN*. Генетском анализом откривена је одраније позната мутација *p.T528M* (*c.1583C>T*) у егзону 6, и нова мутација *p.Y405D* (*c.1213T>G*) у егзону 3 гена *PANK2*. Анализа *in silico* указује да је новооткривена мутација патогена.

Закључак Приказали смо болесницу са *PKAN* и новом мутацијом *p.Y405D* (*c.1213T>G*) у *PANK2,* која никада раније није описана код болесника са *PKAN*.

Кључне речи: неуродегенерација са таложењем гвожђа; неуродегенерација удружена са пантотенат-киназом; *PANK2*

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Exogenous lipoid pneumonia mimicking multifocal subpleural tumors

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SUMMARY

Introduction Exogenous lipoid pneumonia (ELP) is caused by inhalation or aspiration of different oily substances of animal, vegetable, or mineral origin. It can be in acute or chronic form. Herein, we report a case of ELP in its chronic form, confirmed in surgical lung biopsy.

Case outline A 47-year-old male locomotive engineer, former smoker, without clinical symptoms, with a history of pneumonia two years previously, was referred to our institution. The operating diagnosis of multifocal subpleural tumors was made based on the chest computed tomography. A surgical lung biopsy confirmed a diagnosis of ELP.

Conclusion Diagnosis of ELP is frequently made after surgical biopsy performed for suspected neoplasm, because of neglecting profesional exposure to mineral oils.

Keywords: exogenous lipoid pneumonia; machine oil; surgical biopsy

INTRODUCTION

Lipoid pneumonia is an uncommon lung disease caused by the presence of lipids in the alveoli. It is classified as exogenous or endogenous. The endogenous type occurs secondary due to pulmonary alveolar proteinosis, chronic pulmonary bacterial or fungal infections, lipid storage diseases, and bronchial obstruction by tumors or broncholithiasis. Exogenous lipoid pneumonia (ELP) type is associated with the inhalation or aspiration of different oils. The clinical symptoms and radiological findings of ELP are nonspecific, depending on the patient's age, the amount of oily substances, and the length of the inhalation or aspiration period [1–5]. Different pulmonary diseases can resemble ELP. The diagnosis of ELP is based on a history of exposure to oil and the presence of lipid-laden macrophages in sputum or bronchoalveolar lavage or histopathology specimens [1, 5, 6].

There are no standard protocols for the treatment of ELP, but recommendations include discontinuing exposure to the oily agent, oxygen therapy, lung lavage, systemic corticosteroids, and surgical resection of lung tissue unresponsive to medical treatment [1, 4, 5, 6]. Various complications of ELP that can be found in the literature [7].

In the text below, we report a case of chronic form ELP confirmed by surgical lung biopsy.

CASE REPORT

A 47-year-old male who worked as a locomotive engineer was referred to our institution for the evaluation of lung disease which was initially diagnosed as a multifocal subpleural tumor (lipoma or fibroma). The patient, who was a former smoker, had no clinical symptoms and his only medical condition was pneumonia diagnosed two years previously. Chest computed tomography (CT) was performed. Nodular masses were present, measuring 30 mm in the upper and 34 mm in the left middle lobe, with fat density (Figure 1). Bronchoscopy samples were nondiagnostic. Two CT scans and two bronchoscopies were performed afterwards, but the nature of the disease was not clarified and the patient was admitted to our institution.

A physical and cardiovascular examination and routine blood tests showed no abnormal findings. A high-resolution CT scan was performed and showed persistent radiological findings. At a consultative meeting, a decision was made to perform video-assisted thoracoscopic surgery (VATS). VATS was performed and lung biopsy from the middle lobe showed a nodule with cavitation (Figure 2). There were multinucleated giant cells and lipid-laden macrophages in the cavity wall. Chronic interstitial lymphoplasmacytic inflammation formed well-circumscribed aggregates around airways in multiple areas of bioptic sample (Figure 3). Additional immunohistochemical analysis (panCK, vimentin, CD68, EMA, CD1a, CD10, and S100) excluded malignant diseases and set the diagnosis of ELP (Figure 4).

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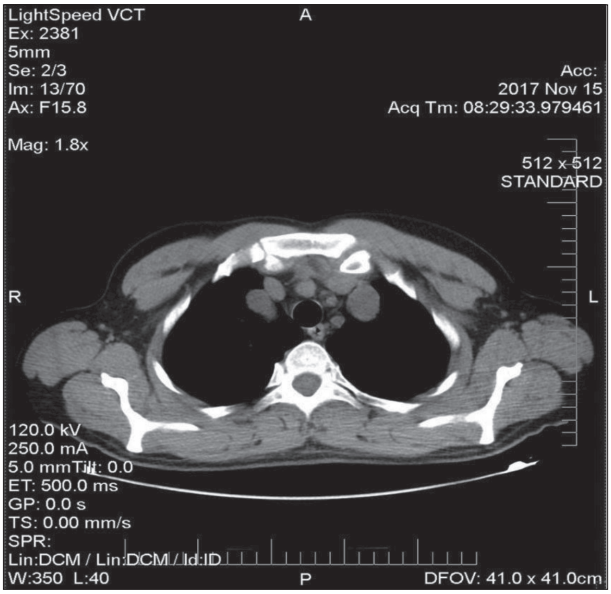

Figure 1. Chest computed tomography showing bilateral nodules

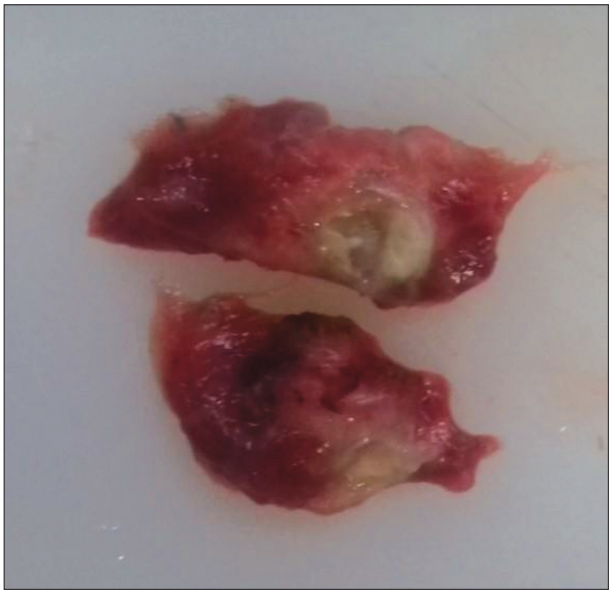

Figure 2. A node with a cavity in the surgical biopsy sample

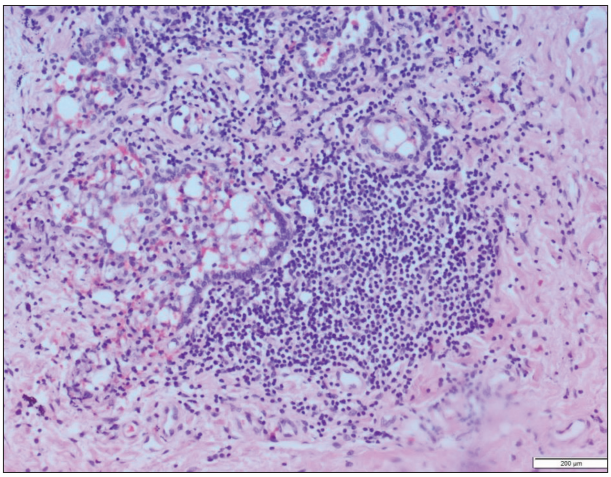

Figure 3. Chronic interstitial inflammation consisting of dense bronchocentric lymphoplasmacytic infiltrates (H&E, 10×)

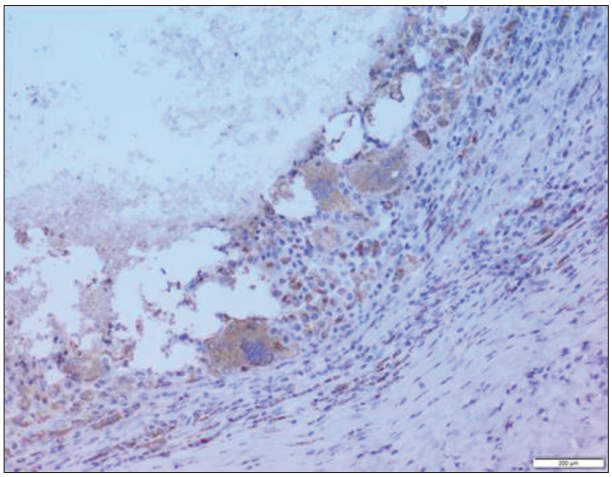

Figure 4. CD68 positivity in multinucleated giant cells (immunohistochemistry, 10×)

Six months after surgery, there was no radiological regression of other described lesions and pulmonary function tests were not modified.

This case report was approved by the institutional ethics committee, and written consent was obtained from the patient for the publication of this case report and any accompanying images.

DISCUSSION

ELP is the most common type of lipoid pneumonia and has been reported as a result of aspiration or inhalation of oil substances (animal, vegetal, or mineral origin) [4, 5, 6, 8]. In our case, no exogenous source was found initially. Once the histologic diagnosis was made, we asked the patient precisely if he had been exposed to mineral oils or had some risk factor for aspiration. The patient sad he had been working as a locomotive engineer for the last 20 years and had been in contact with mineral oil every working day. For this reason, we believed that in our case, ELP was the result of professional exposure to mineral oil.

ELP can be classified as acute or chronic. The acute form of ELP is caused by accidental aspiration of a large quantity of mineral oil in a short period of time. CT scan opacities are typically ground-glass or consolidative and can be seen in most patients within 24 hours [9, 10, 11]. The chronic form typically occurs in older patients with predisposing anatomic or functional abnormality in swallowing, but it has also been reported in children with cleft palate and mental retardation. The diagnosis of chronic form of ELP is set on average 38 days after the onset of nonspecific clinical symptoms such as cough, fever, weight loss, vomiting, and recurrent respiratory infections [12, 13, 14]. In contrast to other cases, our patient had no symptoms.

ELP may occur in all ages, most commonly in patients with gastroesophageal reflux, palpitations, swallowing dysfunction, and after administration of drugs [1, 2, 12, 13]. This opinion was confirmed by Sias et al. [1], who analyzed ELP formed as a consequence of the use of laxatives as a

result of intestinal obstruction due to Ascaris lumbricoides. This study involved 15 girls and 13 boys aged 1–108 months. In contrast to the above data, our patient was older, as in most published papers with individual ELP case reports [2, 3, 6].

Radiological changes are non-specific, mostly localized to the right lung [1, 3]. Changes seen on CT are also nonspecific and may be unilateral, bilateral and multifocal, consolidation with air bronchogram, crazy-paving, interlobular septal thickening, cavitation, and calcification [1, 14, 15, 16]. Jin et al. [4] analyzed 18 cases and found this change in 13 patients: geographical lobular distribution of ground-glass, miliary changes on both sides, interstitium thickening, cavitation, and mediastinal pneumatosis. The fact that it is difficult to set the ELP diagnosis to 18F-fluorodeoxyglucose positron-emission tomography is confirmed by the case of a patient suffering from Kaposi sarcoma. Two spicular changes (25 mm and 9 mm, respectively) in the upper right lobe showed fat density (-30–150 HU) on the CT scan, but the value of SUV 5 without local and distal expansion induced suspicion for a malignant tumor; therefore, lobectomy was multidisciplinary suggested and performed. Pathohistological examination confirmed ELP [8]. A differential diagnosis of ELP includes the following: nonspecific interstitial pneumonia, collagen vascular diseases, chronic eosinophilic pneumonia, idiopathic pulmonary fibrosis, hypersensitive pneumonitis, sarcoidosis, lung tumors (benign and malignant), bacterial pneumonia (acute and chronic), and pulmonary alveolar proteinosis [5, 17]. In our case, nodular lesions registered on CT were initially diagnosed as a bilateral subpleural lipoma or fibroma as their mean density was -30 HU.

ELP may be indicated by the following: data on aspiration or inhalation of oily substances, radiological findings and the presence of lipid-laden macrophages in the sputum, bronchoalveolar lavage, or histological sample [1, 3, 9]. Upon macroscopic examination, lung parenchyma is usually consolidated and yellowish stained, while cavitations are rare [18]. Pathohistological examination can show bronchocentric lymphoplasmacytic cell infiltration with multinucleated giant cells with cholesterol crystals in the cytoplasm, intraalveolar clumps of alveolar macrophages, giant cell granulomas, chronic inflammatory reaction, and interstitial fibrosis [5, 6]. Long-term exposure to an oily material can lead to the development of lung fibrosis with the destruction of normal parenchyma and the development of pulmonary heart, while bacterial superinfections and pulmonary aspergillosis are rare [16, 19]. In our case, ELP was confirmed on permanent paraffin sections and additional immunohistochemical analysis of a surgical sample obtained by VATS. We found a 15-mm-diameter nodule with the cavity filled with friable yellowish-white content and multinucleated giant cells and lipid-laden macrophages in the cavity wall.

The prevention of exposure to oily substances, supportive oxygenotherapy, multiple bronhoalveolar lavage, steroid therapy, and surgical resection represent several modalities of ELP treatment [1, 6, 7]. It was suggested to our patient to avoid machine oil, with a recommendation that the remaining nodular changes should be surgically removed after complete recovery, which he accepted.

Conflict of Interest: None declared.

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Егзогена липоидна пнеумонија која опонаша мултифокалне субплеуралне туморе

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САЖЕТАК

Увод Егзогена липоидна пнеумонија (ЕЛП) узрокована је инхалацијом или аспирацијом различитих уљаних супстанци животињског, биљног или минералног порекла. Може да буде акутна или хронична форма.

Приказујемо случај хроничне форме ЕЛП који је потврђен у хируршком биопсијском узорку.

Приказ болесника Мушкарац стар 47 година, машиновођа, бивши пушач, без клиничких симптома, две године после прележане пнеумоније јавио се у нашу установу. Радна дијагноза мултифокалних субплеуралних тумора је била заснована на компјутеризованој томографији грудног коша. Дијагноза ЕЛП је потврђена у хируршком биопсијском узорку.

Закључак Дијагноза ЕЛП се често поставља на хируршкој биопсији учињеној због сумње на неоплазму, услед занемаривања података о професионалној изложености минералним уљима.

Кључне речи: егзогена липоидна пнеумонија; машинско уље; хируршка биопсија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Pickwickian syndrome – "the tip of the iceberg" in extremely obese patients

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SUMMARY

Introduction Pickwickian syndrome (PS), also known as hypoventilation syndrome in adults, consists of three factors: obesity [Body Mass Index (BMI) > 30 kg/m^2], daytime hypercapnia and sleep-disordered breathing, after ruling out other disorders that may cause alveolar hypoventilation. Timely recognition of PS is of utmost importance because such patients have significant morbidity and mortality. However, recent data indicate that PS is under-recognized and under-treated. We report a case of early-identified PS prehospitally with a favorable outcome after hospital treatment.

Case outline A 67-year-old female patient was diagnosed prehospitally, and the diagnosis was later confirmed in hospital. Diagnostic criteria were as follows: BMI > 45,7 kg/m^2 (height 170 cm, weight 132 kg), hypercapnia, hypoxemia and respiratory acidosis (pCO_2 – 41 mmHg, pO_2 – 56 mmHg, pH 7.45) in the absence of other causes of hypoventilation. During hospitalization, the following diagnostic procedures were performed: standard laboratory analyses, chest radiography, electrocardiography, abdomen and heart echocardiography. An attempted sleep study (polysomnography) was interrupted due to a drop in oxygen saturation levels. Non-invasive mechanical ventilation and a diet were used as the first line of therapy. However, due to the development of a global respiratory insufficiency, the patient was intubated and placed on a mechanical ventilator. After 30 days of hospital treatment, the patient was released in a satisfactory general condition with recommendations for weight reduction and symptomatic therapy.

Conclusion As obesity is becoming an epidemic of modern society, early recognition and treatment of PS is of crucial importance.

Keywords: obesity; Pickwick syndrome; early recognition; treatment

INTRODUCTION

The increase in the prevalence of extreme obesity in the last decade is a health, economic and demographic problem of global proportions. Overweight and obesity cause 3.4 million deaths a year [1]. Classification of obese adults based on body mass index (BMI) (obese class I: BMI 30–34.9; obese class II: BMI 35–39.9; obese class III: BMI ≥ 40) and relative risk assessment of morbidity (elevated, moderately elevated and highly elevated) was made by World Health Organization in 1997. [2]. According to the results of the 2013 Health Survey in the Republic of Serbia, based on the measured BMI, more than half (56.3%) of the population was overweight (35.1% pre-obese and 21.2% obese) [1]. The average BMI value in the adult population of Serbia is 26 ± 4.74 kg/m^2.

Among the many complications of obesity, respiratory tract disorders are in the shadow of metabolic and cardiovascular complications, so they have been extremely rarely mentioned in our surroundings [3]. Types of respiratory disorders in obese people may be different:

1. respiratory function disorders without alveolar hypoventilation;
2. obesity hypoventilation syndrome (OHS);
3. obstructive sleep apnea syndrome (OSAS);
4. risk during and after surgical interventions [3].

OHS, also historically described as the Pickwickian syndrome (PS), is defined as daytime hypercapnia and hypoxemia ($PaCO_2$ > 45 mmHg and PaO_2 < 70 mmHg at sea level) in an obese patient (BMI > 30 kg/m^2) with sleep-disordered breathing in the absence of any other cause of hypoventilation [4]. OHS is a diagnosis of exclusion. Other causes of hypoventilation, such as chronic obstructive pulmonary disease, severe interstitial lung disease, mechanical respiratory limitation (for example, chest wall disorders such as kyphoscoliosis), myopathies (such as myasthenia gravis), neurological diseases, central causes (such as cerebrovascular disease and untreated hypothyroidism), and congenital causes (such as Ondine's syndrome), should be ruled out. OHS often remains undiagnosed until late in the course of the disease. Its exact prevalence is unknown, but it has been estimated that 10–20% of obese patients with obstructive sleep apnea have hypercapnia [5]. Early recognition is important because these patients have significant morbidity and mortality. Effective treatment can lead to significant improvement in patient outcomes, underscoring the importance of early diagnosis and early treatment [6].

We report a case of prehospitally identified PS with a favorable outcome after hospital treatment.

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CASE REPORT

An emergency medical service team intervened because of severe choking of a 67-year-old female patient. She lived alone. The emergency medical service doctor found that the patient is extremely centrally obese (android type), BMI 45.7 kg/cm^2 (height 170 cm, weight 132 kg), moving with difficulty. In medical history, the patient previously stated difficulties breathing, worsening in the lying position, fatigue even during minor activities, as well as all-day drowsiness. The problems have been more pronounced over the previous seven days. Furthermore, she has urinary incontinence and "swollen stomach". The patient treats hypertension with fosinopril, which she takes irregularly. She had been smoking for the last 40 years (three packs a day). The patient denies any loss of consciousness, allergies, previous pulmonary, otorhinolaryngological, neurological, cardiological, metabolical (diabetes mellitus) and endocrinological diseases, as well as chronic use of sedatives. On examination, the patient is mildly somnolent (Glasgow Coma Score 13), oriented, afebrile, dyspnoic (respiratory rate 20/min.), facial plethora, cyanotic (central cyanosis), anicteric. Patient's aspect was severe. There was weakened respiratory noise above the lungs, crackles basal left, oxygen saturation (SaO_2) 44%. Heart rate was rhythmic, sounds somewhat quieter. Blood pressure was 160/90 mmHg. The palpation of internal abdominal organs was difficult due to pronounced obesity. No peripheral edema. Electrocardiography (ECG): sinus rhythm, heart rate 110/min., S wave in D1 and from V1 to V6, without acute changes in the ST segment. Hundred-percent O_2 is applied through an oxygen mask at a dose of 6 L/min. The patient was transported to hospital under the diagnosis of suspected PS.

On admission to hospital, the patient's status remained unchanged, with slightly corrected SaO_2 (58%). Gas analyses with no oxygen therapy when awake were: pO_2 56 mmHg, pCO_2 41 mmHg, pH 7.45. Due to the development of a global respiratory insufficiency (pO_2 34 mmHg, pCO_2 67 mmHg, pH 7.24) and the need for ventilatory support, the patient was moved to the Respiratory Unit. She is initially connected to non-invasive mechanical ventilation. Because of inefficient gas exchange, the patient was intubated and placed on a mechanical ventilator. After being stabilized, the patient was extubated, connected to non-invasive mechanical ventilation and then put on oxygen therapy.

Table 1 shows diagnostic procedures performed during hospitalization. Laboratory test results are shown in Table 2.

The treatment included: therapeutic diet (very-low-calorie diet), crystalloid infusions, electrolytes, antibiotics (ceftazidime, moxifloxacin, vancomycin), anticoagulants (low-molecular-weight heparin, then oral), angiotensin converting enzyme inhibitors, Ca antagonists, gastrointestinal agents and other symptomatic and supportive therapies.

Due to paroxysms of atrial fibrillation (Figure 1), amiodarone was included. The patient was converted to sinus rhythm with occasional paroxysmal atrial fibrillation.

After 30 days of hospital treatment, the patient was released in a satisfactory general condition with recommendation of the following therapy: lifestyle interventions (dietary changes and physical exercise), amiodarone 200 mg 1 × 1 (five days), enalapril 10 mg 2 × 1, amlodipine 5 mg 1 × 1, furosemide 40 mg 1 × 1 with 1 gr KCl, acenocoumarol 1 × 1/2 until international normalized ratio medical check-up (goal international normalized ratio between 2 and 3), pantoprazole 20 mg 1 × 1. The patient had scheduled appointments with a pulmonologist and a cardiologist 15 days after hospital release, and glycemia and Hemoglobin A1c tests after a month.

DISCUSSION

A high suspicion of PS is critical for setting the PS diagnosis [7]. Our patient fulfilled the clinical criteria (SpO_2 44%, dyspnea on exertion, but also at rest, in unbecoming and uncomfortable positions of the body, facial plethora, elevated level of bicarbonates in the blood). According to

Table 1. Diagnostic test and results

Diagnostic test	Results
Chest *x-ray*	Left basal accentuated vascular markings with suspected initial signs of consolidation. Slightly voluminous chylous fluid. No signs of pleural effusion.
Echocardiographic examination of the heart complicated by the constitution.	Left pulmonary artery dilated 46 cm seems free. Mitral valve degenerately altered velum, mild mitral regurgitation and transmitral flow in the type of pseudonormalization are recorded. Left ventricle of normal dimensions, hypertrophic walls 14/12mm, preserved global systolic functions ejection fraction 45–50%. The assessment of regional kinetics is difficult but there seems to be no regional asynergy E/e` 13.6. Aortic bulb of normal diameter at the root and the ascendant part with atherosclerotically altered walls. Aortic valve of degenerately altered velum, partly sclerotic, preserved coaptation and disturbed separation. Increased flow rates over aortic valve Vmax 2.26 m/sec, mild aortic stenosis. Dilated right cavities, limit functions right ventricle tricuspid annular plane systolic excursion 22 mm, mild tricuspid regurgitation and systolic pressure in the right ventricle of about 35 mmHg registered. The pericardium is not split. Fat pad in front of the right ventricle.
Abdominal echocardiographic examination	In the accessible part the liver is homogenous, steatosis, without focal lesions. The gallbladder, bile ducts, pancreas, aorta, and retroperitoneal space (not fully visualized) without visible changes. Spleen and both kidneys no appreciable disease. Bladder almost empty, a catheter placed. No free fluid in the abdomen.
Polysomnography	As there was suspicion of sleep apnea syndrome, a sleep study (polysomnography) was attempted, which was interrupted due to a drop in SaO_2 levels and the necessity to resume oxygen therapy.

Table 2. Laboratory test details

Laboratory test	Result		Laboratory test	Result	
Reference ranges	max.	min.	Reference ranges	max.	min.
WBC 3.9–10 × 10^9/l	10.8	6.1	Urea 2.8–7.2 mmol/l	10.1	3.7
Neu 40–70%	84.7	70	Cre 53–124 µmol/l	158	86
Eo 0–6%	10		CK 26–192 U/L	2511	33
RBC 3.86–5.08 × 10^9/l (women)	4.51		CKMB 24 U/L	66	44
Hgb 110–180 g/l	154		ALT 8–41 U/L	63	57
PLT 140–450 × 10^9/l	189		AST 7–36 U/L	86	33
CRP < 5 mg/l	57.9	10.9	LDH < 241 U/L	353	188
TPI < 0.75 mmol/l	< 0.20		gGT 5–35 U/L (women)	122	51
Na 136–145 mmol/l	140		TP 66–81 g/L	1.36	
Ca 2.25–2.75 mmol/l	1.9		Fe 8.9–30 µmol/l	7	
K 3.5–5 mmol/l	5.2	3.7	UIBC 2 5–59 µmol/l (women)	56	
HCO_3 24–29 mmol/l	32		TIBC 49–75 µmol/l (women)	62	
Glu 3.5–6.1 mmol/l	10.1	5	Pro BNP < 125 pg/ml	2800	243
Oncomarkers and thyroid gland hormones are in the reference range					
Microbiological analysis			*Corynebacterium spp*. – diphtheroids in the smear in the tip of the tubus		
Urine culture sterile					

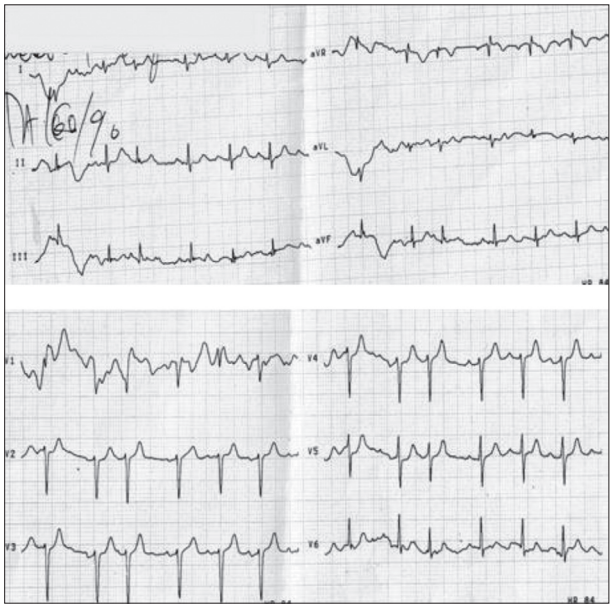

Figure 1. Paroxysmal atrial fibrillation electrocardiography

some data, targeted anamnesis and/or heteroanamnesis have a high sensitivity of 90–100%, but significantly lower specificity: 50–70% [8]. Obesity per se leads to a greater likelihood of diseases such as systemic arterial hypertension, diabetes, dyslipidemia, and hypothyroidism [4]. Additional questions are directed towards sleep, snoring, daily somnolence, possible cyanosis, and pulmonary and cardiovascular symptoms. In physical examination, respiratory noises are mostly reduced due to a thick layer of subcutaneous tissue on the thorax. In uncomplicated cases, early inspiratory basal crackles can be detected (in our patient on the left side). Heart tones are usually quiet, but during the aggravation of the illness, there may be arrhythmia. The ECG finding in our patient indicates an atrial fibrillation that has been arrested with amiodarone. Frequent finding is arterial hypertension due to obesity, smoking, hypoxemia (in our case SaO_2 was 44%), and other factors. Evidence of right ventricle enlargement from pulmonary hypertension that complicates advanced OHS may be seen on ECG and echocardiogram [9].

History and examination cannot diagnose OHS alone, but it requires the demonstration of daytime hypercapnia [5]. Certain laboratory results complete the anamnesis and physical examination [elevated serum bicarbonate (> 27 mEq/L), hypercapnia (arterial pressure of carbon dioxide $PaCO_2$ > 45 mmHg), hypoxemia (PaO_2 < 70 mmHg), polycythemia]. Patients suspected of having OHS can initially be screened by pulse oximetry and by determination of serum levels of venous bicarbonate. SpO_2 values < 93% on pulse oximetry would be suggestive of hypoventilation. A serum bicarbonate level ≥ 27 mEq/L had a sensitivity of 92% and a specificity of 50%, justifying its use in screening [10]. A raised bicarbonate (> 27 mmol/L) or base excess (> 3 mmol/L) in the absence of another cause for a metabolic alkalosis in an obese individual with a $PaCO_2$ < 45 mmHg may be an early indicator of OHS,

warranting closer investigation [11]. We noted similarly. Blood tests are also recommended for the identification of hypothyroidism and polycythemia. A chest radiograph should be performed to exclude parenchymal lung disease, chest wall disease, asymmetrical elevation of a hemidiaphragm (diaphragm paralysis), and cardiomegaly.

The gold standard for diagnosing OSAS is polysomnography, which involves non-invasive measurement of vital parameters during sleep. According to published allegations 90% of PS patients have coexisting OSAS, however, due to unsuccessful polysomnography and missing heteroanamnesis (the patient lived alone), we were unable to confirm this theory [9]. Because symptoms are nonspecific, the diagnosis of PS is frequently delayed. It is commonly misdiagnosed as asthma or chronic obstructive pulmonary disease, and some patients are not diagnosed until hospitalization for acute-on-chronic respiratory failure occurs [12]. However, recent data indicate the OHS is under-recognized and under-treated [13].

In our case, the diagnosis of PS was based on: BMI > 45.7 kg/m^2, hypercapnia, hypoxemia and respiratory acidosis (pCO_2 41 mmHg, pO_2 56 mmHg, pH 7.45) in the absence of other causes of hypoventilation. Comorbidities such as heart failure, coronary artery disease, and *cor pulmonale* are more common in patients with OHS, and the likelihood that such patients will require invasive mechanical ventilation or intensive care unit admission is also increased. Non-invasive positive airway pressure, together with weight loss are the initial first line therapies for patients with OHS [14, 15]. After a global respiratory failure had developed, our patient was intubated and placed on a mechanical ventilator. Mortality rate in PS is increased due to the respiratory and cardiac consequences of obesity as such.

It is critical for physicians to be able to recognize and treat obesity-associated diseases because obesity has become a national epidemic. OHS is still a poorly recognized entity in Serbia. Delayed diagnosis of OHS is associated with an increase in morbidity, mortality, and costs of care of patients who are more severely ill.

Informed consent

Written informed consent in Serbian was obtained from the patient for this case report publication, including the accompanying images, case history and data.

Conflict of interest: None declared.

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Пиквиков синдром – „врх леденог брега" код екстремно гојазних болесника

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САЖЕТАК

Увод Пиквиков синдром (ПС), познат и као хиповентилациони синдром одраслих, чини тријада: гојазност [*Body Mass Index* (БМИ) > 30 *kg/m*^{2}], целодневна хиповентилација и поремећај дисања током спавања у одсуству алтернативних узрока алвеоларне хиповентилације. Благовремено препознавање ПС је од изузетног значаја јер овакви болесници имају знатан морбидитет и морталитет. Међутим, новији подаци указују на то да је ПС недовољно препознат и недовољно лечен. Приказујемо случај рано препознатог ПС на прехоспиталном нивоу са повољним исходом после болничког лечења.

Приказ болесника Шездесетседмогодишњој болесници постављена је прехоспитална дијагноза ПС, која је потврђена и у болници. Дијагностички критеријуми били су: БМИ > 45,7 *kg/m*^{2} (висина 170 *cm*, маса 132 *kg*), хиперкапнија, хипоксемија и респираторна ацидоза (pCO_2 – 41 *mmHg*, pO_2 – 56 *mmHg*, *pH* 7,45) у одсуству других узрока хиповентилације. Током хоспитализације урађене су следеће дијагностичке процедуре: стандардне лабораторијске анализе, радиографија грудног коша, електрокардиограм, ултразвук срца и абдомена. Покушана студија спавања (полисомнографија) прекинута је због пада SaO_2 (засићеност крви кисеоником). Као прва линија терапије примењене су неинвазивна механичка вентилација и дијета. Међутим, због развоја глобалне респирацијске инсуфицијенције болесница је интубирана и стављена на механички вентилатор. После 30 дана болничког лечења отпуштена је кући у задовољавајућем општем стању са препоруком за редукцију телесне тежине и применом симптоматске терапије.

Закључак Гојазност постаје епидемија савременог друштва, те је од кључног значаја рано препознавање и лечење ПС.

Кључне речи: гојазност; Пиквиков синдром; рано препознавање; лечење

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Large choledochal cyst initially interpreted as Mirizzi syndrome – case report and literature review

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SUMMARY

Introduction Choledochal cysts are congenital anomalies manifested as focal or diffuse cystic dilatation of the bile ducts. They are mostly diagnosed in childhood. The magnetic resonance and surgical management are the gold standard diagnostic and treatment modality.

Case outline We present a male patient who was presumed to have Mirizzi syndrome. This presumption was discarded by additional imaging procedures and by later surgical treatment. It was confirmed that it was a large choledochal cyst filled with stones. Considering the diagnosis and good patient's general condition, we opted for surgical treatment.

Conclusion There are several surgical techniques that can be used in the treatment of choledochal cysts, wherein each is intended as a complete resection of the cyst with histological confirmation. Operative techniques do not affect the outcome of the treatment, but the time and extent of surgical resection, as well as any metaplasia of the epithelium, do.

Keywords: choledochal cyst; Todani; common hepatic duct; icterus; Mirizzi syndrome

INTRODUCTION

Choledochal cysts are congenital anomalies that manifest as focal or diffuse cystic dilation of the biliary tree. It is a rare clinical entity with an incidence of 1/150.000 births approximately [1]. There is a significant female predominance with a female to male ratio of 4:1 [2]. Five types of cysts have been described, of which a choledochal cyst type I has around 68% of percentage coverage out of all subtypes [1, 2].

The majority of patients are usually diagnosed in childhood, in the first decade of life. Only 20% of cases are diagnosed in adults [3]. Symptomatology is different in children and in adults. In children, a classical triad of symptoms in the form of abdominal pain, a palpable mass in the upper right quadrant of the abdomen and obstructive jaundice frequently occur. In adults, the ailments are related to biliary and pancreatic symptoms, accompanied by abdominal pain. The ruptures of the cysts are rare and such an occurrence is reported only in neonates [4].

In a differential diagnosis, biliary lithiasis, sclerosing cholangitis, and pancreatic pseudocyst may be taken into account. Biliary atresia is often associated with choledochal cysts and therefore should be excluded at neonatal obstructive jaundice [2, 4].

Choledochal cyst Type I treatment involves complete surgical resection with reconstruction with an isolated small bowel segment by the method of Roux-en-Y. Sometimes, the scope of surgical resection may be much more radical. When the cyst is spreading into the head of the pancreas a cephalic duodenopancreatectomy is preferred surgical option [5].

The objective of this paper is to present the rare disease, the surgical technique, and the literature review.

CASE REPORT

In our paper, we present a male patient, 53 years of age, who was admitted to our hospital because of pain in the upper abdomen, subicterical and afebrile. The initial abdominal ultrasound was performed. Distended gallbladder, with a thickened wall and with multiple stones in the lumen were seen. Choledochus was with concrements inside the lumen, and in close relationship with the gallbladder, Mirizzi syndrome differential diagnosis. A magnetic resonance imaging (MRI) and magnetic resonance imaging cholangiopancreatography (MRCP) were performed as a part of additional diagnostics, in which a large fusiform ductus choledochus cyst was seen. A large choledochal cyst (CC), 7 cm in longitudinal diameter and 4 cm in transverse diameter, was localized at about 2 cm below the primary biliary confluence, without communication with the lumen of the gallbladder, in close contact with the portal vein and with proper hepatic artery. The cyst was filled with numerous stones of different sizes (Figure 1). The laboratory work-up showed elevated WBC 18 × 10^9/L, CRP 65 mg/L, total bilirubin 49 mg/L, and alkaline phosphatase 197 IU/L.

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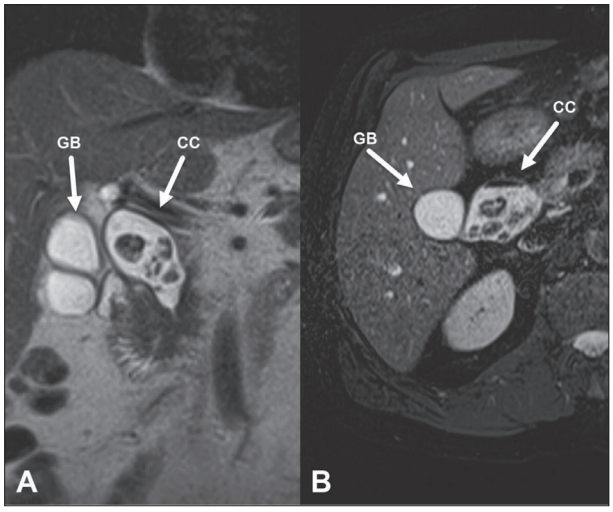

Figure 1. Abdominal magnetic resonance imaging – gallbladder (GB) and Type I choledochal cyst (CC) in sagittal (A) and axial (B) plane

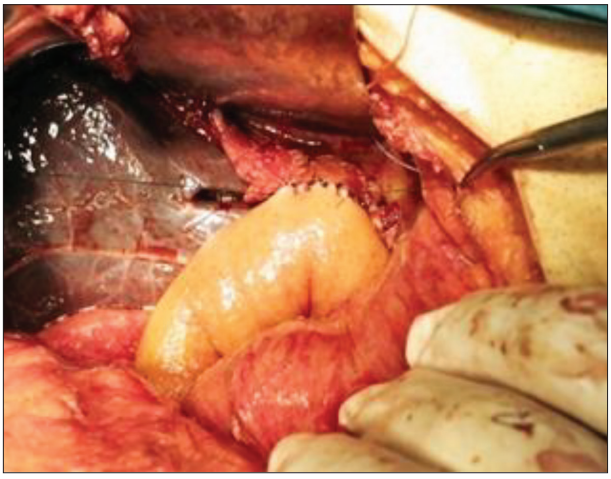

Figure 2. Intraoperative photograph showing hepaticojejunostomy performed using interrupted suture at about 1 cm below the primary biliary confluence

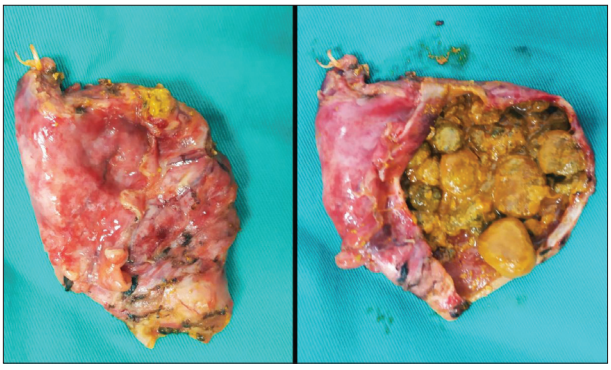

Figure 3. Macroscopic image of the specimen, before and after opening, showing multiple stones in its lumen

In his medical history, the patient provided information that gallbladder calculus was verified by abdominal ultrasound more than 10 years previously. In addition to the examinations that were performed several times during this period, no additional diagnostic procedures were performed. The patient stated that he repeatedly felt difficulties in the form of biliary colic and sometimes spontaneously resolved mild jaundice, which were treated in a conservative manner.

The patient was in good general condition, so we opted for surgical treatment. Given the size, the position of the cyst, and its proximity to surrounding structures, primarily vascular, we applied the open surgical approach. In terms of general endotracheal anesthesia, the abdomen was opened with the right subcostal laparotomy. After adhesiolysis and the inspection, the preoperative diagnosis was confirmed with an inflammatory block surrounding the hepatoduodenal ligament. Hard adhesions were obscuring normal anatomy, cholecystectomy and a careful dissection of the hepatoduodenal ligament was performed, with difficult separation of the vascular structures from the cystic structures. Resection of the common bile duct along with the large cyst was performed. The upper and lower resection was at a distance of 1 cm with respect to both ends of the cyst. Resection margins were sent to an *ex tempore* histopathologic examination. In the meantime, we performed an extensive lavage of the bile ducts, proximal part first and then the distal part from previously resected CC. Using choledochoscope, the proximal part relative to the branch of the left and the right hepatic duct, and then the distal stump of the resected hepatic ductus to the papilla of Vater were inspected. The finding was normal, with no residual stones. *Ex tempore* findings were negative for malignancy. Hepaticojejunostomy was performed using interrupted, monofilament, slowly absorbable suture (4/0) at about 1 cm below the biliary confluence (Figure 2). The abdomen was drained with two abdominal drains and the operating incision was reconstructed. The tissue of the cyst (Figure 3) and of the gall bladder was sent to histopathological examination.

Histopathological examination revealed only mild inflammatory changes in the wall of the gallbladder, with no dysplasia or other significant epithelial changes. A specimen with a cyst measuring 75 × 48 mm in diameter was extensively reviewed. It was seen that the areas of papillated and partly atypical epithelial proliferation were present in the bile duct, but coupled with poorly expressed atypia, so there were only focuses of light epithelial dysplasia (grade I–II). In the surroundings, chronic inflammation with some of the sporadic multiplicity of the diverticula of the same epithelium was present, as well as the light multiplication of such tubulo-glandular structures. None of the sections showed any invasiveness elements. Cystic part of the sections showed mostly flattened and only reactively altered, but mostly non-dysplastic epithelial changes.

The postoperative course was uneventful. Abdominal drains were removed on the third postoperative day and the patient was discharged on the seventh postoperative day. A month later, an abdominal ultrasound was performed and the finding was normal, as well as laboratory analysis. Six months after surgery, MRI and MRCP were performed showing that the anastomosis is passable and that the other findings in the abdomen were normal. The patient's condition is still monitored.

DISCUSSION

CCs were first reported by Vater and Ezler [6]. This congenital malformation is characterized by dilatation of the biliary tree without acute obstruction of the flow of bile. The cyst may be present in any part of the biliary tree. According to the volume, it may be complete or segmented, and according to the shape, it can be saccular or fusiform [7]. In our case, it was a fusiform cyst, filled with numerous concrements.

Etiology of CCs is still unclear and there is still no clear expert consensus. The most frequently mentioned hypothesis in the current literature suggests that CCs occur after partial obstruction of the bile duct, which produces an increase in pressure in the proximal part of the bile duct, which leads to the dilatation of this part [7]. CC prevalence is much higher in Asian countries such as in Japan and predominantly occurs in females [8].

The first classification of CC was announced by Alonso-Lej et al. [9] in 1959. This initial classification was completed by Todani et al. [10], wherein the choledochal cysts were classified into five types. Type I cysts represent the dilatation of the extrahepatic bile ducts. Also, they represent the most common type according to the previously mentioned Todani classification, where the incidence is 1:1,000, compared to western countries, where the incidence ranges from 1:150,000 up to 1:1,000,000 births. Type I CCs have the greatest frequency of occurrence (75–85%) compared to other types [1, 10]. Isolated cystic dilatation of the cystic duct was added to the Todani classification in 1991 as type VI [11].

CCs are presented with different symptoms, but they can often be asymptomatic. In symptomatic patients, they are commonly presented as abdominal pain, nausea, and vomiting. In these patients, biliary stones, cholangitis, liver abscess, and biliary cirrhosis are present in 60–80% of the cases [4, 12].

Ultrasound examination of the abdomen is the first diagnostic procedure, particularly in children. As an additional diagnosis, computed tomography is used. Both methods cannot always provide sufficient information. The gold standard is MRI, as well as MRCP, with an efficiency of 96–100% [1, 12]. Endoscopic retrograde cholangiopancreatography and percutaneous transhepatic cholangiography represent very reliable diagnostic procedures, but both are invasive procedures and their application is not routinely performed [12, 13].

In our case, only after completed MRI and MRCP were we able to remove the dilemma on the possible presence of Mirizzi syndrome. There was no communication of CC dilation with the lumen of the gall bladder, nor any expressed compression in the relations of biliary structures, as originally seen in the abdominal ultrasound examination. Since the only reliable and correct treatment modality of these biliary tree anomalies is complete surgical resection, surgical treatment should not be delayed, especially if you take into account that these anomalies represent the premalignant condition. Malignant alteration depends on the cyst type and that percentage is the largest for Type I cysts (about 70%), followed by Type IV cysts (about 20%), Type II cysts (about 5%), and Type III cysts (about 5%) [14].

Within frequent cholangitis and inflammatory processes of the surrounding structures and their consequences, sometimes it is difficult to achieve complete excision of the CC, especially because of the close relationship with the blood vessels, especially the portal vein. CC sometimes spreads into the parenchyma of the pancreas and it is necessary, in order to apply the adequate and radical surgical approach, to perform a cephalic duodenopancreatectomy with all the risks that this procedure can cause [5, 7].

After the excision of the cyst, the reconstruction can be done in two ways: hepatico-duodenal anastomosis or hepatico-jejunal anastomosis by the Roux-en-Y method [15]. The success of operational procedures and of the selection of anastomosis is measured by the ease of implementation, as well as by short- and long-term results of the surgical treatment. The data from the current literature suggest that the success of hepaticojejunostomy is about 92% with the complication rate of 7%, compared to hepatico-duodenostomy, with the complication rate about 42% [7, 15].

Surgical management can be carried out by using several surgical techniques. These include the classical operational approach, which we also apply, then minimally invasive or laparoscopic access, and the most modern, robotic-assisted surgical approach [16, 17].

Depending on the technical equipment and the training of the surgical team, in institutions where this type of surgery is performed, it is possible to effectively apply several surgical techniques, whose ultimate objective is the same – the complete excision of the CC with the appropriate reconstruction of the biliary ducts. After reviewing the literature and bibliographic databases (PubMed, Scopus), we came to the conclusion that the short- and long-term results of surgical treatment outcomes of patients operated on for Type I CC are similar, regardless of the applied surgical technique. It can be concluded that the applied surgical technique does not affect considerably the final result of the treatment, but the period required to diagnose the disease, the extent of surgical resection, and the presence of the bile duct epithelium metaplasia do.

Informed consent: Written informed consent was obtained from the patient for this case report publication, including the accompanying images, case history, and data.

Conflict of interest: None declared.

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Циста холедоха иницијално дијагностикована као Миризијев синдром – приказ болесника и преглед литературе

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САЖЕТАК

Увод Цисте холедоха су урођене аномалије које се манифестују као фокалне или дифузне дилатације жучних водова. Најчешће се симптоматски испоље и дијагностикују током детињства. Магнетна резонанца је златни стандард за постављање дијагнозе, а хируршко лечење је једини сигуран и ефикасан начин лечења.

Приказ болесника Болесник стар 53 године примљен је на клинику због болова у трбуху, мучнине и иктеруса. Из медицинске документације се сазнало да је 10 година уназад знао за калкулозу жучне кесе, те се после иницијалне ултразвучне дијагностике посумњало на Миризијев синдром. После спроведене допунске сликовне дијагностике утврђено је да се заправо радило о великој цисти холедохуса која је испуњена масом калкулуса. Имајући у виду добро опште стање болесника, одлучено је да се болесник лечи хируршки.

Закључак Описано је неколико оперативних техника за хируршки третман цисте холедоха. Без обзира на примењену технику, циљ је био да се одстрани комплетна циста и хистопатолошки прегледају маргине ресекције, као и сам препарат. Избор оперативне технике не утиче на исход лечења. С обзиром на познату тенденцију метапластичне промене епитела холедоха код болесника са цистом холедоха, од суштинског је значаја да се оперативном лечењу приступи на време.

Кључне речи: циста холедоха; Тодани; заједнички хепатични вод; иктерус; Миризијев синдром

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Rare case of an anorectal leiomyoma

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SUMMARY
Introduction Leiomyoma is a kind of benign tumors of smooth muscle origin, occurring mostly throughout the genitourinary system. They are rarely found in the anorectal region, where they represent less than 0.1% of the tumors of the rectum. Due to this exceptional occurrence, we report a case of leiomyoma arising in the anorectal region.
Case outline A 43-year-old otherwise healthy woman presents with the big mass in analorectal region. Major complain is pain and discomfort while sitting. Magnetic resonance imaging is done and pathohistology report confirmed large leiomyoma. Abdominoperianeal resection of the rectum was performed due to the size of the tumor. Postoperative course was uneventful.
Conclusion There is no gold standard technique, and decision about the best surgical approach for all pathohistological types of tumors in the anorectal region. The decision sometimes has to be made according to size and major complains.
Keywords: anal canal; leiomyoma; surgery

INTRODUCTION

Leiomyoma are benign tumors of smooth muscle origin occurring throughout the genitourinary system [1]. They are usually present as an intrauterine tumor, but may occasionally be extrauterine. The leiomyomas, which evolve to lose their attachment from the uterus and become adherent to other organs (such as the broad ligament, omentum, or retroperitoneal connective tissue) and acquire blood supply from them, are call parasitic leiomyomas. They can be found anywhere in the abdomen and pelvis. Extrauterine leiomyoma can be classified according to the place of origin as a piloleiomyoma (derived from erectile muscles of hair follicles), an angioleiomyoma (from blood vessel smooth muscle wall), or a genital leiomyoma (derived from) tunica dartos of the scrotum [2]. In the digestive tract, the most common presentations are in the stomach, followed by the small intestine, but are rarely found in the anorectal region, where they represent less than 0.1% of the tumors of the rectum, with a rare presentation in soft parts, mainly in the perianal topography [3, 4]. Literature shows that the anal leiomyoma is a rare finding: isolated cases have been reported, and the most common presentation is a painless tumor in this area [4]. Due to this exceptional occurrence, we report a case of leiomyoma arising in the anorectal region.

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CASE REPORT

A 43-year-old otherwise healthy woman came to the office because of anal region disorders and uneasiness while sitting. She noticed a bump in the anal region, which got bigger in size through the course of one year. This was her first visit. She denied other symptoms except pain during defecation. Her medical family history was unremarkable. Physical examination revealed a large mass protruding from the anal canal and the anus itself (Figure 1). On rectal examination, the anus was passable for a fingertip; endoscopy or endoanal ultrasonography could not be performed due to extreme discomfort and size of the lesion. The laboratory and biochemical results were within the referential values, findings on abdominal ultrasonography were normal. The magnetic resonance imaging (MRI) was done followed by a biopsy. Pelvic MRI showed an expansive tumor of the rectum of the anus without the uterus and the bladder infiltration (Figure 2). Pathohistology report confirmed leiomyoma. Due to the size of the tumor and infiltration of muscles, abdominoperineal resection was performed (Figure 3). The postoperative course

Figure 1. Clinical presentation of the anorectal leiomyoma

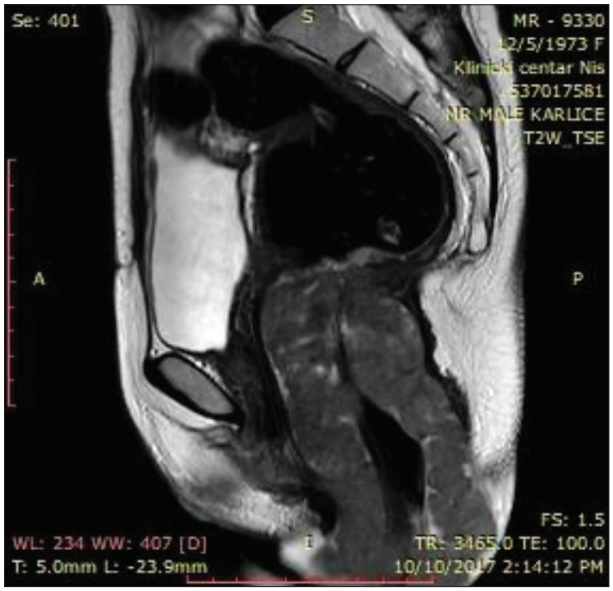

Figure 2. Magnetic resonance imaging finding of the large anorectal leiomyoma

was uneventful. One year after the surgery, control examination results were good, and the patient gave written consent for this case presentation, including the accompanying images, case history, and data.

DISCUSSION

In 1845, the first description of leiomyoma was made by Virchow, who defined it as a benign tumor of mesenchymal origin developing from smooth muscle fibers. The leiomyoma can develop in any place where smooth muscle is present. In the previous classification, it belonged to the so-called gastro-intestinal stromal tumor (GIST). Nowadays, the GIST is considered a soft tissue tumor, as well as the leiomyoma, but it has its own identity based on specific immunohistochemical pattern, while many tumors previously defined as leiomyoma are now classified as GIST and even have a different type of treatment [5, 6]. The leiomyoma have exhibited positivity for smooth muscle actin and negativity for CD117 and CD34 (c–Kit). Desmin may be absent, or it may be expressed later [4, 7]. Histological patterns are similar to those of leiomyosarcoma, but in leiomyosarcoma, a higher degree of cellular atypical activity with local pleomorphic and increased mitotic activity is expressed. There are no well-established criteria to determine malignancy in these tumors, with some characteristics suggesting a malignant behavior: tumor size (> 5 cm), histological appearance (necrosis, ulceration, or cells with atypical) and increased number of mitoses (> 2 mitoses per field, with a 10-fold increase) [4, 8]. In the case presented here, the growth and the size of the tumor suggested its malignant behavior.

It is classified as superficial or deep. The latter is further divided in somatic and retroperitoneal [2]. The superficial variant usually affects the extremities with the same incidence in both sexes whereas the retroperitoneal generally involves the pelvic region in fertile women, like in this case

Figure 3. Speciment after abdominoperineal resection for anorectal leiomyoma

[2, 9]. The organs mainly involved in gastrointestinal tract are the stomach and small bowel, less frequent regions are esophagus, colon, and anorectal localization which is fairly uncommon [5, 10]. In the anorectal region, they represent only 3% of leiomyomas of the gastrointestinal tract and less than 0.1% of rectum tumors, rarely found in soft tissues, mainly in the perianal area, and 3.8% incidence of all soft tissues benign tumors [11].

Considering their growth type, these tumors are divided into three variants: intraluminal, extraluminal, and intramural. Intraluminal leiomyomas are usually located in the posterior wall of the distal part of the rectum as in the case presented here, and they may be sessile or pedunculated. On the other hand, extraluminal leiomyomas generally grow from the colonic wall inside the abdomen and they often resemble a GIST [12]. Sometimes the tumors grow in both directions, forming an "hour glass," which could be said for this case according to the MRI [5, 6].

Preoperative diagnosis is difficult because most patients are asymptomatic and these tumors are commonly identified during endoscopy and imaging examination. The symptoms related to the presence of leiomyoma vary widely. They may include pain, rectorrhagia, tenesmus or they can be confirmed by a biopsy and the treatment depends on their alteration, which is confirmed by medical examination. Biopsy is often non-informative because it does not involve the entire tumor, including the full evolution of histological characteristics [3, 6]. The radiological imaging, such as MRI or 360° tridimensional transrectal ultrasound, are useful to identify and localize the mass, to provide information on its relationship with the adjacent structures such as the anal sphincter or uro-gynecological structures and to indicate the operative strategy [5].

The treatment of perianal leiomyoma consists in the complete surgical resection, ensuring tumor-free margins [3]. Since local resection was impossible due to the size of the lesion and sphincter involvement, abdominoperineal resection was performed as suggested in other cases reported. The recurrence rate is low when complete local excision is performed, but the extended postoperative follow-up with clinical and complementary examinations is important to

confirm the absence of the disease or to detect any recurrences and\or malignant transformation [12, 13]. All perianal lesions need a final diagnosis, leiomyoma should be considered in differential diagnosis of tumors in anorectal region. The best surgical approach depends not only on nature of the tumor, but sometimes decision has to be made according to size and major complains.

Conflict of interest: None declared

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Редак случај аноректалног лејомиома

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САЖЕТАК

Увод Лејомиоми су бенигни тумори порекла глатких мишића који се углавном јављају на генитоуринарном тракту. Ретко се срећу у аноректалној регији, где чине мање од 0,1% свих тумора ректума. У раду је приказан изузетно редак случај лејомиома аноректалне регије.

Приказ болесника Четрдесеттрогодишња, иначе здрава жена јавила се због велике масе у аноректалној регији. Жалила се на бол и нелагодност током седења. Магнетна резонанца уз патохистолошки налаз показала је велики лејомиом. Због величине тумора и локализације изведена је абдоминоперинеална ресекција ректума. Постоперативни ток протекао је без компликација.

Закључак Не постоји златни стандард који се тиче хируршког лечења за све патохистолошке типове тумора у аноректалној регији. Одлука се понекад доноси у односу на величину тумора и главне тегобе болесника.

Кључне речи: анални канал; лејомиом; хирургија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Focal brain lesions as a consequence of an obscure neurosurgical treatment in a drug-addicted patient

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SUMMARY

Introduction Infectious or non-infectious noxae may occur in drug-addicted patients who have clinical presentation of meningeal syndrome with a spectrum of possible complications, such as a diffuse or focal brain lesions.

The objective of this report is to present a rare case of a drug-addicted male patient, initially suspected of mycosis of the central nervous system, but computed tomography (CT) and magnetic resonance imaging (MRI) showed the signs of an invasive neurosurgical operation that the patient underwent during the treatment of drug addiction.

Case outline A 37-year-old male patient was hospitalized at the Clinic for Infectious Diseases, Clinical Center of Vojvodina, with a meningeal syndrome, initially suspected of mycosis of the central nervous system. He was diagnosed at the Center for Radiology. Neuroimaging – CT and MRI were used in order to prove or disprove the presence of brain infection. These diagnostic procedures ruled out the presence of brain infection, but opened questions about the type of neurosurgical treatment performed out of legal institution, due to the presence of craniotomy and focal glial brain lesions in the frontal lobes.

Conclusion In drug-addicted patients, meningeal syndrome can be connected with diffuse or focal brain infections. Together with laboratory and clinical analysis, imaging methods contribute to the decision making and optimal treatment of patients. In our case, CT and MRI made a significant contribution in the detection of the focal brain lesions and clarification of their etiology.

Keywords: magnetic resonance imaging; tomography, X-ray computed; substance-related disorders; neuroimaging; craniotomy; nervous system

INTRODUCTION

Intravenous (IV) drug addicts are prone to various blood-borne infectious diseases. Most common are human immunodeficiency virus (HIV) and hepatitis B and C infections [1]. Due to a high coincidence of IV drug use with HIV, malnutrition, and immunodeficiency, we used laboratory tests, serological and molecular biological tests to differentiate these conditions. IV addicts can suffer from numerous infectious and non-infectious diseases of the central nervous system (CNS). Patients with HIV develop neurological complications in 40–80% of cases [2]. These complications arise from opportunistic infections, tumors or HIV encephalitis [2, 3]. They are more often caused by bacteria and fungi than by viruses [4]. These diseases can have a clinical presentation of meningeal syndrome with a spectrum of possible complications, such as diffuse or focal brain lesions [4, 5]. Unexplained and constant headaches in IV drug addicts should raise suspicion of intracerebral abscess along with other life-threatening pathologies [6]. Neuroimaging methods such as computed tomography (CT) and magnetic resonance imaging (MRI) are of crucial importance in setting a diagnosis of parenchymal brain lesions.

The objective of this report is to present a rare case of a 37-year-old drug-addicted male patient, initially suspected of mycosis of the CNS, but after both CT and MRI have been done, it showed the signs of an invasive neurosurgical operation that the patient underwent during the treatment of drug addiction.

This study was conducted in accordance with the Helsinki Declaration and Guidelines for Good Clinical Practice and was approved by the Ethics Committee of the Faculty of Medicine, University of Novi Sad.

CASE REPORT

A 37-year-old intravenous male patient, addicted to drugs for 15 years, came to the emergency department complaining of photophobia, vomiting, and extreme headaches – signs of the meningeal syndrome. The patient was disoriented and uncooperative – thus, medical history was difficult to obtain. He still used heroin from time to time, the last time being 3–4 weeks before admission. He said that he did not have any recent viral or bacterial infection nor loss of body mass. Laboratory analysis showed HCV-positive infections, but HIV status

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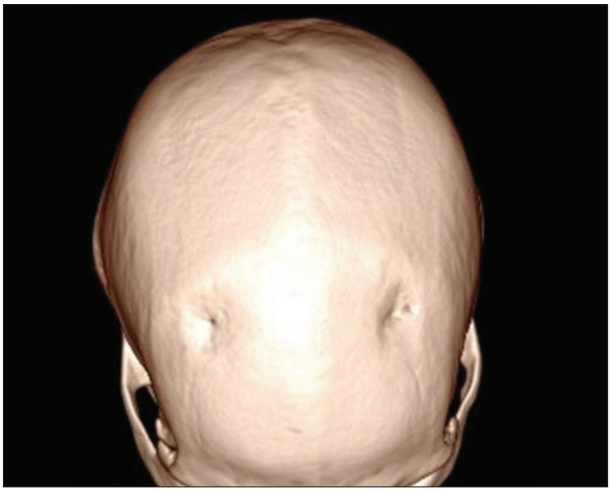

Figure 1. Computed tomography axial bone window displays bone defects of the frontal bones

of the patient was unknown. Clinical examination indicated that the patient was afebrile, somnolent, with old scars on both sides of the frontoparietal scalp. Neurological examination showed that the right pupil was slightly more dilated, meningeal signs were negative, and deep tendon reflexes on the lower extremities were brisker than normally. Other internal and neurological findings were without a coarse outburst. In laboratory findings, slightly elevated inflammation parameters were recorded – sedimentation rate 38/70 mm/h, C-reactive protein 30.1 mg/l (ref. 0–5), fibrinogen was in the normal range, as well as all the other biochemical blood and urine analyses. Acute bacterial and fungal nasopharyngeal infections were excluded (bacterial nasal and pharyngeal cultures, as well as fungal cultures were sterile). Hemoculture, bacteriological, and fungal findings were negative. Serological testing for toxoplasmosis (ELISA method) was negative. Chest X-ray, abdominal and pelvic ultrasonography were made during hospitalization and they were without any pathological findings. Initially, parenteral antibiotics, antimycotics (aminopenicillin, vancomycin, metronidazole, fluconazole), antiedematous therapy (mannitol), nonspecific hepatoprotective drug (Silymarin caps), infusion, and symptomatic (analgesic) therapy were included. The patient remained afebrile the entire time and his headaches had a tendency to regress. Lumbar puncture showed normal cerebrospinal fluid. During his hospitalization, head CT and MRI were done.

Non-enhanced head CT was performed. The contrast was not applied because the peripheral vein could not be accessed. It showed focal hypodense brain lesions in both frontal lobes. An infection of CNS was firstly suspected, but in differential diagnostics malignant tumors could not be excluded. On the bone window, bone defects from previous trepanation were seen on both sides of frontal bones (Figure 1). Acute pansinusitis was detected, most prominently in the right aspect of the frontal sinus, as well as sinusitis of the right maxillary sinus, which was described as mycotic (fungal ball) sinusitis.

Brain MRI and time of flight angiography showed oval T1 weighted (T1W) hypo/T2 weighted (T2W) hyperintense zones (Figure 2) with marginal T2W/FLAIR hyperintensity, without restriction of water molecules diffusion, without postcontrast enhancements frontally parasagittally bilaterally and with no pathological signal on proton density. Symmetrically from these zones throughout the postoperative skull defects there were linear T2W/FLAIR hyperintensities that suit gliosis. These findings are primarily fields of encephalomalacia and gliosis due to a nonstandard neurosurgical procedure. Infectious, malignant, and inflammatory processes were excluded with great certainty.

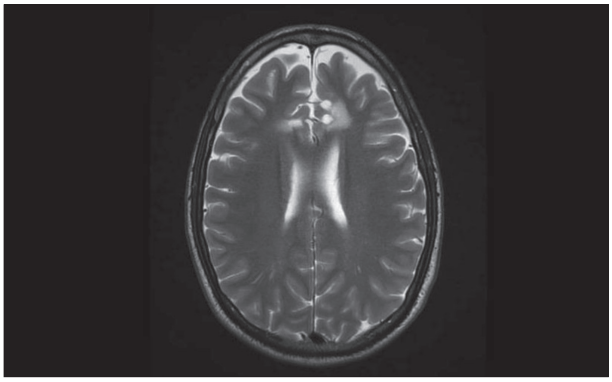

Figure 2. Magnetic resonance imaging T2W axial plane sequence – hyperintense zones frontally parasagittally bilaterally

Just after we presented our results to the patient, he revealed that he underwent a neurosurgical procedure in order to treat his addiction, but he did not have any medical documentation to prove the claim.

DISCUSSION

A persistent headache in IV drug users imposes consideration of infectious complications of the CNS [6]. An afebrile state with normal or slightly elevated inflammatory parameters (leukocytosis, sedimentation, C-reactive protein) do not exclude infection of the CNS [7]. The type of narcotics, route, and length of application significantly affect the etiology, localization, and the form of CNS infection [6]. The severe headache which our patient had could be prescribed to acute pansinusitis detected on CT since we excluded CNS infections.

Focal brain infections in IV drug addicts are among possible complications and are usually caused by *Staphylococcus aureus*, *Streptococcus spp*., and *Cryptococcus spp*. [6, 8]. Bacterial sinusitis can also be the source of meningitis, cerebritis, and focal parenchymal lesions of the CNS [9]. Given the suspicion of the fungal etiology of focal brain lesions in CNS, we firstly suspected *Cryptococcus neoformans*, which is the most common causative agent of focal brain lesions. But after a non-enhanced CT was done, it made a huge reversal in our diagnosis. We found bone defects on both sides of the frontal bones, and although they could have been caused by a fungus (fungal osteomyelitis), these holes looked like trepanation defects. These facts opened a question about the type of non-documented neurosurgical treatment which was performed out of

a legal institution, due to the presence of a craniotomy. Stereotactic neurosurgical procedure had been initially performed for treating psychiatric complaints [10]. It later extended and was used as a treatment for pain and movement disorders [11]. A recent research has exposed the fact that it can be very useful in patients with an addiction refracted to therapy and for treating Parkinson's disease as well [12, 13]. Nowadays, with the development of deep brain stimulation (DBS), it is mainly used in implanting the intracerebral electrodes [14]. DBS presents an adaptable, adjustable, helpful neurosurgical procedure which delivers electrical pulses to the specific areas in the brain using implanted electrodes [11]. Considering all this, DBS was among our most probable causes, but we had to think of others as well.

Different surgical procedures have been performed to treat drug addiction, but without precise data from a large sample and randomized controlled trials concerning possible complications and the efficiency of the treatment [13]. Frontal lobe plays an important role in cognitive, behavioral and emotional processes with significant interactions between mesolimbic and mesocortical circuits, especially in drug-addicted patients [15]. Dopaminergic dysfunction in neural circuits also plays a specific role in prefrontal and anterior cingulate cortices in drug-addicted patients, and these specific areas are the target sites for neurosurgical interventions [12]. Bilateral cingulotomy and DBS are shown to be possible treatments in the therapy of addiction, psychiatric diseases, and essential tremor [16]. For the first time in 1973, Balasubramaniam et al. analyzed the results of 28 addicted patients treated with bilateral stereotaxic cingulotomy with no long-term complications [17]. Later, Medvedev et al. [18] also investigated the characteristics and effect of the cingulotomy treatment in 348 heroin-dependent patients in 2003 [13]. Until the 2000s, 300 heroin-addicted patients were treated with bilateral cingulotomy in Russia. DBS can have adverse events, such as infections (1.7%), transient confusions (15.6%), seizures (1.5%), and can lead to death (0–0.4%). Implantation of the electrode, other than minor gliosis, do not cause any other damage to the brain tissue [19].

Brain imaging methods CT and MRI are essential for differential diagnosis and give more information about the types of brain lesions [4, 20]. The significance of radiological imaging modalities in our drug-addicted patient, especially MRI, was to show that the bilateral focal lesions in frontal brain regions did not originate from infections, but were rather a result of an invasive neurosurgical treatment.

In drug-addicted patients; meningeal syndrome could be connected with diffuse or focal brain infections. Together with laboratory and clinical analysis, imaging methods contribute to the decision-making and optimal treatment of patients.

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Фокалне лезије мозга као последица нејасног неурохируршког третмана код интравенског корисника психоактивних супстанци

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САЖЕТАК

Увод У основи менингеалног синдрома код интравенских корисника психоактивних супстанци диференцијалнодијагностичка размишљања иду у правцу нокси инфективног и неинфективног порекла, са могућим компликацијама као што су дифузне или фокалне лезије мозга.

Циљ рада је приказ случаја интравенског корисника психоактивних супстанци са иницијалном сумњом на микозу централног нервног система, где су компјутеризована томографија (КТ) и магнетна резонанца (МР) показале знакове неурохируршке операције, којој се болесник подвргао у циљу лечења зависности.

Приказ болесника Мушкарац стар 37 година примљен је на Клинику за инфективне болести Клиничког центра Војводине са знацима менингеалног синдрома. Прво се сумњало на микозу централног нервног система, а КТ и МР су се спровеле да би се проверило да ли постоји инфекција. Дијагностичке процедуре су искључиле постојање инфекције, али се отворило питање о врсти неурохируршке процедуре која је спроведена ван легалне институције, с обзиром на постојање краниотомије и фокалних глијалних лезија у фронталним режњевима.

Закључак Код интравенских корисника психоактивних супстанци менингеални синдром може бити повезан са дифузним или фокалним инфекцијама мозга. Заједно са лабораторијским и клиничким анализама, сликовне методе доприносе доношењу одлука и оптималном третману болесника. У овом случају, КТ и МР су дале значајан допринос у откривању фокалних лезија мозга и разјашњавању њихове етиологије.

Кључне речи: магнетна резонанца; компјутеризована томографија; поремећаји повезани са уносом психоактивних супстанци; неуроснимање; краниотомија; нервни систем

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Multidisciplinary treatment of patients with chronic odontogenic maxillary sinusitis – a case series

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SUMMARY

Introduction The treatment of chronic odontogenic maxillary sinusitis remains an important problem for medicine due to the presence of numerous available techniques, number of complex surgical approaches, performed by an ENT or maxillofacial surgeon or both.

This study aims to analyse different methods of treatment of chronic maxillary sinusitis by several specialists for the choice of the optimal treatment technique.

Outline of cases We describe two clinical cases of multidisciplinary treatment of patients with chronic odontogenic maxillary sinusitis with the involvement of different specialists – the ENT and the maxillofacial surgeon. One patient was treated with endoscopic technique, and other underwent classic open sinusotomy using local tissues and xenogenic collagen membrane for removing an oroantral fistula. For assessing the condition before and after the treatment, clinical examination and computed tomography were used.

Conclusion According to the results of our study, the endoscopic technique is the preferred method of treatment of patients with chronic maxillary sinusitis when there is no connection with the oral cavity. If an oroantral fistula is present, it is necessary to perform an open operation by a maxillofacial surgeon.

Keywords: chronic odontogenic maxillary sinusitis; surgical treatment; multidisciplinary treatment

INTRODUCTION

Chronic odontogenic sinusitis is a disease that requires the involvement of several specialists in its diagnosis and treatment: an ENT, maxillofacial, and dental surgeon [1, 2]. Moreover, their intervention will depend on the patient's condition and the well-organized collaboration of specialists. In the case of chronic odontogenic sinusitis, both endoscopic sinus sanitation and open surgery in the volume of traditional sinusotomy are possible [2, 3]. Treatment of patients with chronic perforated maxillary sinus (MS) is complex because of the absence of the primary substrate for neo-osteogenesis and the presence of an oroantral fistula. The process of healing and tissue regeneration is extremely slow due to persistent microbial contamination. Disease recurrence is frequent, which then leads to the need for reoperation and reduction in the overall quality of the patient's life.

There is no one common opinion among specialists on how odontogenic maxillary sinusitis should be treated, and by whome – ENT or maxillofacial or oral surgeon. This is one of the reasons why the results are often quite controversial.

To demonstrate our collective work at the Sechenov University, we hereby present two clinical cases with different modalities of surgical treatment.

The subjects' written consent was obtained, according to the Declaration of Helsinki, and the study was approved by the competent ethics committee (protocols of Local Ethics Committee N8 from May 26, 2014, and N10-12 from October 18, 2012) and conforms to the legal standards. Both patients have given oral and written agreement for using their computed tomography (CT) images and medical data.

CASE REPORT #1

Patient D., a 38-year-old woman, was admitted to the Clinic for ENT Diseases at the Sechenov University with bilateral nasal obstruction, intermittent mucopurulent discharges, mainly from the left side, and intermittent "pulling" pain in the left cheek.

The patient had dental treatment of the left upper jaw about 10 years previously, re-endodontic treatment of 2.5–2.6 teeth, followed by their extraction after one year because of exacerbation of chronic apical periodontitis and poor success after therapeutic dental treatment.

The dental implantation in the area of these teeth was planned. After cone beam CT of the paranasal sinuses, the patient was sent by a surgical dentist to the ENT clinic for the treatment of chronic left-side maxillary sinusitis. During

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the examination, nasal breathing was difficult through both halves of the nasal cavity and the mucous membrane of the nasal cavity was pink and moist. The nasal septum was deviated in both directions, more to the right with the formation of a crest in the bone and cartilage and compensatory diffuse hypertrophy of the left inferior nasal concha.

At the time of examination, there was no pathological discharge in the nasal cavity. When examining the oral cavity, teeth 2.5 and 2.6 were missing. The remaining ENT findings were normal. Based on the results of clinical examination and CT, the patient was diagnosed with chronic left-side odontogenic maxillary sinusitis, foreign body of the left MS, nasal septum deviation and hypertrophic rhinitis (Figure 1).

At the Clinic for ENT Diseases at the Sechenov University, the patient underwent septoplasty with a single-step endoscopic operation on the left MS with removal of a foreign body and radio wave correction of the conchae under combined endotracheal anesthesia. The postoperative period was uneventful. On the first day, the operation tampons were removed from the common nasal passages. On the second day, nasal packings were removed from the middle nasal meatus. Nasal and sinus irrigation through the extended natural ostium and applications of the vasoconstrictors were performed.

The patient was discharged on the fourth postoperative day with improvement. Giving the possible presence of postoperative edema of the mucous membrane of the nasal cavity and the left MS, dental implantation was recommended two to three months after surgery.

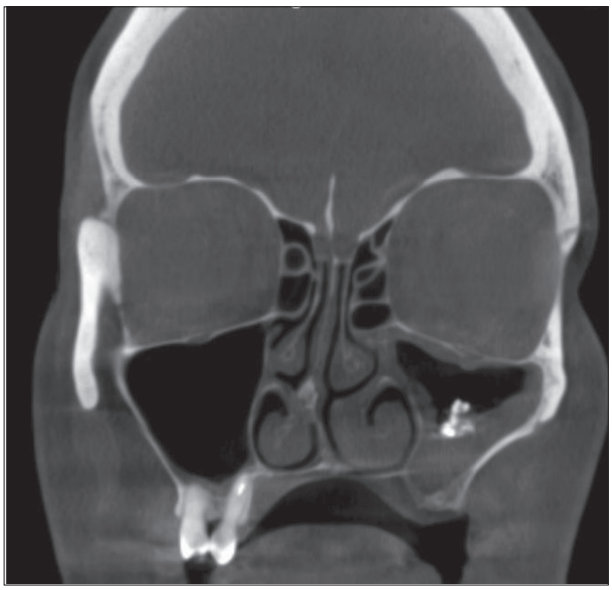

Figure 1. Patient D.; cone beam computed tomography of the paranasal sinuses before surgery; the crest of the nasal septum, the decrease in pneumatization of the left maxillary sinus, and the shadow of high density in the middle sections of the sinus (filling material and dense fungal inclusions) are determined

Figure 2. Computed tomogram of patient S; before surgery: the defect of the alveolar process of the right upper jaw, the fistula of the right maxillary sinus with the oral cavity, and thickening of the sinus mucosa are visualised

CASE REPORT #2

Patient S., a 21-year-old man, reported to the Sechenov University at the Department of Surgical Dentistry with facial edema on the right side, and air and food getting from the oral cavity to the nose while eating.

The patient had tooth 1.8 removed three weeks previously. A week later, he noted the appearance of these symptoms. His doctor at the dental clinic sutured the area of socket of the previously removed tooth 1.8 with a temporary positive effect. A week later, the buccal edema appeared on the right side of the face and his body temperature rose to 38°C. The patient had again turned to the clinic, where antimicrobial therapy was prescribed. His body temperature returned to normal, but the buccal edema remained.

On the orthopantomogram, prior to the extraction of the tooth 1.8, the root tips were present in the MS.

During the examination, swelling of the cheek on the right side was present, with skin moderately hyperemic, gathered in the fold. On palpation, the temperature of this area was higher compared to the other side. The symptom of fluctuation was negative. Mouth opening was moderately limited to 3.5 cm and painful due to swelling of the cheek on the right side. Swallowing was free and painless. Palpation marked a moderate increase in size of submandibular lymph nodes, more to the right. On examining the oral cavity, the mucous membrane of the right buccal region was swollen, hyperemic and painful during palpation, and the symptom of fluctuation was positive. When examining the area of a previously removed tooth 1.8, the defect of the alveolar process of the upper jaw was visualized in the retromolar region, with a transition to the vestibular side up to 1.5–1.8 cm. The nasal test was positive. Puncturing the line of mucous membrane closure of the right buccal area, pus was obtained. Abscess of the buccal region on the right and chronic odontogenic sinusitis with oroantral fistula on the right were diagnosed.

Under conditions of local infiltration and conductive anesthesia, a purulent focus was reorganized: an opening

of the abscess of the right buccal region, wound revision, washing with antiseptics, and its drainage. The patient was under dynamic observation and underwent a course of antibacterial therapy. Daily dressing was performed. After the patient's condition improved, a course of physiotherapy was performed (magnetic therapy) to reduce the swelling of the soft tissues of the buccal region. After three weeks (Figure 2), the patient underwent sanitation of the sinus through the bone defect area. Plasty of the oroantral bone fistula was done with local tissues, the buccal flap and collagen xenogenic membrane. The postoperative period was uneventful. During the entire period of dynamic observation of the patient, signs of recurrent oroantral fistula or exacerbation of chronic sinusitis were not detected.

DISCUSSION

The method of treatment of patients with chronic maxillary sinusitis and oroantral fistula remains a very important problem for otorhinolaryngology, maxillofacial, and oral surgery.

This chronic sinusitis is usually odontogenic in nature and is frequently iatrogenic. For example, Philipsen et al. [4] reported odontogenic cause of chronic sinusitis in 4.7% of 788 patients after their treatment in dental clinics. Tooth extraction is the most common dental procedure that leads to sinusitis, in approximately 30%. The most common reason of perforated form of sinusitis during recent years was the open sinus lifting. Surgeons can damage Schneider membrane during the detachment, thus leaving the connection of the MS with the oral cavity in the most severe cases. According to the literature, the rate of similar situations is not less than 30%.

Due to the complex character of chronic odontogenic maxillary sinusitis (COMS), surgical treatment requires a multidisciplinary approach. There is no method that can be considered the standard of treatment for chronic maxillary sinusitis. Endoscopic surgical techniques promote the transition from extensive type of surgery to minimally invasive. Endoscopic approach allows for saving important anatomical structures of the area operated on and maintaining physiological function in the MS in the postoperative period [5].

On recognized odontogenic nature of maxillary sinusitis and the absence of signs of acute inflammation, there is an opportunity for the primary endodontic preparation of the canals, followed by endoscopic sanitation of the MS by an ENT surgeon. In other cases, when tooth roots do not penetrate mucous membrane of the MS, maxillofacial surgeon (or dental surgeon) can work in collaboration with the ENT surgeon during a single procedure. The first doctor performs tooth extraction, while the second one removes changed mucous membrane of the MS as well as foreign bodies. However, endoscopic treatment has its own limitations associated with the angle of working instruments and endoscopes. This problem can be solved with changing the approach from transnasal to microsurgical intraoral approach under the upper lip.

For example, Karpischenko et al. [6] presented a case report of surgical treatment in a patient with exacerbation of chronic maxillary sinusitis. Due to multiple previous surgeries (two endoscopic surgeries and one radical sinusotomy of the left MS) 3D CT of paranasal sinuses presented multiple cells with abnormal contents, a front-wall sinus defect, and scar retractions. The complex anatomy of the MS forced the authors to use electromagnetic navigation system during the surgical treatment of the patient. This device allowed for the assessment of the sinus anatomy and adjacent structures and adequate surgical opening of all sinus cells. On control 3D CT scan of the reconstruction of paranasal sinuses, all cells of MS on the side of operation were not damaged [6].

The more difficult case for surgical treatment is the partial location of the upper third molar in oral cavity where there isn't enough soft tissue to provide good impermeability of the surgical wound. This could lead to perforation after tooth extraction and then formation of oroantral fistula. Dental and maxillofacial surgeons must be prepared for these situations and have available different additional instruments, suture and osteoplastic or barrier materials to prevent formation of oroantral fistula.

The difficulties of using different techniques for closing of acute perforation after tooth extraction are connected with wide spreading of microorganism in oral cavity and easy migration through the surgical wound to MS. Appearance of area inflammation, especially in the zone of intraoperative bleeding with later formation of hematoma, leads to development of acute maxillary sinusitis in postoperative period. The presence of microorganism increases the risk of suture failure and the inability of secondary wound healing during persistent infection. Thus, methods and materials for closing of acute perforation of MS and oroantral fistula should be chosen very carefully.

There are various techniques of repairing odontogenic perforations of the bottom of the MS using mucosal flap from the palatine and the vestibule side of the oral cavity, flaps from the lateral surface of the tongue, the mucous membrane of the cheek and the nasal cavity. All of them have their advantages and disadvantages and require further investigation.

Thus, the issue of treating patients with chronic odontogenic sinusitis remains open due to a rather large number of conditions, which makes the selection of the leading specialist complex. With COMS with a foreign body present, if the sinus anatomy is preserved and there is no communication with the oral cavity, it is preferable to conduct endoscopic sanitation of the MS by an ENT specialist. In the presence of an oroantral fistula, additional involvement of the maxillofacial surgeon or dental surgeon is necessary to conduct a full-fledged sinusotomy and to perform reconstructive techniques using osteoplastic materials and flaps.

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Мултидисциплинарно лечење болесника са хроничном одонтогеном упалом максиларног синуса – прикази болесника

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САЖЕТАК

Увод Лечење хроничног одонтогеног максиларног синуситиса и даље је важан проблем за медицину због бројних расположивих техника, сложених хируршких приступа, које обављају оториноларинголошки или максилофацијални хирург или обојица.

Циљ ове студије је да се анализирају различите методе лечења хроничног максиларног синуситиса од стране различитих специјалиста како би се начинио избор најпогоднije технике лечења.

Прикази болесника Описују се два клиничка случаја мултидисциплинарног лечења болесника са хроничним одонтогеним максиларним синуситисом уз учешће различитих специјалиста – оториноларинголошког и максилофацијалног хирурга. Један болесник је лечен ендоскопском техником, а други је подвргнут класичној отвореној синусотомији коришћењем локалних ткива и ксеногене мембране колагена за уклањање ороантралне фистуле. За процену стања пре и после лечења коришћени су клинички преглед и компјутеризована томографија.

Закључак Према резултатима ове студије, ендоскопска техника је пожељна метода лечења болесника са хроничним максиларним синуситисом када није повезан са усном шупљином. Ако постоји ороантрална фистула, потребно је да отворену операцију изведе максилофацијални хирург.

Кључне речи: хронични одонтогени максиларни синуситис; хируршко лечење; мултидисциплинарни третман

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Mineral trioxide aggregate for the treatment of external root resorption in an avulsed immature tooth – ten years of follow-up

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SUMMARY

Introduction Root resorption may occur as a consequence of avulsion injury and may lead to the progressive loss of tooth structure. The aim was to report the outcome of root resorption treated with mineral trioxide aggregate in a replanted immature permanent incisor after 10 years of follow-up.

Case outline This case presents external root resorption that was detected 18 months after the avulsion injury in a nine-year-old child. Apical portion of the canal was filled with mineral trioxide aggregate and the rest of the canal was filled with a canal sealer and gutta-percha. Control examinations were performed six months after the completion of the endodontic treatment and afterwards yearly. The tooth was asymptomatic clinically and radiographs did not show progression of root resorption up to four years of follow-up. Infraposition of the injured tooth was detected five years after the replantation, but without significant radiographic changes, until the eight-year follow-up, when root resorption was detected again. However, the tooth was still hard and symptomless at the 10-year follow-up.

Conclusion Mineral trioxide aggregate may have an important role in the preservation of replanted immature teeth for a prolonged period.

Keywords: immature teeth; avulsion injury; root resorption; mineral trioxide aggregate

INTRODUCTION

Traumatic injuries to permanent anterior teeth are common during childhood and 0.5–16% of the 7–10-year age group experience tooth avulsion [1]. Prolonged inadequate storage has been identified as a crucial factor for the survival of an avulsed tooth [2]. Ideally, tooth should be replanted within five minutes after the injury, but in clinical practice, teeth are frequently stored in unphysiological media and replantation is delayed [2]. Therefore, loss of pulpal vitality could be considered to be an expected clinical finding in avulsed teeth [3]. As a further complication, root resorption may occur and may lead to the progressive loss of tooth structure.

The treatment of avulsed immature permanent teeth presents a challenge in the contemporary clinical practice. Despite the fact that teeth with open apices have a potential to revascularize and continue root development, these teeth usually have poorer prognosis in comparison to mature teeth, particularly due to delayed replantation after unphysiological storage [3]. Unfortunately, in the majority of clinical cases, the objective of endodontic treatment is to eliminate the infection or arrest the root resorption.

Mineral trioxide aggregate (MTA) is endodontic cement that consists of tricalcium oxide, tricalcium silicate, tricalcium aluminate, silicate oxide, bismuth oxide, and other hydrophilic particles, which set in the presence of moisture. This is a biocompatible material which provides good sealing [4, 5]. It has been reported to stimulate osteoblasts/odontoblasts, thus inducting hard tissue formation, and its use has been recommended in complex endodontic cases [6, 7, 8]. The use of MTA for endodontic treatment of immature teeth implies the formation of an apical plug that acts as an artificial barrier providing an immediate obturation of the open apex.

The purpose of this report was to describe the outcome of root resorption treated with MTA in a replanted immature permanent incisor after 10 years of follow-up.

CASE REPORT

A nine-year-old girl was referred from the public dental health service to the Clinic for Paediatric and Preventive Dentistry, School of Dental Medicine, University of Belgrade. Serbia, in August of 2004. The night before the referral, after an accident during play, she suffered an avulsion injury of a permanent left central maxillary incisor and primary left lateral maxillary incisor and uncomplicated crown fracture of a permanent right central maxillary incisor.

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Figure 1. Initial radiograph

Figure 2. Calcium hydroxide dressing

Figure 3. External root resorption after 18 months

Replantation of the avulsed permanent tooth was performed after three-hour dry storage at the public health service facilities (Figure 1) and the injured tooth was stabilized at our clinic with a passive, flexible acid-etched composite splint after a total of 14 hours. Although tetracycline was recommended, the girl was placed on 250 mg of penicillin to be taken every six hours for one week. Tetanus coverage was evaluated and suitable oral hygiene and dietetic regime were recommended. Informed parental consent for the future treatment was obtained in writing and consent was also obtained from the child.

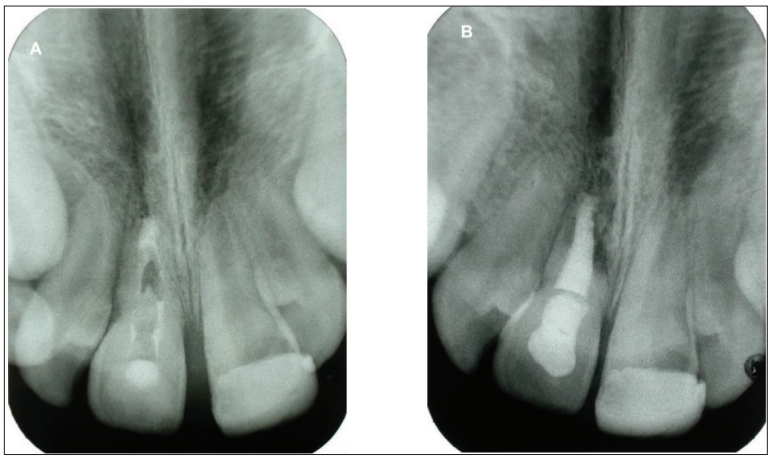

Figure 4. Definitive obturation with: (A) MTA; (B) root canal sealer and gutta-percha

Ten days after the injury, endodontic treatment of the replanted tooth was initiated. Following pulpectomy, a non-setting calcium hydroxide paste (Kalcipast®, ICN Galenika, Belgrade, Serbia) was placed in the root canal. The splint was removed 14 days after the injury.

According to guidelines at that time, endodontic treatment consisted of periodical changes of a calcium hydroxide dressing for apexification at the following regime: seven days, one month, and when radiographs revealed a loss of material from the root canal (Figure 2) [9]. Periapical radiographs were taken every three months during the first year, and every six months thereon. Eighteen months after the injury, external root resorption was detected radiographically (Figure 3). At this point, the patient and her parents were informed that initial treatment was not successful, and that progredient root resorption would eventually lead to tooth loss. They were not particularly motivated to continue with the treatment that is "temporary," especially in the situation when the complication occurred. Taking into account all clinical considerations, a definitive endodontic treatment was suggested.

Calcium hydroxide was removed and the apical portion of the canal was filled with mineral trioxide aggregate (ProRoot MTA, Dentsply Tulsa Dental, Tulsa, OK, USA; Figure 4a). The material was allowed to set into the canal and after three days the rest of the canal was filled with a canal sealer (Acroseal, Septodont, Saint Maur des Fosses, France) and gutta-percha (Figure 4b). The crown of the tooth was then restored with glass-ionomer cement (Fuji IX, GC Int, Tokyo, Japan) and composite material (Gradia Direct, GC Int).

Control examinations were performed six months after the completion of the endodontic treatment and yearly thereon. The tooth was asymptomatic clinically and radiographs did not show progression of root resorption up to four years of follow-up (Figures 5a–5c). Infraposition of the injured tooth was detected five years after replantation, suggesting that replacement resorption was developing (Figure 5d). At this point, decoronation was not considered as a treatment option since alveolar contour was still preserved. To improve aesthetic appearance, the tooth was built-up with composite material. At the six-year (Figure 5e) and seven-year (Figure 5f) follow-up, no significant resorption progression was observed clinically or radiographically. Starting from the eighth year, root resorption developed again (Figures 5g–5i). However, the tooth was still hard and symptomless at the 10-year follow-up.

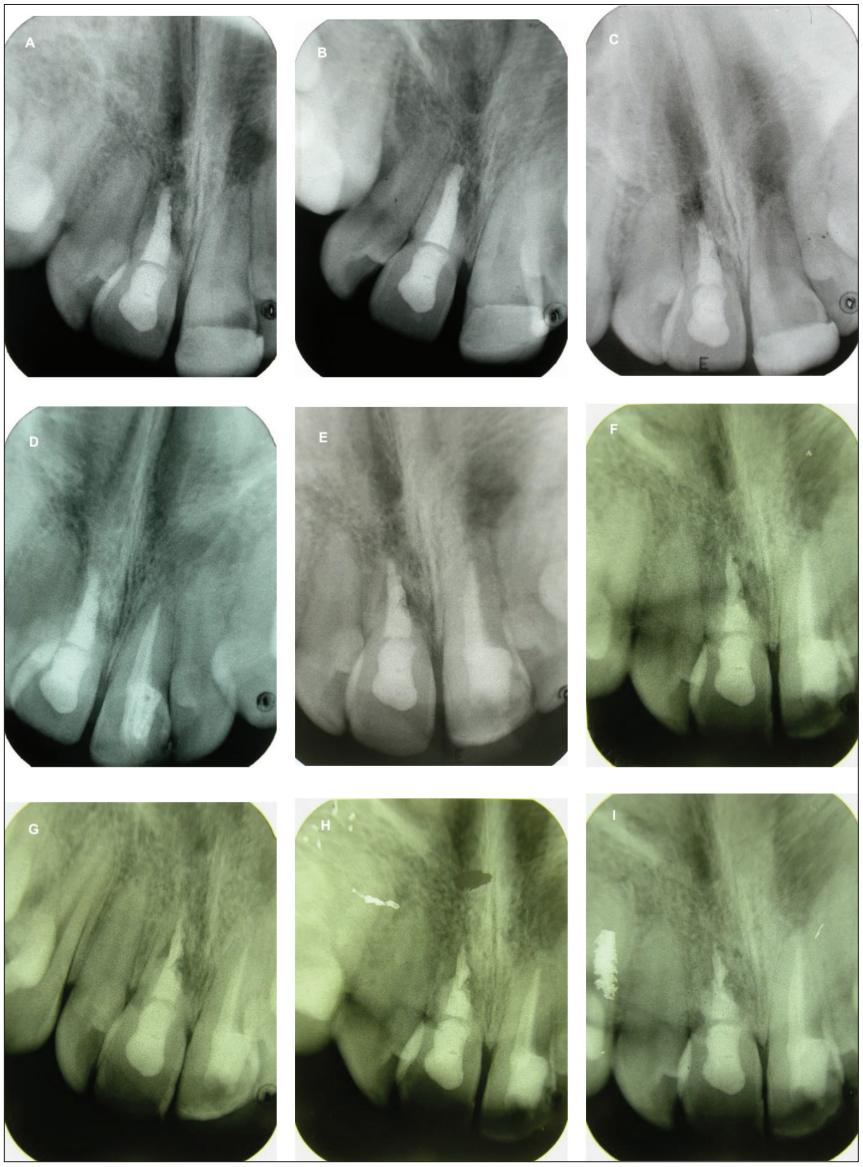

Figure 5. Radiographic follow-up after: (A) two years; (B) three years; (C) four years; (D) five years; (E) six years; (F) seven years; (G) eight years; (H) nine years; and (I) 10 years

Unfortunately, the patient was not followed longer as she had moved and the tooth was extracted by another dentist.

DISCUSSION

Most avulsion injuries occur at an age which is crucial to facial growth and the psychosocial development of a child. The treatment of an avulsion injury is rather demanding, considering the fact that several factors, such as the mechanism of the injury, apical maturity of the root, extra-alveolar storage, the patient's compliance, etc. may influence therapy and prognosis of these injuries [3]. Nevertheless, the maintenance of the injured tooth is of utmost importance in young patients until growth reaches its full potential.

Favorable healing after an avulsion injury requires quick emergency intervention, followed by an evaluation and possible treatment at decisive times during the healing phase [10]. The maintenance of periodontal ligament cellular viability is essential for longevity of the replanted tooth [11]. However, if excessive drying occurs before replantation, the damaged periodontal ligament cells will elicit a severe inflammatory response over a diffuse area on the root surface. Treatment strategies should always be considered in the context of limiting the extent of the peri-radicular inflammation, thus tipping the balance toward favorable (cemental) rather than unfavorable (osseous replacement or inflammatory root resorption) healing [10].

Apical maturity of the root presents an important factor that determines the outcome of a replanted tooth. Although a tooth with uncompleted root development possesses a strong reparatory potential and a thicker periodontal ligament that desiccates more slowly, the postreplantation outcome is usually poorer compared to a mature tooth. Andreasen et al. [2] in a study of 400 replanted teeth reported higher failure rates in teeth with open apices. According to the current guidelines, the removal of the necrotic pulp tissue and the use of calcium hydroxide in the root canal are mandatory to stimulate apical closure in a developing tooth and to prevent root resorption [12]. However, it has been reported that prolonged treatment with calcium hydroxide exhibits a high complication rate [2, 3]. This approach comprises repetitive changes of calcium hydroxide dressing which may last for several months leading to the difficulties in patient follow-up. In addition, the canal is susceptible to reinfection because it is covered by a temporary coronal seal [13]. For those reasons, the use of calcium hydroxide for apexification is no longer supported [14].

Currently, MTA is recommended for the treatment of immature teeth with necrotic pulp [5]. Erdem and Sepet [13] performed early treatment with MTA in traumatized immature incisors and showed resolution of the periapical lesions and apexification in four out of five teeth during the two-year follow-up period. Sarris et al. [15] investigated MTA as an apical barrier in 17 non-vital immature permanent incisors and reported a decrease in the size of the periapical lesion without radiographic signs of root resorption in 76.5% of cases. Moore et al. [16] evaluated 22 non-vital traumatized teeth treated with MTA and showed

95.5% clinical and 90.9% radiographical success during the minimum 18-month follow-up. Pace et al. [17] evaluated 17 immature teeth treated with MTA during 10 years and reported 94% healing rates. Ćetenović et al. [18] assessed two MTA products in both immature and mature traumatized necrotic teeth, and showed significant reduction or complete regression of chronic periapical lesions. Data on comparison between calcium hydroxide and MTA clinical efficacy in immature teeth apexification are still limited. It has been shown that both materials had similar clinical (asymptomatic tooth without pain, swelling, and luxation) and radiographical (apical barrier formation, normal periapical space, no root resorption/fracture) success rates, with apical barrier formation with MTA requiring significantly shorter time [19–23].

The evidence on use of MTA for the treatment of teeth with root resorption is low [5]. Correspondingly to the present case, Aggarwal and Singla [24] reported that treatment with MTA successfully discontinued external root resorption after avulsion and replantation during a four-year follow-up. It has been shown that MTA has high pH and the ability to release calcium and phosphate ions for a longer period, which may deactivate the inflammatory process and retard the odontoclastic activity [24, 25].

To conclude, maintenance of an anterior tooth is of the utmost importance in pediatric patients until growth reaches its full potential. MTA may have an important role in the preservation of a replanted immature tooth for a long period.

Informed consent: Written informed consent was obtained from the patient for this case report publication, including the accompanying images, case history, and data.

Conflict of interest: None declared.

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Минерални триоксидни агрегат у терапији екстерне ресорпције корена избијеног зуба са незавршеним растом корена – исход после десет година

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САЖЕТАК

Увод Ресорпција корена зуба се може јавити као последица повреде и може водити прогресивном губитку зубних структура.

Циљ рада је био да се прикаже клинички исход репланта-ције избијеног сталног зуба са незавршеним растом корена после десет година.

Приказ болесника Представљен је случај екстерне ресорпције корена зуба детектоване 18 месеци после авулзионе повреде код деветогодишњег детета. Апикални део канала корена зуба је напуњен минералним триоксидним агрегатом, док је остатак канала оптуриран пастом и гутаперка поенима. Контролни прегледи су обављани шест месеци после завршетка ендодонтског лечења и, после тога, једном годишње. До четврте године праћења зуб је био без клиничких и радиографских знакова прогресије ресорпције корена. Инфрапозиција повређеног зуба уочена је пет година после реплантације, али без значајних радиографских промена све до осме године праћења, када је уочено напредовање ресорпције корена. Ипак, после десет година зуб је и даље био клинички без симптома.

Закључак Минерални триоксидни агрегат може имати значајну улогу у дуготрајном очувању реплантираних зуба са незавршеним растом корена.

Кључне речи: незавршен раст корена; авулзија; ресорпција корена; минерални триоксидни агрегат

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REVIEW ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Myoma pseudocapsule – a biological and surgical structure to respect during myomectomy

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SUMMARY
Uterine fibroids affect almost one in two patients, causing many pelvic problems and requiring pharmacologic and surgical treatment. For many years, the importance of the fibroid was emphasized as uterine pathology, without focusing on the complex myometrial biology peripheral to fibroid. Moreover, the traditional surgical technique in fibroid removal has not been investigated for years. In recent years, on the contrary, morphological, neuroendocrine and anatomical studies have demonstrated the importance of a biological and surgical structure surrounding myoma, rich in neurotransmitters and neurofibres, the myoma pseudocapsule. This structure is formed in the womb peripheral to fibroid onset, it separates the fibroid from the myometrium and acts as a tissue regenerator after the removal of the fibroid from the uterus. The translation of scientific research on pseudocapsules into surgical practice has allowed us to identify new techniques of myomectomy, removing the myoma inside the pseudocapsule and promoting the pseudocapsules sparing surgery. All this to favor the subsequent biological process of uterine scarring and healing, by activating the neurotransmitters and neurofibres present in the myometrial fovea. The correct healing after fibroid removal restores the uterine anatomy, with a positive impact on subsequent reproductive function, reducing problems related to the muscle scar.
Keywords: myoma pseudocapsule; myomectomy; neurovascular bundle; reproductive surgery; pregnancy; fertility

INTRODUCTION

Uterine myomas, leiomyomas, or fibroids are the most common pathology in female genital organs; most of them are asymptomatic, but they can also cause severe symptoms [1]. The prevalence of uterine fibroids ranges 5.4–77% [2]. Fibroids negatively affect female reproductive function, as they are recognized as an important cause of infertility. Moreover, women affected by fibroids are at a higher risk for complications during pregnancy, labor, and delivery [3]. Fibroids are preferentially treated by surgery, by vaginal, laparoscopic, laparotomic, or hysteroscopic approach [4, 5, 6].

Currently, minimally invasive techniques based on biologically reasoned myomectomy allow the preservation of the myoma pseudocapsule, in order to spare the muscular and fibro-neurovascular myometrial fibers, ensuring the complete and bloodless removal of the myoma [7, 8].

PHYSIOLOGY OF UTERINE MUSCLE REPAIR

Myometrial scar after myomectomy requires a correct and physiological healing. The healing process, once the fibroid is enucleated, is one of the fundamental steps that restores the uterine reproductive function. Nevertheless, the muscle damage itself may promote the signaling activities to trigger regeneration of the myometrium. The synthesis and release of signaling molecules, initiated by neurotransmitters and neurofibres, after the cellular damage, can prompt itself the cell activation, inducing muscle regeneration and healing [9]. After the muscle damage, the biological "injury–repair–regeneration" sequence leads to complete functional recovery during the days or weeks after the initial injury [10].

The fundamental process in the regeneration of a damaged muscle is the revascularization of the injured muscular tissue. The neoangiogenetic network in the myometrial fovea (where the fibroid was located) is the first sign of tissue repair, as a necessity for the later morphological and functional healing. New capillaries grow from the surviving stems of the blood vessels into the center of the myometrial fovea; the neoangiogenesis in the damaged myometrium provides a sufficient supply of oxygen, improving aerobic energy metabolism for the muscular tissue repair [11]. In such a regenerative process, the neuropeptides and neurotransmitters have a significant role in wound healing.

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Moreover, there is evidence that the nervous system and its neurotransmitters, such as neuropeptide Y (NPY), substance P (SP), vasoactive intestinal peptide (VIP), oxytocin, vasopressin (VP), growth-hormone-releasing hormone (GHRH), protein gene product 9.5 (PGP 9.5) and calcitonin gene-related peptide (CGRP) play a crucial role in mediating inflammation and healing processes [9, 12, 13]. Among these neurotransmitters, a slight distribution of oxytocin neurofibres into the uterus has been demonstrated, with their high presence into the cervico-isthmic area [14].

Analyzing the biology of the myometrial scar after myomectomy, the spared neuropeptides enhance the correct myometrial healing at the hysterotomic site, and most of the abovementioned neuropeptides have been found in the fibroid pseudocapsule, as a neurovascular bundle surrounding the fibroid [14–17]. To date, it has not been clarified if the pseudocapsule vasculature network could be sustained by mechanical and inflammatory effects of the fibroid on myometrium, or produced by a sort of "neoplastic-type" neoangiogenesis, due to the myoma growth or even to a muscle and tissue healing process [18].

In the human uterus, obstacles in attaining serial specimens of hysterotomic scar after myomectomy are the main problems of biological and surgical investigations. Thus, the post-cesarean section and post-myomectomy remodeling processes in the womb are currently an unsolved puzzle. They can be monitored only by ultrasound or magnetic resonance imaging [19].

Healing of the wound is a vibrant process which involves neuromediators, neuropeptides, angiogenetic factors, blood cells, extracellular matrix, and parenchymal cells. It follows three composite and coinciding phases: inflammation, tissue formation, and remodeling [20]. In these phases, the intra-pseudocapsule growth factors can be very useful in enhancing the muscle repair process after myomectomy [21].

Nonetheless, the biogenesis of a myoma pseudocapsule requires further investigations, either on the analysis of the hormonal and pharmacological effects of drugs on the pseudocapsule, based on reducing fibroid growth without compromising pseudocapsule characteristics, or on the preoperative therapy and the post-operative follow up, in order to preserve womb functionality, as much as possible [22, 23].

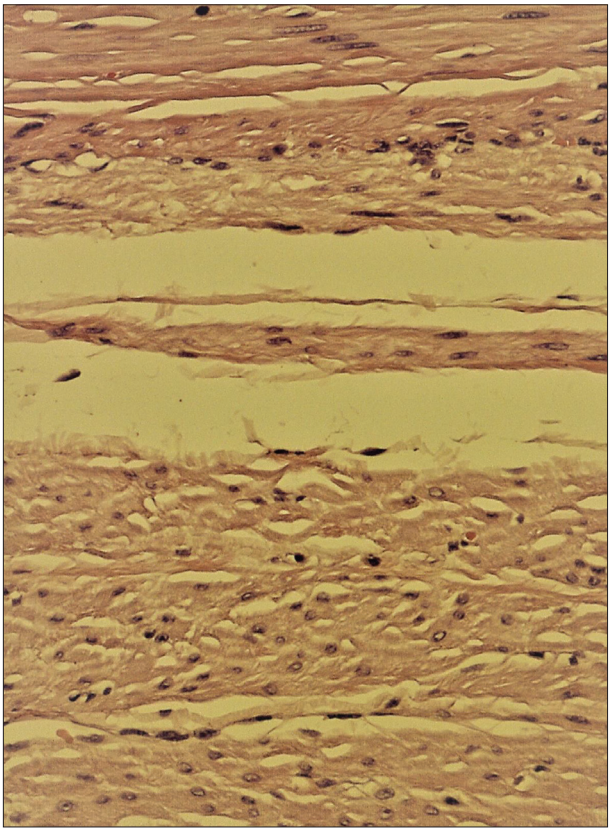

Figure 1. Hystology of the myoma pseudocapsule

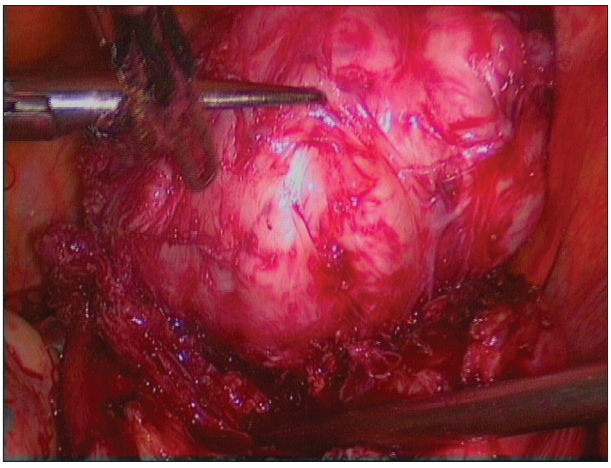

Figure 2. Laparoscopic intracapsular myomectomy

THE "INTRACAPSULAR MYOMECTOMY"

The rationale for the fibroid removal starts with the preservation of its pseudocapsule (Figure 1), the neurovascular bundle which surrounds the myoma itself [15, 16, 17]. This modern technique of removing fibroids was adapted from urology, in which the removal of the prostate gland must occur with the conservation of the neurovascular bundle above it. Thus, concerning the significance of the prostatic capsule-sparing and the physiological role of nerve-sparing techniques for prostatectomy, it was possible to translate it on the pseudocapsule-sparing during myomectomy.

There are also some passages that can be borrowed from urology to gynecology during prostatectomy and myomectomy. During laparoscopic/robotic prostatectomy, if bleeding occurs, meanwhile, insufflation pressure can be increased and local compression can also be performed by a hemostatic gauze, directly on the prostate neurovascular bundle (the source of bleeding). The same surgical passages can be repeated during laparoscopic myomectomy (Figure 2), in case of bleeding. Then, the hemostasis by high-wattage diathermocoagulation should always be avoided during dissection near the prostate neurovascular bundles, as it has been shown that it can be harmful to the cavernous nerve function in the canine model, with indirect damage to the pelvic nerves of sexual function after prostatectomy [18, 24].

Figure 3. Macroscopic exposition of the pseudocapsule

Returning to the fibroid enucleation technique, the possibility to perform myomectomy by removing the myoma from its pseudocapsule was called "intracapsular myomectomy" [16, 18, 19]. In this technique, the fibroid is taken out, by surgeon, from its muscular fovea, by stretching and enucleating the myoma directly from the adjacent myometrium, avoiding the damage to the adjacent fibromuscular skeleton, breaking up the fibrous bridges (Figure 3) [4, 6, 25, 26]. As a general surgical recommendation, in robotic, laparoscopic, laparotomic, vaginal, or hysteroscopic setting, the surgical enucleation of each fibroid always needs to be gently performed in order to enhance and improve the successive myometrial healing, trying to correctly restore the uterine anatomy and biology [27, 28]. Thus, the myoma pseudocapsule neurovascular bundle needs to be preserved and spared during myomectomy, avoiding any damaging surgical maneuvers, such as extensive and high-wattage diathermocoagulation or unnecessary tissue manipulation or muscular injury. This physiological surgical technique to remove fibroids largely respects the fibroid neurovascular bundle, and its neuropeptides and neurofibers, since the iatrogenic pseudocapsule damage

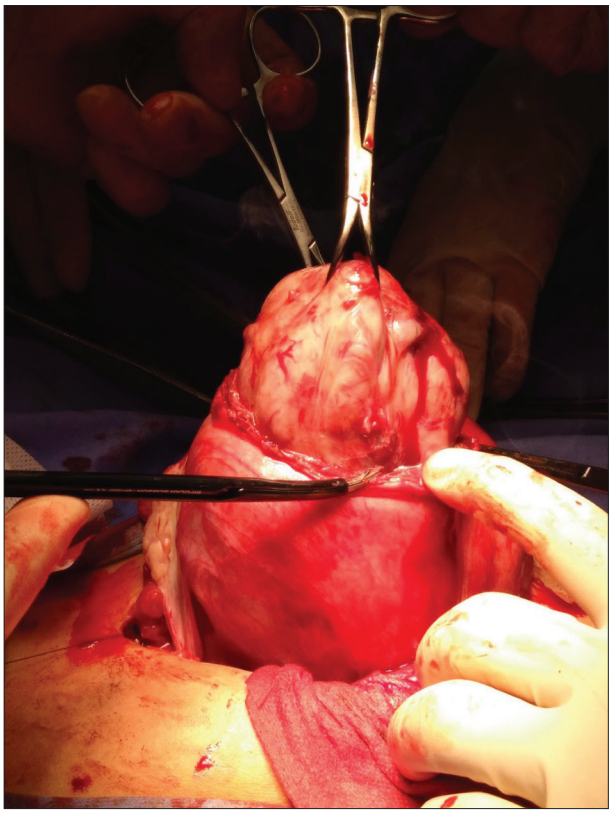

Figure 4. Laparotomic intracapsular myomectomy

may change the successive neurotransmitters' function in muscle repair, thus impairing uterine healing [8, 18].

The operative consequences of an inappropriate myomectomy technique performed with pseudocapsule injury may lead to the following: 1) a reduction in the number of neuropeptides and neurofibres at the hysterotomic site, with a negative impact on physiological myometrial healing; 2) an increase of the fibrotic process at the hysterotomic site; and 3) insufficiency of either myometrial neurotransmission or of muscular impulse and contractility. All of these features can lead to an altered post-surgical uterine physiology, as well as to a reduced uterine musculature functionality. This process could be the main one responsible for impaired healing with uncorrected myometrial functionality, with unfavorable effects on a subsequent pregnancy, including a possible dramatic uterine rupture [29, 30].

LAPAROTOMIC/LAPAROSCOPIC INTRACAPSULAR MYOMECTOMY

The laparotomic and laparoscopic intracapsular myomectomy techniques do not differ substantially, except for the major surgical benefit of laparoscopy in case of subserous and intramural fibroids. The principal benefits of the endoscopic approach are significant reduction of perioperative operative blood loss, lower dosage requirements for analgesic drugs, and shorter hospitalization. Additionally, laparoscopic intracapsular myomectomy results in slightly enhanced short-term outcomes in terms of postoperative fever, myometrium scar hematoma formation, ileus and

antibiotic treatment, in comparison to laparotomic surgery (Figure 4). Laparoscopic myomectomy has reduced blood loss for the hemostatic CO_2 pressure, a part of selective gentle coagulation of the pseudocapsule vessels. The CO_2 insufflation can reduce blood loss during laparoscopy, as increased intra-peritoneal pressure can lead to the occlusion of small blood vessels and capillaries during myomectomy [30]. This effect, combined with fewer traumatic endoscopic micro-manipulations, could result in overall positive surgical results [2, 7, 25].

Once the visceral peritoneum is incised at the midline longitudinal plane, by monopolar scissors, by the harmonic scalpel, monopolar scissors, or crochet needle electrode, the myoma pseudocapsule is easily recognized during dissection and then cut to expose the fibroid surface. The identification of the "cleavage plane" between the fibroid and the pseudocapsule is important for correct intracapsular myomectomy, in order not to preserve muscular fibers and to selectively coagulate blood vessels of the pseudocapsule. Atraumatic clamp or irrigator cannula should be used to gently dissect the myoma from its pseudocapsule. Hemostasis of the small vessels bleeding can be selectively achieved by low-wattage bipolar clamps, a harmonic scalpel, hook electrode, or monopolar scissors, always at low wattage (not more than 30 watts), to progressively expose the hidden part of the fibroid into the uterus (the myometrial fovea), incorrectly called "the base of the myoma", and the pseudocapsule fibrovascular bridges (selectively coagulated). This technique allows for a complete minimally traumatic removal of the fibroid from its pseudocapsule, with minimal blood loss and pseudocapsule sparing.

HYSTEROSCOPIC INTRACAPSULAR MYOMECTOMY

The development of hysteroscopic myomectomy in 1976 represented a revolution in the treatment of submucous fibroids, replacing the laparotomic approach, significantly improving patients' surgical outcomes [31]. Nowadays, the hysteroscopic myomectomy represents the gold standard in the treatment of submucous myomas [32]. Nevertheless, the submucous myomas' treatment is probably a hysteroscopic procedure that caries greater risk than others, due to the potential for complications it has, such as cervix laceration, hemorrhage, uterine perforation, or clinical intravasation syndrome [33, 34]. It is not easy to estimate the right frequency of the abovementioned complications as it is of high variability according to pathology characteristics, surgeon skills, and employed technique [35].

Wamsteker et al. [36] and Lasmar et al. [37] investigated and assessed the characteristics of submucous fibroids able to influence surgical outcomes in hysteroscopic myomectomy. The treatment of intracavitary fibroids with an intramural extension of 50% or more has always been represented as a challenge for hysteroscopic surgeons, as it is burdened with an increased risk for intraoperative complications, sometimes requiring a multiple-step procedure [5, 36, 38]. Moreover, in cases of multiple myomas, the risk is even higher.

Currently, resectoscopic slicing is still the most commonly used technique for treating submucous myomas, and probably for this reason myomectomy represents the hysteroscopic procedure with a higher complication rate. Unfortunately, the classical slicing technique, even in expert hands, does not respect the pseudocapsule and the adjacent healthy myometrium. During the resection of the intramural component of a myoma, the "pseudocapsular tissue" should be visualized all over the resected area. Sometimes, due to fibroid dimensions and bleeding, it can be challenging to make a distinction between the myoma tissue, the pseudocapsule, and the healthy myometrium. The problem is the direct action of the electrical loop during the myoma slicing and the incorrect dissection of the anatomical planes. The traditional technique altered the intrauterine anatomy and it is almost impossible to avoid the injury of the myometrial fibers, causing a direct (by cutting) and indirect (by thermal spreading) damage to the pseudocapsule and the surrounding healthy myometrium. This fundamental issue is responsible for all the intraoperative complications during the hysteroscopic myomectomy. Moreover, the role of surgical trauma to the healthy myometrium during hysteroscopic surgery can lead to synechiae and adhesions' formation [28, 39].

The ideal hysteroscopic myomectomy ideally should be performed in one surgical step, a safe and effective procedure, which is simple and well-tolerated [32]. In the last decades, several techniques have been proposed in order to overcome the limits represented by the classical slicing for the treatment of the myometrial portion of submucous myomas [5]. The main objective of these techniques was the detachment of the intra-myometrial part of the fibroid, in order to enable the sliding from the myometrium into the uterine cavity. Some authors proposed the use of uterine contractions, induced by manual massage, drugs, or changing intrauterine pressure [40–43]. A combination of multiple techniques and ultrasound monitoring during myomectomy was also described [44, 45]. Authors proposed the detachment of intramural component of fibroids by electrical incision of the fibroconnective bridges, anchoring the myoma to the pseudocapsule [46].

A technique that allowed us to overcome the limits represented by the classical slicing was described in 1995, as the cold-loop hysteroscopic myomectomy [46]. This method allowed to change the approach to the myoma, from the progressive reduction or electrical power using and its switching to a mechanical enucleation of the myoma from the pseudocapsule, by the physiological contraction of the myometrium. It represented a revolution in the hysteroscopic treatment of submucous fibroids, since it distinguished the anatomical planes, respected the anatomical and functional integrity of the myometrium and of the pseudocapsule, at the same time ensuring a safe and effective procedure. By the cold-loop myomectomy, the fibroconnective bridges that anchor the myoma to its pseudocapsule are mechanically disconnected, enucleating the intramural component of the fibroid, without any effect to the adjacent healthy myometrial tissue. Moreover, the cold loops are applied between the myoma and the pseudocapsule, allowing to avoid uterine

perforation by electrical loop and injury to abdominal organs or vessels. Noteworthy, in case of perforation by cold loops, the damage induced can be considered to be the same as that with a Hegar dilator [8].

Finally, the respect of the myometrium allows uterine contractions, facilitating the sliding of the intramural part of the fibroid into the uterine cavity and at the same time increases the free myometrial margin thickness [47]. The uterine contraction and the respect of the myometrial muscular fibers decreases the risk of bleeding and the absorption of the distension medium, enhancing the possibility to accomplish the treatment in a single operation [48].

The respect of the pseudocapsule promotes better healing of the myometrium, avoiding scarring and reducing the intrauterine adhesions and dramatic complications, such as subsequent uterine rupture [5, 28, 49, 50].

CONCLUSION

The morphological and molecular investigation performed on the fibroid pseudocapsule changed the current surgical and biological scenario of myomectomy, due to interesting scientific results. The discovery of the fibroneurovascular structure surrounding the fibroid, rich in neuropeptides and neurotransmitters, translated the new concept of myomectomy in reproductive surgery and in fertility-sparing procedures, even for giant fibroids and during pregnancy.

Much evidence on the presence of angiogenetic properties in a few millimeters of the biological structure, the pseudocapsule, underline the necessity to preserve it as much as possible while performing myomectomy, especially to preserve myometrial integrity near the fibroid site, indirectly enhancing myometrial healing after myomectomy, and reducing surgical bleeding.

Intracapsular myomectomy should also enhance the post-operative adhesions' reduction. In our opinion, the intracapsular myomectomy, with pseudocapsule sparing by endoscopic "microsurgical" magnification, as a safe and feasible minimally invasive technique, should be performed in all myomectomies.

Conflict of interest: None declared.

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Псеудокапсула миома – биолошка и хируршка структура коју треба поштовати током миомектомије

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САЖЕТАК

Миоми материце се јављају код готово сваке друге болснице, узрокују бројне интрапелвичне тегобе и захтевају фармаколошко или хируршко лечење. Дуги низ година значај миома је посматран као болест материце, без фокусирања на комплексну биологију миометријума који их окружује. Уз то, традиционална хируршка техника уклањања миома није истраживана годинама. Насупрот томе, у новије време, морфолошке, неуроендокрине и анатомске студије су показале значај биолошке и хируршке структуре која окружује миом, богате неуротрансмитерима и нервним влакнима, односно псеудокапсуле миома. Ова структура се формира у материци периферно од миома, одваја миом од миометријума и функционише као регенератор ткива после уклањања миома из материце. Примена научних истраживања псеудокапсула у хируршкој пракси омогућила је настанак нових техника миомектомије, које подразумевају уклањање миома унутар псеудокапсуле и промовисање поштедних операција у односу на псеудокапсулу. Све ово погодује накнадним биолошким процесима ожиљавања и зарастања материце, активацијом неуротрансмитера и нервних влакана присутних у миометријалној ложи миома. Правилно зарастање после уклањања миома успоставља анатомију материце, са позитивним утицајем на касниј репродуктивну функцију, смањујући компликације повезане са ожиљком у миометријуму.

Кључне речи: псеудокапсула миома; миомектомија; неуроваскуларна петељка; репродуктивна хирургија; трудноћа; фертилитет

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REVIEW ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Imaging of the temporomandibular joint – contemporary clinical and radiological implications

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SUMMARY
The temporomandibular joint (TMJ) represents a biomechanically and morphologically complex structure, tightly connected with the development and growth of mandible and craniofacial complex.
The aim of this article is to comprehensively present contemporary diagnostic modalities and clinical implications for imaging of the TMJ.
Contemporary imaging modalities, if used properly and according to adequate clinical implications and criteria, are able to depict different pathological processes and play a crucial role in establishing the right diagnosis and monitoring therapeutic effect. The key to right diagnosis, however, still lies in good knowledge of the TMJ developmental and functional anatomy, as well as the TMJ dysfunction related to the jaws, surrounding muscles, teeth, and cranial base.
Keywords: temporomandibular joint; temporomandibular joint disorders; magnetic resonance imaging; craniomandibular disorders

INTRODUCTION

The temporomandibular joint (TMJ) represents a biomechanically and morphologically complex structure, tightly connected with the development and growth of mandible and craniofacial complex [1]. The superior part of the joint is formed by articular eminence (part of the temporal bone), while the inferior part is formed by the mandibular condyle [2, 3]. The understanding of growth and development of the TMJ is necessary for understanding complex pathophysiological fundaments of the TMJ dysfunction [4]. Furthermore, it is essential for comprehensive radiological evaluation of the joint and establishing a clinically valuable diagnosis based on the imaging.

One of the most common symptoms that induce the patient to seek the treatment is pain. Chronic pain is associated with some psychological disorders such as depression and somatization, which makes the evaluation and treatment even more complicated [5]. Chronic pain in the TMJ is considered to be the part of central sensitization syndrome (CSS), meaning that even subtle anatomical derangements can lead to the chronic regional pain [6]. There is a clinical instrument used as screening for CSS, considered to be a useful tool in the hands of an experienced clinician [7, 8, 9].

Imaging of the TMJ was initially performed using methods of conventional radiography, such as panoramic, transcranial radiography, and cephalometry [10, 11, 12]. Conventional radiography is of limited use since the anatomy the TMJ requires three-plane imaging [13]. These methods are limited, due to distortions, superimpositions of tissues, and poor tissue contrast. Disadvantages of these two-dimensional radiologic methods opened the door for three-dimensional imaging modalities in the TMJ evaluation. Computed tomography (CT) is an imaging method that provides useful information about osseous morphology of the joint, but high radiation dose and high cost make it unfavorable for the TMJ evaluation [14]. Cone-beam computed tomography (CBCT) is a recently developed imaging modality and has already become a method of choice for evaluation of the TMJ osseous morphology [15]. It enables obtaining submillimeter slices in all three planes, with shorter scanning time, lower radiation dose, and at lower cost than CT [16]. However, soft tissue imaging remains a problem for CBCT, making space for magnetic resonance imaging (MRI), which is the imaging modality of choice for assessment of disc and soft tissue pathology [17, 18].

Imaging of the TMJ requires a dedicated patient posturing. Adequate mandible positioning is essential both for soft tissue and osseous imaging. It is necessary to obtain images both in positions of the closed jaw and fully-opened jaw. Fully-opened jaw position is essential for evaluating condyle position and disc status. If the position of fully-opened jaw is suboptimally

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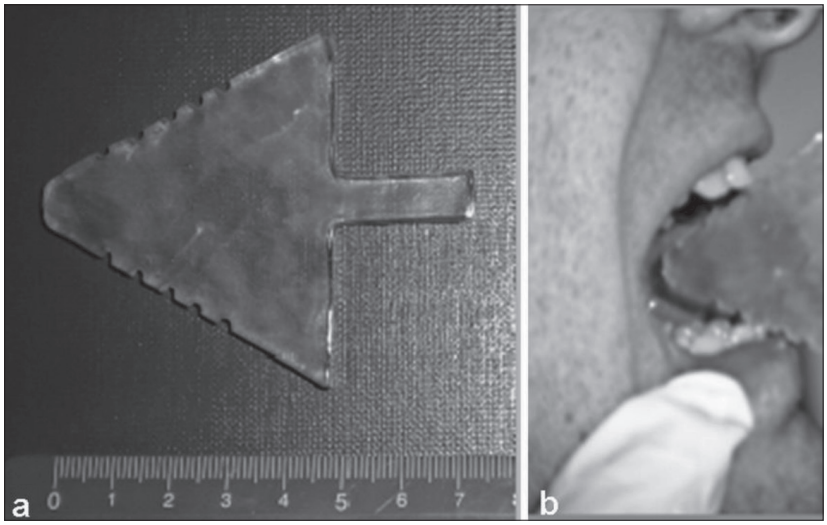

Figure 1. (a) Applicator – mouth opener; (b) the position of the applicator during the opening of the jaw in the middle position

Figure 2. The position of parasagittal images of temporomandibular joint that follow the axis of the mandibular body

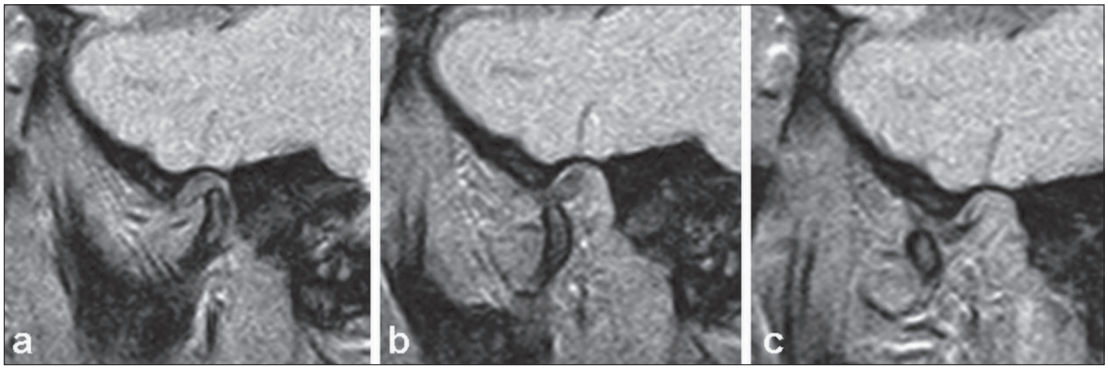

Figure 3. Parasagittal magnetic resonance images of the temporomandibular joint in the closed jaw (a), semiopened jaw (b), and fully opened jaw (c) positions with the use of the applicator; the translation of the mandibular condyle and the disc movements can be observed in the 12 o'clock position

accomplished, spatial relationships in the TMJ cannot be reliably examined and the proposed diagnosis is not accurate [1]. However, a number of patients are not able to remain adequately positioned during the time of scanning (especially with MRI), so prefabricated mouth openers (applicators) are commonly used to maintain the position of opened jaw (Figure 1). Also, sometimes, a scan with a splint placed between the teeth is necessary to evaluate the effect of the splint on the position of the condyle.

Clinical implications for the imaging of the TMJ are various, covering a broad span of possible etiologies, including developmental (hemifacial microsomia, hypoplasia, hyperplasia) [19], traumatic (fractures) [20], inflammatory (juvenile idiopathic arthritis, rheumatoid arthritis, pigmented villonodular synovitis) [13], degenerative [21, 22], neoplastic (benign and malignant tumors) [23], or vascular disorders [24, 25].

The aim of this article is to comprehensively present contemporary diagnostic modalities and clinical implications for the imaging of the TMJ.

IMAGING MODALITIES

Magnetic resonance imaging

MRI of the TMJ is established as an internationally recognized standard for the evaluation of position, shape, and mobility of the disc and condyle, as well as for the evaluation of soft tissues surrounding the joint [26]. The advantage of MRI is free choice of the plane in which the images are obtained. Classical planes used for the TMJ assessment are corrected sagittal oblique and coronal oblique, through the axis of the mandibular body (Figure 2). These planes are used in order to detect correct spatial relationship between the disc and the condyle, as well as between the condyle and the glenoid fossa. MRI offers a palette of different sequences enabling different tissue contrasts, necessary for detecting pathological processes of different structures. Conventional MRI protocol of the TMJ consists of T1-weighted (T1W), T2-weighted (T2W), and proton density-weighted (PDW) tomograms both in opened- and closed-jaw positions (Figure 3). T1W and PDW tomograms depict anatomical relationships and morphology of structures, while T2W tomograms depict the presence of abnormal fluid collections and bone marrow edema.

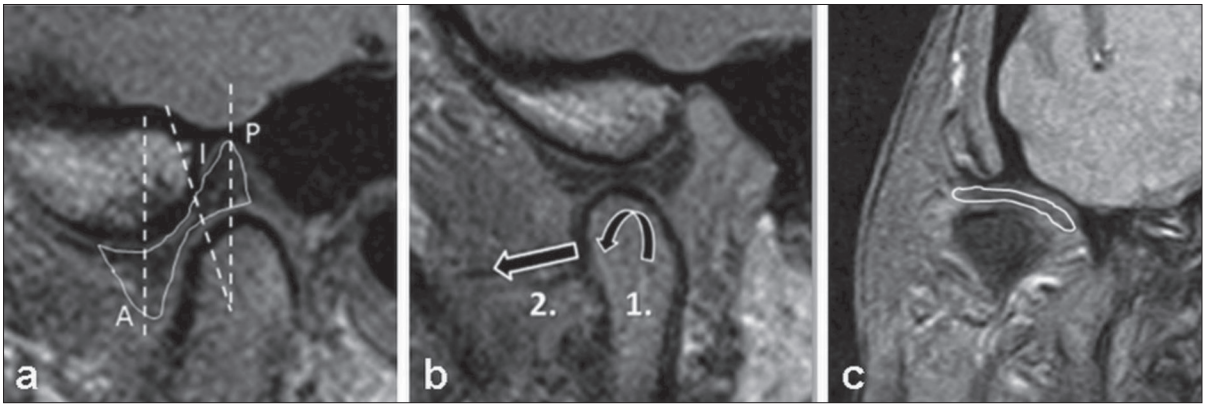

Figure 4. The disc at the 12 o'clock position in the closed jaw position in the sagittal view; (a) the anterior zone (A) is below the articular eminence, the intermediate zone (I) is between the condyle and the posterior slope of the articular eminence, and the posterior zone (P) is above the tip of the condyle; (b) in the opened jaw position, the condyle (1) performs rotation and then translation anteriorly (2); (c) in the coronal view, the disc is situated on the condylar head without medial or lateral dislocation

Postcontrast imaging is performed by injecting intravenous paramagnetic contrast agent (GdTPA) and obtaining T1W tomograms, preferably suppressing the fat signals. Postcontrast imaging is indicated when there is clinical suspicion of inflammatory or neoplastic disorder [3].

MRI represents a non-invasive diagnostic module that does not imply the use of ionizing radiation and therefore carries little or no risk for the patient's long-term well-being. However, the examination is time-consuming (over 30 minutes), not widely available, and expensive. Furthermore, there are absolute (cochlear implants, pacemakers, presence of metal foreign bodies, obesity) and relative (pregnancy, claustrophobia) contraindications for MRI that prevent a substantial group of patients from undergoing this examination. Finally, MRI is superior in evaluation of soft tissue pathology, while the evaluation of osseous structures and the presence of fracture is rather limited. However, clinical implications regarding the status of bone marrow (edema, infiltration) should be examined using MRI, since it is able to clearly and reliably depict these pathological processes [13, 20].

Cone-beam computed tomography

CBCT is a diagnostic modality mostly used for imaging in dentistry. Imaging protocol using CBCT includes images axially corrected perpendicular to and along the long axis of the mandibular condyle. These images are obtained as three-dimensional volume format and can be reformatted in the sagittal oblique and coronal oblique planes using postprocessing on digital workstations. Furthermore, curvilinear reconstructions along the curve of the mandible (panoramic reformation) and three-dimensional surface renderings in frontal and lateral views can be obtained. This enables the visualization of the maxillofacial complex and the evaluation of the effect of the TMJ disorders on the mandible and the teeth [15]. CBCT provides useful information about osseous morphology of the TMJ by obtaining submillimeter slices in all three planes. The scanning time is short and radiation dose is lower than that of helical CT, so CBCT has been established as the method of choice for the assessment of osseous structures and the TMJ morphology [16].

Computed tomography

CT has a small role in the imaging of the TMJ. Although it has a superior spatial resolution and short scanning time, it implies a high radiation dose and excessive cost, so it is not considered a standard imaging tool for evaluating the TMJ pathology [15, 16].

CBCT and CT both represent fast-performance diagnostic modules that obtain high-quality images of the TMJ. Nevertheless, both modalities imply ionizing radiation and therefore should be used carefully and with clear clinical implications and questions [16]. Finally, those imaging modalities are superior for examining osseous pathology and anatomical disorders, while the utility for evaluating soft tissue processes remains limited [27].

IMAGING EVALUATION OF NORMAL ANATOMY

The examination should start from the position of the condyle contour in relation to the glenoid fossa. Cortex of the condyle is thinner on the curvature (above the neck) both on posterior and anterior surfaces. In a normal condyle, the posterior height of the contour is inferiorly positioned compared to the anterior one and this distance grows with growing of the patient (until reaching the adult size). Equator of the condyle can be observed on coronal oblique views as the line that passes through medial and lateral heights of the condyle contour. Loss of bone tissue in the articular surfaces (above the equator) indicates a degenerative or inflammatory disorder, and can be key differential diagnosis between condylar hypoplasia and degenerative joint disease [10, 28].

Articular surfaces of the condyle and the glenoid fossa are not congruent. The disc is a fibrocartilaginous structure used for amortizing this incongruency. The normal shape of the disc is biconcave, with rounded surfaces, normally positioned between the anterior aspect of the

condylar curvature and the posterior slope of the articular eminence. The irregularity of the disc shape, length, or position speaks for dysfunction of the TMJ as a structural unit. The normal posterior zone of the disc (the thickest part) is situated at the 12 o'clock position on the sagittal oblique view (with the anterior aspect on the left-hand side) (Figure 4). Thin intermediate zone is positioned above the maximum height of the condyle curvature. The anterior zone is positioned on the superior head of the lateral pterygoid muscle at the point of insertion in the pterygoid fovea. In the opened jaw position, a normal condyle translates forward to the point inferior to the eminence crest (1–3 mm anteriorly or posteriorly) but always remaining below the eminence, with enough space for the intermediate zone of the disc between the articular surfaces (Figure 4b) [10, 11]. CBCT is not able to depict the disc itself, contrary to MRI. On MRI, the disc is a low-to-intermediate signal intensity biconcave structure both on T1W and T2W, due to high amount of fiber tissue. A small, laminar amount of free joint fluid surrounding the disc is considered normal [11].

The articular surface cortex should be eggshell-thin and continuous (in children, the cortex is often invisible), presented as high-density line on CBCT/CT. On MRI, the articular cortex is hypointense and slightly thicker (compared to CBCT) due to the presence of low-signal fibrocartilaginous cap indistinguishable from bony cortex [27]. Thickening of the cortex is observed in degenerative joint diseases, while the absence of continuity is observed most commonly in fractures. Increased bone density on CBCT/CT and T1W and T2W low signal intensity indicates bone sclerosis, while T1W low and T2W high signal intensity indicates the presence of excess fluid in the form of bone marrow edema or cystic fluid collections. The presence of calcifications can reliably be detected only by using CBCT/CT, since MRI is not specific enough for the presence of calcium (at least conventional MRI) [28].

TRAUMATIC CHANGES

Neonatal fractures

Neonatal fractures occur seldomly, specifically with forceps delivery. The fracture is located in the condylar neck, with anterior dislocation of the fragment resulting in acute mandibular notch with a classical pair-of-scissors appearance. This dislocation of the condyle results in suboptimal pressure on the fibrocartilage of the eminence. The articular eminence consequently remains flat, due to the lack of stimulating pressure from the condyle. Mild to moderate mandibular asymmetry is always additionally present, since the normal growth of mandible is also compromised [29].

Bifid condyle

Bifid condyle represents a rare condition presented with partial division of the mandibular condyle. The etiology is unclear, possibly due to congenital, developmental, or traumatic reasons. The condyle shape is changed, ranging from a heart-shaped condyle, over vertical depression of the curvature, to the duplication of the condyle observed on sagittal oblique views [29].

Ankylosis

Ankylosis of the TMJ is most often a consequence of a trauma (hemarthrosis) and can be both bony or fibrous. Secondly, it can be due to inflammatory processes and previous surgery or major infection (osteomyelitis). Bony ankylosis presents with completely fused bony structures of the joint, resulting in severely compromised joint function and movement. Fibrous ankylosis is presented with low-density joint space and irregular articular surfaces [29, 30].

IMAGING OF INFLAMMATORY CONDITIONS

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disorder affecting various synovial joints, including the TMJ. Inflammation of the capsular synovia in the TMJ results in forming granulomatous pannus that further erodes articular fibrocartilage cap and underlying bone structures of the joint. The process is often unilateral or bilateral with one-side predominance. The beginning of the process is characterized by joint effusions and synovial proliferation, followed by osteopenia and resorption of the articular surface of the bones. Anterior and posterior aspects of the condyle flatten (sharpened-pencil appearance). MRI is a modality of choice in depicting changes connected to RA (Figure 5). Pannus is observed as an intermediate signal intensity structure displacing the temporal posterior attachment inferiorly and the condylar posterior attachment posteriorly. Condylar height is reduced, mandible rotates and

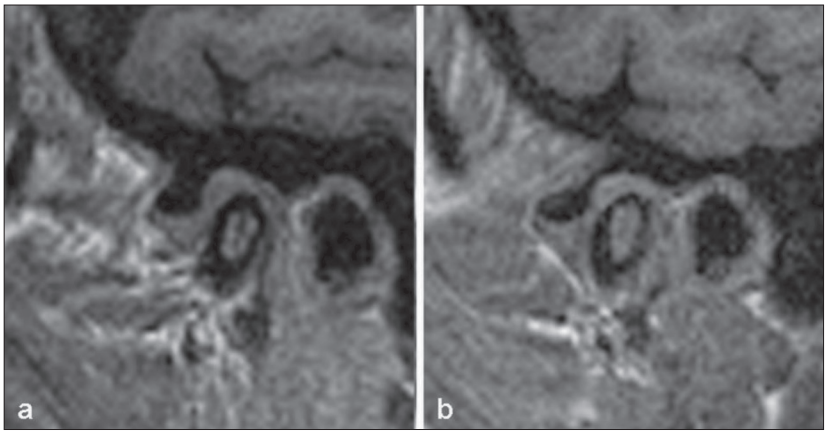

Figure 5. The contrast enhancement of the synovial thickening (a), due to synovial proliferation, is observed inside the articular fossa in a patient with rheumatoid arthritis (b)

the patient presents with anterior open bite. The advanced phase of the disease is represented with severely limited function and movements of the TMJ, as well as fibrous or bony ankylosis [31].

Juvenile idiopathic arthritis

Juvenile idiopathic arthritis (JIA) is an autoimmune inflammatory disorder presenting primarily in large joints, during the childhood. Rarely it can also affect the TMJ. The imaging modality of choice is contrast-enhanced MRI. Inflammation usually affects the condyle, resulting in a flat and wide glenoid fossa. The condyle is displaced anteriorly and superiorly. When there is an active inflammatory process present, the contrast enhancement is present in joint compartments on MRI preceding radiographically (and tomographically) visible bone destruction [32].

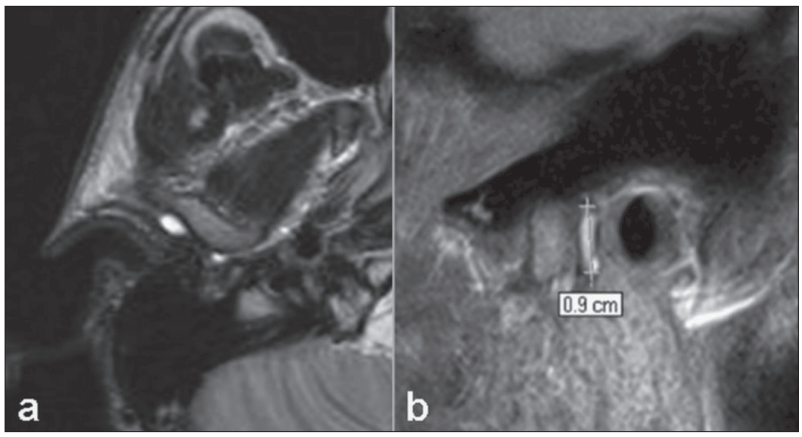

Figure 6. Incidental synovial cyst of the left temporomandibular joint observed in a 40-year-old female patient

Pigmented villonodular synovitis

Pigmented villonodular synovitis (PVNS) is an inflammatory, locally aggressive tumefactive disorder seldomly affecting the TMJ. The imaging modality of choice is contrast-enhanced MRI, since this condition has a radiologically aggressive appearance with destroying of the condyle and invasion of the middle cranial fossa. Low signal intensity on T1W and T2W sequences with peripheral no-signal intensity is observed in the lesion, representing pigmented portions of the inflammatory proliferating tissue. After contrast injection, some portions of the mass may enhance mildly [33].

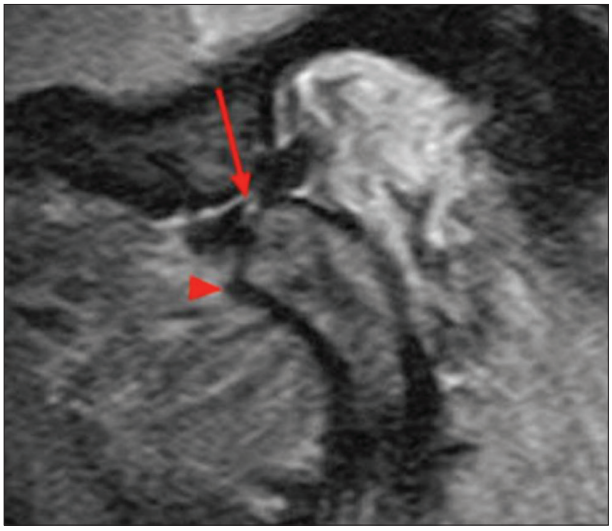

Figure 7. Perforation of the disc in the parasagittal view, proton density-weighted sequence, in the fully opened jaw position; the perforation of the intermediate zone (long arrow) is observed along with the condyle deformation and osteophytes in the anterior part (arrow tip)

Cysts

Cyst of the TMJ represents a very rare condition that can be observed in two forms: ganglion cyst and synovial cyst. Ganglion cyst is a pseudocyst, covered by fibrous tissue, while synovial cyst is a real cyst, with synovial cysts constituting the inner membrane (Figure 6). Most often it is found in female population, 20–40 years old. The etiology remains unclear, potentially traumatic or congenital [34].

IMAGING OF DEGENERATIVE DISORDERS

The TMJ is an anatomically and functionally complex joint, designed to withstand high multidirectional mechanical forces. At the point when those forces exceed the biomechanical threshold of the disc, the derangement of disc integrity and attachments occur [35]. Disc displacement from its normal anatomical position is a consequence of gradual translation of the posterior zone from its normal 12 o'clock position. Disc dislocation can occur in two planes: sagittal and transversal. Disc dislocations in the sagittal plane are termed anterior and posterior, while in the transversal one they can be lateral or medial [26]. Posterior dislocation is somewhat disputable, since it might also represent pseudodisc due to the thickening and fibrosis of the posterior attachment (retrodiscal fibrosis due to inappropriate loading of the bilaminar zone), having the same or even lower signal intensity as that of a disc [27, 33]. Rupture or perforation of the disc represents a subgroup of the posterior dislocation, according to Westesson et al. [36] (Figure 7). Anterior disc dislocation is present if the posterior zone of the disc can be observed on MRI anterior to the normal 12 o'clock position [37, 38]. Due to clinical implications, it is important to assess disc mobility during jaw movements. If the normal disc–condyle relationship is restored in the opened jaw position, this type is called disc dislocation with reduction (DDWR) (Figure 8); if not, it is called disc dislocation without reduction (DDWOR) (Figure 9) [39, 40]. DDWOR may result in a locked joint, with complete restriction of movement [39]. The imaging modality of choice in establishing diagnosis of disc dislocation is native MRI in closed and fully opened jaw positions. Osseous changes occur consequently to soft tissue changes [41]. The morphology of the condyles at the end-stage of a degenerative

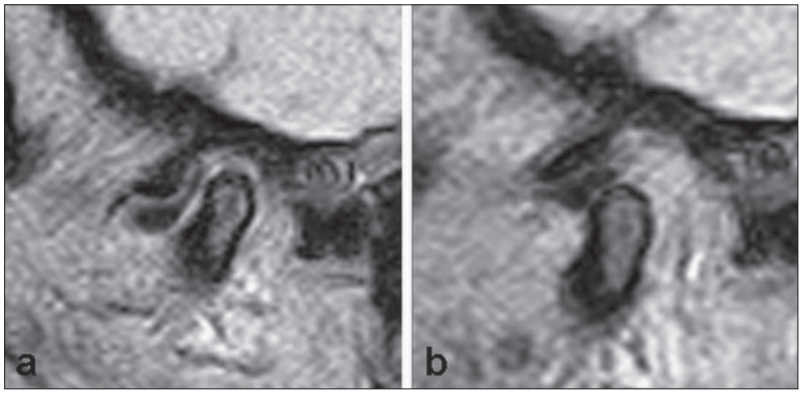

Figure 8. Disc dislocation with reduction in the closed (a) and fully opened jaw position (b); in the fully opened jaw position, the disc is dislocated anteriorly, and in the closed jaw position it returns to its normal position

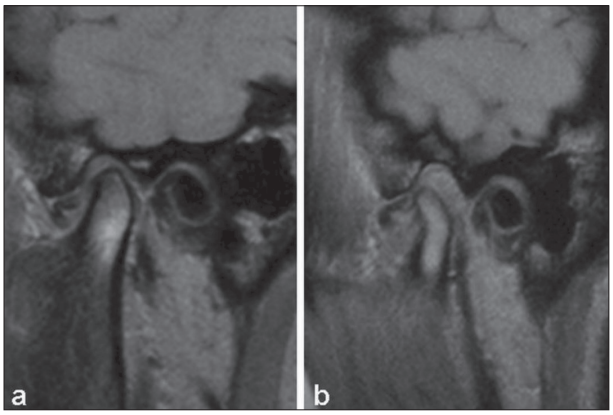

Figure 9. Disc dislocation without reduction in the closed (a) and fully opened jaw position (b); in the fully opened jaw position, the deformed disc is dislocated anteriorly and it does not return to its normal position during the closing of the jaw

process is different in children (this form of joint disorder is called progressive or idiopathic condylar resorption) and adults. However, the process of osseous destruction is similar. When biomechanical stress exceeds the threshold of fibrocartilage and articular surfaces, cortical thickening and subchondral sclerosis of the articular bone surfaces occur as a response to stress distribution. With further biomechanical stress, erosions of the cortical bone occur, destroying the articular surface and reducing its volume. The condylar height is reduced and the relationship with glenoid fossa is disturbed. Repair process in adults consists of forming marginal osteophytes as an attempt to increase the surface area for load distribution. The imaging modality of choice for depicting osseous changes is CBCT [15, 18].

According to the motion range of the condyle, the TMJ can be of normal mobility, hypermobile, or hypomobile (Figure 10). Hypermobility of the condyle is defined as the condyle motion of more than 120° anterior and superior to the eminence crest [42]. This condition occurs with the elongation of the posterior attachments, sphenomandibular and stylomandibular ligaments, in early cases of internal derangement of the TMJ or in Ehlers–Danlos syndrome [43]. If the condyle returns to the anatomical position with closed jaw, it is manifested as subluxation, but if it remains in the pathological place, it is considered dislocation (open lock). Condylar restriction represents the state where the condyle is located posteriorly and superiorly to the eminence crest. DDWR usually presents with normal motion range, while DDWOR presents with open lock in its acute phase. With entering the chronic phase, the normal range of motion could be restored. Hypomobility of the condyle usually appears as the consequence of disc adhesions secondary to trauma or disc dislocation, or synovitis, as well as in the end-stage phase of inflammatory conditions (such as RA, JIA, etc.) [31, 32].

Temporomandibular disorders (TMD) consist of several conditions related to pain and dysfunction in the TMJ and masticatory muscles. The two most common types of TMD are TMJ-associated disorders (disc dislocations and degenerative processes) and pain-related disorders (myalgia, TMD-associated headache, and arthralgia). This condition is associated with impaired general health, psychological disorders, and chronic pain, representing an important cause of reduced quality of life [44, 45, 46].

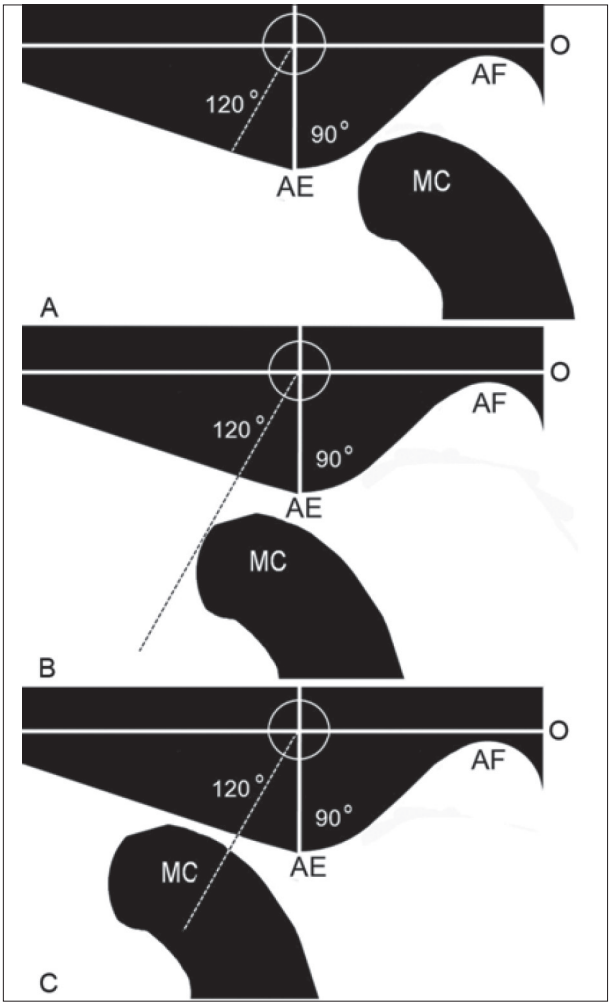

Figure 10. Scheme of hypomobility (a), normal mobility (b), and hypermobility (c) of the condyle

IMAGING OF BENIGN NEOPLASTIC PROCESSES

Osteochondroma

Osteochondroma is a benign exophytic bony lesion with a cartilaginous cap, arising from the side of the bone. It can arise either from the condyle or from the coronoid process [47]. On the imaging, it presents as a pedunculated mass attached to the condyle, often extending from the anterior surface of the condyle in the direction if the fibers of the lateral pterygoid muscle. When it grows large, it is able to displace the mandible contralaterally, resulting in contralateral posterior crossbite and ipsilateral posterior open bite [48].

Osteoma

Osteoma is a benign bone tumor characterized with slow growth and proliferation of compact or cancellous bone. Usually it originates from the non-articular surface of the condyle, which is covered with periosteum. On imaging, it appears as pedunculated, well-defined bone density mass with homogenous structure and normal bone pattern. It can also cause mandibular displacement if the dimensions are large enough [35].

IMAGING OF MALIGNANT NEOPLASTIC PROCESSES

Chondrosarcoma

Chondrosarcoma is a malignant cartilaginous tumor located centrally in the temporal bone or the condyle, parosteally or in soft tissues of the TMJ. A non-enhancing, lobulated, low-density mass with flocculent calcifications is observed in the condyle, temporal bone, or in the widened joint space. The condyle may also appear enlarged or remodeled. Periosteal reaction is variable. On MRI, the mass is of high signal intensity on T2W with hypointense foci representing calcifications. Postcontrast enhancement is heterogeneous [49].

Metastases

Metastases to bone structures of the TMJ are rare, reported as single case reports in the literature. Most commonly, the origin of metastases to the bones is breast, followed by lungs, prostate, colon, and kidneys [49, 50]. Symptoms at presentation include pain, swelling, and trismus. Although a rare condition, one must bear in mind the possibility of metastatic lesion in differential diagnosis [50].

CONCLUSION

The TMJ is anatomically, embryologically, and physiologically a complex structure, functionally tightly connected to the rest of the craniomandibular complex. Contemporary imaging modalities, if used properly and according to adequate clinical implications and criteria, are able to depict different pathological processes and play the crucial role in establishing the right diagnosis and monitoring therapeutic effect. The key to the right diagnosis, however, still lies in thorough familiarity with the TMJ developmental and functional anatomy, as well as with the TMJ dysfunction related to the jaws, teeth, and cranial base.

Conflict of interest: None declared.

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Снимање темпоромандибуларног зглоба – савремене клиничке и радиолошке импликације

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САЖЕТАК

Темпоромандибуларни зглоб представља биомеханички и морфолошки сложену структуру, која је уско повезана са растом и развојем мандибуларног и краниофацијалног комплекса.

Циљ овог рада је да се свеобухватно прикажу савремени дијагностички модалитети и клиничке импликације дијагностике сликом темпоромандибуларног зглоба.

Савремени начини снимања, ако се правилно користе и према адекватним клиничким импликацијама и критеријумима, могу да прикажу различите патолошке процесе и играју кључну улогу у успостављању адекватне дијагнозе и праћења терапијског ефекта, уз адекватан клинички протокол. Кључ за исправну дијагнозу, међутим, и даље лежи у добром познавању функционалне анатомије темпоромандибуларног зглоба, као и дисфункција темпоромандибуларног зглоба које су повезане са максилом, мандибулом, околним мишићима, зубима и базом кранијума.

Кључне речи: темпоромандибуларни зглоб; обољења темпоромандибуларног зглоба; магнетна резонанца; краниомандибуларне дисфункције

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Bioethical aspects of assisted suicide and euthanasia in people suffering from mental health problems

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SUMMARY
This paper deals with euthanasia and assisted suicide in people with mental health problems, based on the fundamental principles of contemporary medical ethics. In some situations, psychiatric patients are incapable of realizing they are ill and they need to be treated due to the compromise of cognitive functions. It is difficult to establish the relationship of negotiation and joint decision-making with such patients, so it is necessary that the psychiatrist takes responsibility in order to protect both their patient and the environment from any potentially harmful activity.
Keywords: euthanasia; medical ethics; mental health

INTRODUCTION

As a result of the development of medical sciences and raising awareness of human rights, there is a series of bioethical dilemmas concerning the conception and the ending of human life. One of the key questions that intrigue the human mind is the question of legalizing euthanasia [1].

Euthanasia has been legalized by the Netherlands, Belgium, and Luxemburg, whereas in some countries, such as Switzerland, Germany, Canada, Japan, and the USA (Oregon, Washington, Montana, California, Vermont), assisted suicide is allowed [2]. According to the Serbian Criminal Code, both euthanasia and assisted sucide represent criminal acts (Articles 117 and 119) [3].

However, when we discuss euthanasia in people suffering from mental health problems, we should consider the fact that psychiatry, more than any other branch of medicine, places emphasis on working with people who do not feel the need to get professional help or whose cognitive functions might be compromised to such an extent that they are not capable of realizing what their real needs are [4].

THE CONCEPT AND TYPES OF EUTHANASIA

Euthanasia is deliberate and intentional killing of a human being by a direct action, such as a lethal injection, or by withdrawing life support system in order to release that human being from painful life [5].

There are several forms of euthanasia, each with a different set of rights and wrongs. Active euthanasia means that a doctor directly or indirectly causes the patient's death. On the other hand, passive euthanasia means the termination of a medical treatment (switching off the machine that is keeping a person alive), or withholding a treatment which would prolong the dying patient's life (not carrying out surgery that will extend life for a short period of time).

Voluntary euthanasia is done according to the patient's will and upon their exclusive request. Non-voluntary euthanasia involves a situation where a person is unconscious or otherwise unable (for example, a person of extremely low intelligence) to make a meaningful choice between life and death, and an appropriate person decides on their behalf. Involuntary euthanasia occurs when the person who dies chooses life but is killed anyway. This is usually called a murder, but it is possible to imagine cases where killing would count as being beneficial for the person who dies [6].

SUICIDE AND ASSISTED SUICIDE

Suicide is a conscious and deliberate intervention towards ending one's own life. In order to commit suicide, there has to be a suicidogenic disposition, a natural or acquired reduction of vital instincts or increased psychological sensitivity, as well as a suicidogenic motive (i.e. the fact that a suicide takes as the cause and the reason for taking their own life). Suicidogenic motives can be endogenous (e.g. somatic and psychiatric disorders) and exogenous, which can be affective (they originate from misunderstandings in love, fear of punishment, etc.), economic (job loss, impoverishment, etc.), and moral (embarrassment, defamation, etc.) [7].

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This entire definition is given in the monograph *Suicide* by Prof. Dr. Milovan Milovanović, published in 1929.

Apart from the above listed types of euthanasia, assisted suicide is mentioned as a way to end a terminally ill patient's life. This act involves any action that doctors consciously and deliberately do in order to help a person commit suicide, upon that person's explicit request [4].

KILLING AND/OR LETTING DIE

An important part of the euthanasia debate is the conflict between active and passive euthanasia, which is reflected in the moral distinction between killing and letting die.

Shaw [8], in her article, analyzes two arguments about the distinction between killing and letting die. To perform this analysis, she uses an article by James Rachels and the reply by William Nesbitt. She states that James Rachels describes in his essay two actors who share the same intent (murder of a child), the same motive (greed, to inherit money) but in a different way (the first actor does something that causes the child to die directly, and the second does nothing to prevent death). In this way, Rachels shows that killing and letting die are morally equivalent acts, but only when measured isolated, without the influence of other factors. However, euthanasia is not a decision that can be made without examining other relevant factors that we encounter in real life, and one of them is certainly the intention of the physician, which the author himself suggests. On the other hand, William Nesbitt states that, in order to get closer to real situations, he makes a moral difference between "being willing to kill someone" and "being willing to let someone die." Here Nesbitt argues that people tend to think it is worse to be willing to kill someone rather than to just let them die, and that it is this difference which provides justification for the idea that passive euthanasia is morally better than active euthanasia. But as Sarah Beth states, if willingness to kill is equivalent to willingness to help (in most euthanasia cases it is), Nesbit cannot use this distinction to challenge the idea of the substance of the benefits of active euthanasia, which was his intention [8]. It is our opinion, that there is no significant difference between killing and letting die, since both acts are absolutely unacceptable for any medical professional, since the consequence of both acts is death.

There is also the claim that causing death is morally wrong only if it is unjustified and unwarranted. If a person freely chooses death and realizes that it is a personal gain, then fulfilling that person's request does not imply clear moral harm [9]. We recognize that under this assumption, the patient's opinion about personal gain is taken as the only relevant and dominant factor on the basis on which it can be justified to cause death, while the opinion, needs and motives of the executor (physician) are also derived from the motives and principles of the medical profession (*primum non nocere* – do not harm the patient, and *salus aegroti suprema lex* – patient's health is the highest law), completely neglected. In this case, we consider it necessary to pay attention to what we consider crucial: whether the commission of such acts, even if the motive is well-intentioned, is useful in the context of the purpose of the medical profession and the physician himself, since the benefit for the patient should not exclude the expediency and essential role of physicians and the medical profession.

EUTHANASIA AND ASSISTED SUICIDE IN PEOPLE SUFFERING FROM MENTAL DISORDERS

Although there are various debates on defining mental disorders, it is generally accepted that they involve thought disorders, behavioral disorders, and emotional disorders serious enough to compromise people's functioning [10].

Mental health disorders are among the leading causes of disability in the world, as well as a major risk factor for suicide. According to the WHO data from 2014, there are around 800,000 people annualy who commit suicide as a consequence of a spectrum of mental disorders [11, 12]. Therefore, early detection of people at risk of mental disorders is of great importance in the prevention of mental disorders and suicide as a significant public health problem [11, 13].

As the first and foremost argument against euthanasia, we state our opinion based on the fact that the desire for suicide and suicide are expression of the reduced urge to live, that is, a sign of human psychopathology. Therefore, we believe that assisting a patient by a psychiatrist in the act of suicide is a radical counter to the tasks of psychiatry and is a violation of professional and moral responsibility.

According to another important argument, mental disorder is not a terminal illness or an illness which deprives people of physical ability to take their own life if they really want to. Under such circumstances, there is an additional argument according to which no one has the right to involve other people in taking their own life, thus putting an ethical burden on their back [14]. This is especially true of medical professionals who should always be a symbol of fight for health and life, in every moment and in all cases.

However, despite clear arguments, the right to euthanasia in case of psychological suffering is legally regulated in the Netherlands, Belgium, and Luxembourg and it necessarily involves fulfilling essential and procedural criteria envisaged by law.

Belgian law on euthanasia emphasizes essential principles according to which a request for euthanasia has to be voluntary, well considered, repeated and not a result of external pressure. The person must be in medically hopeless and futile condition which is the result of unbearable physical or psychological suffering, and the disorder must be serious and characterized by poor prognosis, without reasonable recovery alternatives [15].

Apart from the mentioned legal regulations, it is necessary to underline that there are various ethical and medical doubts within the essential criteria primarily related to the (in)ability of meeting these criteria in case of mentally ill people [15].

According to many authors, psychiatry is in a less favorable position compared to other branches of medicine

because the course of mental disorders is prone to variations in time, so not even prognoses of psychiatric treatments are precise enough to make a final decision on the curability of an illness, or a definitive prognosis. These are exactly the arguments owing to which euthanasia and assisted suicide are not justified in the field of psychiatry [15, 16].

Respecting autonomy is usually considered the central reason for giving permission to execute these acts and within it an accent is put on the right of a person to decide on their own how they will live their life and how they will end it. However, when we talk about a mentally ill person, we should always be aware of the fact that certain psychiatric disorders (e.g. depressive and manic episodes in the spectrum of mood disorders) can considerably compromise the decision-making capacity, so a certain number of patients are considered incompetent [17]. In case this capacity is preserved, and a wish to die is a symptom of the disease, there is tension between respecting patient's autonomy on one side and preventing suicide and reducing damage to life and health of the patient on the other. In the countries where these procedures are legal, the law requires that the patient's wish is exclusively the result of their own decision, without any external coercion [15]. However, it is well-known that various social circumstances, which worsen the psychological status and could cause suicidal wishes and ideas in those who suffer from depression and other mental disorders, can affect the patient's decision. One study, conducted in the Netherlands, showed that more than half of the requests for euthanasia and assisted suicide were based on social isolation and loneliness. So, difficulties in cases of psychiatric patients do not originate exclusively from the symptoms of their illness, but they also reveal defective reactions of society [18].

Finally, we will provide an example of a young, mentally ill person from Canada who appealed for euthanasia due to unbearable psychological suffering, emphasizing that he was not suicidal, that life was beautiful but his suffering was unbearable. After his request was denied, the young man committed suicide. It follows from the foregoing that the young man denied his statement with his deed. At the same time, he did not need the help of a physician in realizing his own desire for self-destruction. Our position is that his request should be taken as a signal that it is essential for medicine and doctors to be fully engaged in reducing mentally sick person's suffering by treating their basic disease, as well as to (re)activate the network of his social support and strengthen his capacities for a more adequate tolerance of current circumstances.

CONCLUSION

The question of euthanasia and assisted suicide in psychiatry is very sensitive, for several reasons – a relative possibility of precise diagnostic evaluation, doctor's evaluation of the course and prognosis of a psychiatric disorder, and determining the existence of competence for reasoning in people whose psychological functions are compromised owing to the nature of their mental disorder.

In case of patients who suffer from mental disorders, the doctor's role specifically involves removing or reducing existing symptoms of the disease which are the cause of their suffering, developing alternatives, and providing support to the patient in active removal of stressors, development and spreading adequate functional coping styles in relation to the circumstances which are permanent triggers compromising his psychological health. We believe that one of the specific roles of doctors and other medical staff who take care of mentally ill patients involves expanding their network of social support and reducing loneliness which is, as we have mentioned, one of the most important factors for the occurrence of their request for euthanasia and assisted suicide.

In order to answer the question of applying euthanasia and assisted suicide in the field of psychiatry, we would like to emphasize that doctor's basic or fundamental role, a sacred role, is maximum commitment in providing medical help to patients who suffer from mental disorders using all available and scientifically accepted resources. A doctor should always mean hope and salvation, in every moment and for each patient. The task of doctors and medicine is to fight for life as such, for its preservation, because life itself has unconditional value.

Conflict of interest: None declared.

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Биоетички апекти асистираног самоубиства и еутаназије код особа које пате од проблема у вези са менталним здрављем

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САЖЕТАК

У овом раду говоримо о еутаназији и асистираном самоубиству код ментално оболелих особа, ослањајући се на основна начела савремене медицинске етике. Психијатријски болесници, у извесним ситуацијама, због компромитовања когнитивних функција нису у могућности да схвате да су болесни и да им је неопходно лечење. Управо са оваквим болесницима није лако успоставити однос договарања и заједничког одлучивања, већ је неопходно да психијатар преузме одговорност на себе како би заштитио самог болесника, али и околину, од могуће штете.

Кључне речи: еутаназија; медицинска етика; ментално здравље

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 објављен 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 6. 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.

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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.

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