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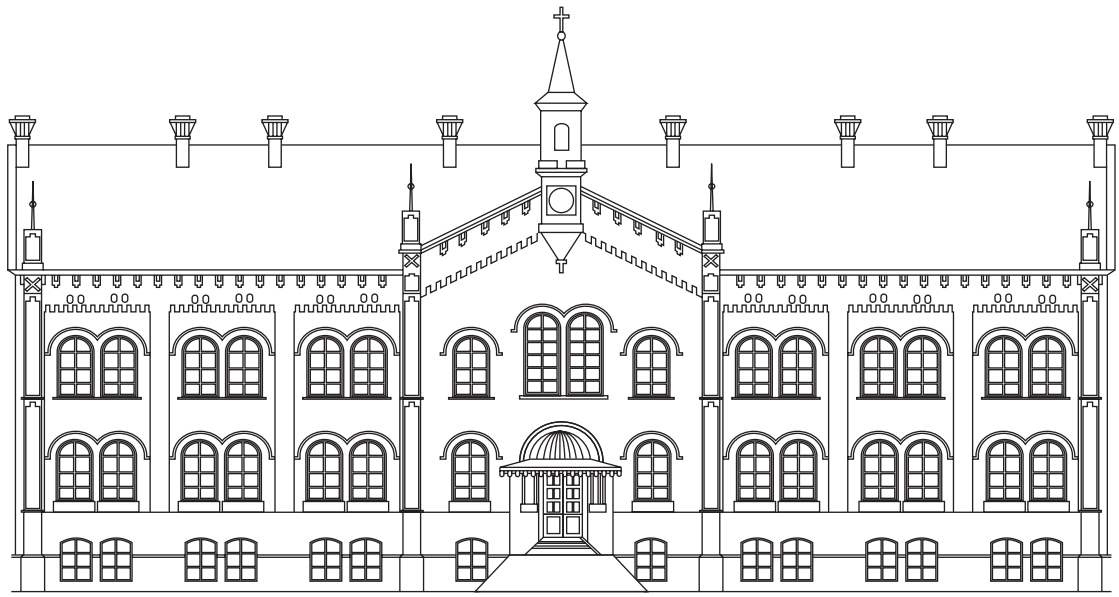

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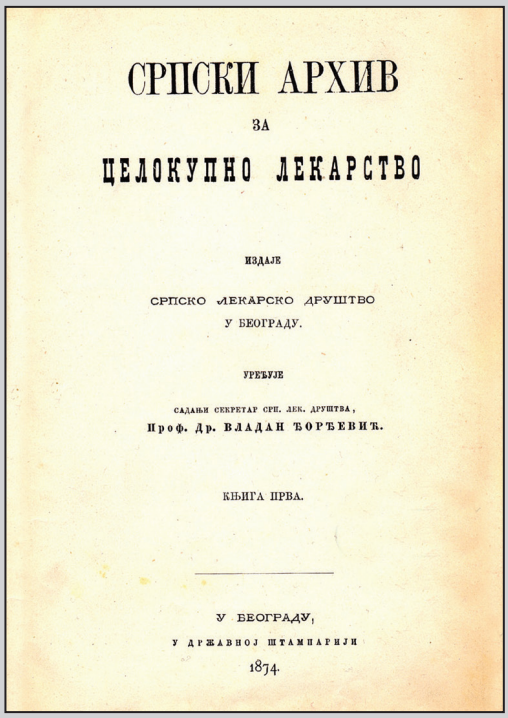

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

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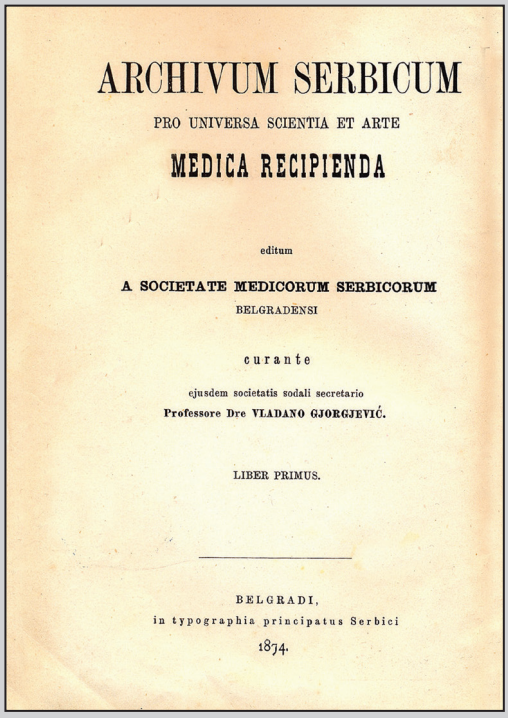
1874.

Прва страна првог броја часописа на српском језику



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1874.

The title page of the first journal volume in Latin

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Prevalence of periodontitis among young adults with mental disorders

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SUMMARY

Introduction/Objective Previous investigations pointed to a notable frequency of periodontitis appearance in persons with mental disorders, but almost none of them were addressed to the periodontitis among young adults with mental disorders as a target group, which can have high public health significance. Therefore, the aim of this investigation was to estimate the prevalence of periodontitis among young adults suffering mental disorders and to determine probable risk factors for their overall periodontal health.

Methods The investigation included two groups of patients, each group having 81 participants-the study group (young adults with mental disorders) and the control group (mentally healthy young adults). The study instruments included a questionnaire (age, gender, psychoactive substances use, and maintaining oral hygiene) and community periodontal index for both groups, and the data concerning primary disease of mentally deceased patients (diagnostic category, mental disorder duration, number of hospitalizations, and psychotropic medications).

Results In terms of psychoactive substances use and maintaining oral hygiene, statistically significant differences were observed between groups in all independent variables. Young adults with mental disorders shown a high prevalence of periodontitis compared to the mentally healthy young adults. Also, gender, smoking habits, and the use of antipsychotics exhibited as possible risk factors contributing current periodontal health of young mentally deceased patients.

Conclusion This study indicates the need for more consideration for periodontal health among people with mental disorders and determination of potential models for its improvement.

Keyword: periodontitis; prevalence; young adults; mental disorders

INTRODUCTION

Periodontitis is a microbe-induced oral disease, characterized by inflammation of periodontal tissues, which may provoke tooth loss and significantly lower quality of life [1, 2]. Although immunological processes are Table crucial for initiation and progression of periodontitis, previous studies have shown that they are influenced by several risk factors, such as smoking habit, alcohol beverage consumption, poor oral hygiene, use of different medications on daily basis, hormonal changes, as well as stress and psychic factors [3, 4]. However, periodontitis can be preventable and treatable if appropriate and timely management is undertaken, especially for modifiable risk factors [3].

Young adulthood is a specific developmental period of human life, which occurs between the ages of 18 and 25 years [4]. This period of life comes after adolescence and it is very important because of a significant increase of depression, anxiety, self-harming traits, and eating disorders, including first episodes of more severe mental disorders, such as psychosis and personality disorders [5]. It is assumed that almost 75% of adults with a diagnosed mental health problem will manifest first symptoms of altered mental health by the age of 24 [5]. Therefore, altered mental health represents a prominent burden for this age group and should represent a priority for health improvement [5]. In addition, a numerous modifiable risk factors, such as psychoactive substance use (alcohol beverage consumption, smoking, cannabis consumption, etc.), starts during this period of life [6, 7].

Previous studies have shown a high prevalence of the two most common oral diseases in population of people with mental disorders – dental caries and periodontitis [8, 9, 10]; however, almost none of them were addressed to periodontitis among young adults with mental disorders as a target group, which can have high public health significance. Therefore, the aim of this investigation was to estimate the prevalence of periodontitis among young adults with mental disorders and to determine possible risk factors for their overall periodontal health.

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METHODS

This observational, epidemiological, and cross-sectional study was conducted at the Dr. Laza Lazarević Clinic for Mental Disorders in Belgrade and Vračar Community Health Center in Belgrade. It was adjusted to the statement "Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)," designed to improve quality of observational studies [11], and conducted according to Declaration of Helsinki [12]. The study was approved by the Ethics Committee of the Dr. Laza Lazarević Clinic for Mental Disorders (No. 2878) and an approval from the director of Vračar Community Health Center (No. 02/900). Before participation in the investigation, all the participants signed the informed consent form before participating in any part of the study.

Two groups of patients were created, both comprising 81 randomly selected young adults. The study group comprised young adults with mental disorders (46 males and 35 females, mean age 21.8 ± 3.6 years), hospitalized at the Dr. Laza Lazarević Clinic for Mental Disorders in Belgrade ("bias-coin" randomization). The inclusion criteria for entering the study were: patients' age (between 18 and 25 years), suffering from mental disorder according to the 10th Revision of the International Classification of Diseases (ICD-10) diagnosed at least two years prior to this investigation. The exclusion criteria were: the patients younger than 18 or older than 25 years, diagnosed with mental disorders in a period shorter than two years prior to the investigation, simultaneous presence of severe somatic illnesses or severe disability, and inability/refusal to cooperate. The control group, also, comprised 81 randomly chosen young adults, age and gender-matched with study group of patients (42 males and 39 females, mean age 22.8 ± 2.6 years). They were suffering from dental caries, without any mental or somatic disorders or conditions. These patients were recruited from patients visiting Vračar Community Health Center in Belgrade for caries treatment. The control group of patients did not use any medication that could affect oral or mental health [13].

A special type of questionnaire was designed for both groups in order to note socio-demographic data (gender and age), oral health habits (maintaining oral hygiene, tooth brushing technique), and psychoactive substances use (smoking habits and consuming alcohol beverages). The data about mental disorder of the study group patients were taken from the medical records and included the type of mental disorder (according to the ICD-10), duration of medical disorder, number of hospitalizations and current psychotropic medication. All patients were subjected to the thorough dental clinical examination according to the World Health Organization (WHO) criteria [14]. The clinical examinations were carried out by two trained examiners at the Dr. Laza Lazarević Clinic for Mental Disorders and the Vračar Health Center in Belgrade, Serbia. The examiners were calibrated twice, by assessing the Community Periodontal Index (CPI) [14], before and during the study, with a degree of agreement being ± 1 mm of 94%. The clinical measurements were performed by using the periodontal probe graded in mm (WHO-621 Trinity probe) on the sextants, scoring on the scale from 0 to 4. All the teeth were examined in each sextant, and only the highest value for each sextant was noted.

All collected data were organized and evaluated using the dedicated software IBM SPSS Statistics for Windows, Version 21.0. (IBM Corp., Armonk, NY, USA) and were analyzed by the descriptive statistical parameters, methods for testing the hypothesis and regression models (uni- and multivariate linear regression analysis). The descriptive statistical methods were represented by the measures of central tendency (mean and median), measure of variability (standard deviation and variation interval) and were expressed in percentages. The methods for testing the difference of numerical data were represented by the Kruskal–Wallis test and Mann–Whitney test. For testing the data of different categories (gender, smoking habits, drinking alcohol beverages, maintaining of oral hygiene, tooth brushing technique, etc.), the χ^2-test was used. The level of significance was set at $p \leq 0.05$.

RESULTS

The use of psychoactive substances and oral hygiene habits among both groups of patients are presented in Table 1. A statistically significant difference between groups was observed in terms of all independent variables (Table 1). Most of the study group patients were smokers (72.8%), alcohol beverages consumers (66.7%), with maintaining oral hygiene several times per month or once a day (55.6%) and with incorrect technique of tooth brushing (76%). On the contrary, most of the control group patients were non-smokers (80%), non-users of alcohol beverages (82.7%), with maintaining oral hygiene twice a day or more times per day and with correct technique of tooth brushing (Table 1).

Table 1. The use of psychoactive substances and oral hygiene habits among the investigated groups

Independent variables	Obtained values		p (χ^2 test / Man–Whitney test)
	Study group n (%)	Control group n (%)	
Smoking habits:			
yes	59 (72.8)	15 (20.0)	0.000*
no	22 (27.2)	66 (80.0)	
Drinking alcoholic beverages:			
yes	54 (66.7)	8 (17.3)	0.000*
no	27 (33.3)	73 (82.7)	
Maintaining oral hygiene:			
no	19 (23.5)	0 (0.0)	0.000*
yes, several times per month or once a day	45 (55.6)	7 (8.6)	
yes, twice a day or more times per day	17 (20.9)	74 (91.4)	
Demonstrating tooth brushing technique:			
correct	18 (24.0)	65 (80.2)	0.000*
incorrect	63 (76.0)	16 (19.8)	

n (%) – number (percentage); p – significance; * – statistically significant

Concerning mental disorders, most of the study group patients were diagnosed to be F_{20}–F_{29} or schizophrenia, schizotypal, and delusional disorder, and F_{30}–F_{39} or mood/affective disorders (Table 2). Mean value of duration of mental disorders among the study group patients was approximately six years, and they were treated with several psychotropic medications, mostly antipsychotics, anxiolytics and mood stabilizers (Table 2).

Statistically significant differences between groups were also observed in terms of mean value of CPI (Table 3). The study group patients had more than twice higher mean value of this periodontal index (1.6 ± 0.7) than the control group patients (0.7 ± 0.5). The patients in the study group had gingival bleeding more often (30.1%) than the control group patients, who had healthy periodontal ligament (PDL) more frequently (48%). Moreover, the periodontal pockets were detected 21.9% in the study group patients, while only in 4% of the control group patients had this pathological finding (Table 3).

Table 2. Medical data of the study group patients

Independent variables	Obtained values Study group
Diagnostic category (ICD-10), n (%)	
F20–F29	42 (51.9)
F30–F39	18 (22.2)
F40–F49	2 (2.5)
F50–F59	3 (3.7)
F60–F69	3 (3.7)
F70–F79	1 (1.2)
F90–F98	12 (14.8)
Duration of mental disorder per patient X ± SD; Med (min–max)	5.8 ± 3.6; 5 (0–9)
Psychotropic medication per patient X ± SD; Med (min–max)	4.2 ± 1.7; 3 (1–5)
Antipsychotics, n (%)	78 (96.3)
Antidepressants, n (%)	21 (25.9)
Anxiolytics, n (%)	63 (77.8)
Hypnotics, n (%)	41 (50.6)
Mood stabilizers, n (%)	57 (70.3)
Anticholinergics, n (%)	9 (11.1)

n (%) – number (percentage); X – mean value; SD – standard deviation; Med – median

Table 3. Community periodontal index values of both groups of patients

Groups	**Obtained values**					
	X ± SD; Med (min–max)	Community Periodontal Index codes, n (%)				
		0	1	2	3	4
Study group	1.6 ± 0.7; 1.5 (0–4)	15 (20)	23 (30.1)	21 (28)	11 (15.2)	5 (6.7)
Control group	0.7 ± 0.5; 1 (0–3)	36 (48)	24 (32)	12 (16)	3 (4)	0 (0)
p (χ^2 test / Man–Whitney test)	0.000*	0.000*				

X – mean value; SD – standard deviation; Med – median; p – significance; * – statistically significant

Analyzing the values of the CPI in relation to the psychoactive substances use and oral hygiene habits in both groups, a statistically significant difference in the study group patients was observed in terms of smoking habits, maintaining oral hygiene and tooth brushing technique (Table 4). The highest values of the CPI were registered among smokers, those who brushed their teeth several times per month or once a day and those who use an incorrect technique of tooth brushing. Similarly, in the control group of patients, a statistically significant difference in the CPI values was observed in terms of maintaining oral hygiene and tooth brushing technique (Table 4). In addition, mentally healthy patients who brushed their teeth several times per month or once a day and those who used an incorrect technique of tooth brushing had highest values of the CPI.

In terms of psychotropic medications of the study group patients, statistically significant differences in the values of the CPI among the study group patients were observed in terms of using antipsychotics and anticholinergics (Table 5). The highest values of the CPI were observed among those who use antipsychotics and those who use anticholinergics.

The impact of psychoactive substances, oral health habits, and characteristics of the primary disease, the CPI values among the study group patients were examined by the linear regression model (Table 6). In univariate regression model, statistically significant factors in terms of the CPI value among the study group patients were gender, smoking habits, drinking of alcohol beverages, maintaining of oral hygiene, tooth brushing technique, the use of antipsychotics and anticholinergics (Table 6). However, multivariate regression model showed that only gender, smoking habits and the use of antipsychotics were statistically significant factors that contributed to the value of the CPI among the study group patients.

DISCUSSION

The main objective of the current investigation was to estimate the prevalence of periodontitis among young adults with mental disorders. In addition, this study also defined the possible risk factors that may contribute to the current periodontal health among this group of people with mental disorders. The principal finding of this study was a high prevalence of periodontitis among young adults with mental disorder compared to the mentally healthy young adults. Also, this study showed that the gender, smoking habits, and the use of antipsychotics are possible risk factors that may contribute to the current periodontal health of young adults with mental disorders.

According to the current study, most of patients of the study group were smokers (72.8%) and alcohol beverages consumers (66.7%) which are known to be risk factors for xerostomia and salivary gland hypofunction [15, 16]. Smoking is recognized as the most relevant risk factor for periodontitis, because it evokes different responses in oral microcirculation, highlighting the importance of many toxic substances beside nicotine [17]. In addition, a study from 2019 reveals that smoking in a period of late adolescence is relevant risk factor for periodontitis in young adulthood [18]. On the other hand, chronic alcohol consumption may increase the severity of periodontitis due to lower local inflammatory response and higher level of

Table 4. Community periodontal index values among both groups of patients in terms of using psychoactive substances and oral hygiene habits

Independents variables	Obtained values of Community Periodontal Index			
	Study group		Control group	
	X ± SD	p (Kruskal–Wallis / Man–Whitney test)	X ± SD	p (Man–Whitney test)
Smoking habits: yes no	 2.05 ± 0.23 1.15 ± 1.38	 0.001*	 0.72 ± 0.85 0.93 ± 0.96	 0.414
Drinking of alcoholic beverages: yes no	 1.45 ± 1.06 1.66 ± 1.24	 0.512	 0.79 ± 0.89 0.62 ± 0.77	 0.570
Maintaining oral hygiene: no yes, several times per month or once a day yes, twice a day or more times per day	 2.14 ± 0.08 1.78 ± 1.26 1.12 ± 0.49	0.003* (1:2) 0.731 (2:3) 0.045* (1:3) 0.002*	 n/a 1.28 ± 0.19 0.12 ± 0.09	0.000*
Demonstrating tooth brushing technique: correct incorrect	 1.17 ± 1.56 2.01 ± 0.41	0.012*	 0.26 ± 0.41 1.13 ± 0.83	0.000*

X – mean value; SD – standard deviation; p – significance; * – statistically significant

Table 5. Community periodontal index values among the study group patients, in terms of psychotropic medications

Independent variables	Obtained values of Community Periodontal Index	
	X ± SD	p (Mann–Whitney test)
Antipsychotics: no yes	 1.07 ± 1.14 2.12 ± 0.76	0.001*
Antidepressants: no yes	 1.67 ± 1.39 1.56 ± 1.61	0.536
Anxiolytics: no yes	 1.40 ± 1.23 1.82 ± 1.01	0.058
Hypnotics: no yes	 1.75 ± 0.97 1.61 ± 0.79	0.729
Mood stabilizers: no yes	 1.58 ± 1.32 1.81 ± 1.02	0.067
Anticholinergics: no yes	 1.15 ± 1.78 2.21 ± 0.46	0.043*

X – mean value; SD – standard deviation; p – significance;
* – statistically significant

Table 6. Community periodontal index among the study group patients examined by linear regression model

Independent variables	Univariate linear regression analysis		Multivariate linear regression analysis	
	#B (95%CI)	*p*	#B (95%CI)	p
Gender	0.222	0.004*	0.519	0.032*
Age	-0.011	0.721	/	/
Smoking habits	0.518	0.004*	0.098	0.016*
Drinking of alcohol beverages	0.410	0.026*	-0.098	0.314
Maintaining of oral hygiene	-0.108	0.018*	0.116	0.315
Tooth brushing technique	0.160	0.032*	0.346	0.455
Diagnostic category	0.006	0.150	n/a	n/a
Duration of mental disorder	0.164	0.139	n/a	n/a
Psychotropic medication	-0.049	0.768	n/a	n/a
Antipsychotics	0.138	0.002*	1.024	0.021*
Antidepressants	0.188	0.490	n/a	n/a
Anxiolytics	-0.490	0.098	n/a	n/a
Hypnotics	0.233	0.399	n/a	n/a
Mood stabilizers	0.064	0.822	n/a	n/a
Anticholinergics	-0.243	0.050*	-0.449	0.421

p – significance; #B – unstandardized Coefficient B; * – statistically significant

alveolar bone resorption [19]. Also, in our study mostly of the study group patients were diagnosed as schizophrenia schizotypal, and delusional disorders (51.9%), with a mean value of duration of mental disorder per patient 5.8 ± 3.6 years and mean value of psychotropic medications per patient 4.2 ± 1.7 (mostly antipsychotics, anxiolytics and mood stabilizers). Hu KH et al. [20] concluded that younger persons with newly diagnosed schizophrenia, female gender and exposure to the antipsychotics were independent risk factors for periodontitis. In addition, hypo-salivation as an adverse effect of first generation of antipsychotics was associated with an increased risk for periodontitis [20]. Skallevold et al. [21] in their review from 2023 conclude that the mutual relationship of oral health and mental disorders, among others, dysregulates microbiome, translocated bacteria, and systemic inflammation.

The periodontal index used in this study was the CPI, recommended by the WHO [14]. According to the studies based on critical review of periodontal indices, the CPI represents a modification of former CPI and treatment needs, which is one of the most common used diagnostic tools in epidemiological types of studies [22, 23]. Modification is done by eliminating "treatment needs" and including loss of attachment category, which avoid the false scoring of pseudo periodontal pockets [22, 23]. The mean value of the CPI in current study was over two times higher in the study group of patients compared to the control group (1.6 ± 0.7 *vs*. 0.7 ± 0.5), with 21.9% of them with registered periodontal pockets (15.2% of shallow periodontal pockets and 5.7% of deep periodontal pockets). Additionally, only 20% of young adults with mental disorders had a healthy PDL. The most common finding among young adults with mental disorders was a gingival bleeding (30.1%), in contrast to the healthy PDL, which was observed in almost 50% of mentally healthy young adults. These findings are in correlation with previous studies [10, 23]. A meta-analysis of the association between periodontitis and severe mental illnesses from 2022 shows that severity of mental disorder is associated with an increased prevalence of periodontitis compared

to general population [10]. According to these findings, Amedari et al. [24] showed that outpatients with mental disorders in most cases had gingival bleeding. An average of almost three teeth in each outpatient with mental disorder was associated with gingival bleeding compared to the average of less than one tooth in the control group [23]. Gingival bleeding is considered a symptom of gingival inflammatory process, and it, if untreated, can progress to periodontitis [24]. The main reason for gingival bleeding is the absence of oral hygiene habits and/or inadequate tooth brushing technique [25]. This can explain our results in terms of high values of the CPI among young adults with mental disorders who do not maintain oral hygiene and those who maintain oral hygiene but with inadequate tooth brushing technique. In addition, 23.5% of the study group patients did not maintain oral hygiene at all. Also, 76% of the study group patients demonstrated inadequate tooth brushing technique. It is known that absence of oral hygiene and inadequate oral health technique are associated with an increased level of periodontitis, mostly because of higher accumulation of dental plaque [26].

This study reveals that the gender, age, current smoking habit and the use of antipsychotics are possible predictors for periodontitis among young adults with mental disorders. In addition, a higher values of the CPI were registered among young males compared to females, smokers compared to the non-smokers and those who have antipsychotics in their daily therapy compared to the patients who do not use antipsychotics. This is similar to the results of other studies. Coelho et al. [8] in their cross-sectional study based on association of periodontitis with common mental disorder show that the occurrence of periodontitis among people with common mental disorder is approximately 50% higher compared to those of persons without common mental disorder, with statistical significance after adjustment of age, gender, family income, current smoking status, alcohol beverage consumption and cardiovascular disorder. Similarly, Kisely [27] concluded that there were interactions between periodontal health and mental disorders, comprising several biological, behavioral, and psychosocial factors.

CONCLUSION

This investigation reveals a high prevalence of periodontitis among young adults with mental disorder and that the gender, smoking habits and the use of antipsychotics exhibited as possible risk factors that may contribute to the current periodontal health of this subgroup of young people. Also, this study indicates the need for more consideration for periodontal health among people with mental disorders and determination of potential models for its improvement.

Conflict of interest: None declared.

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Преваленција пародонтитиса код младих одраслих особа са менталним поремећајима

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САЖЕТАК

Увод/Циљ Претходно спроведене студије показале су високу преваленцију пародонтитиса код особа са менталним поремећајима, али скоро ниједна од њих није била усмерена на пародонтитис међу младим одраслим особама са менталним поремећајима као циљном групом, што може имати велики значај за јавно здравље. Стога је циљ овог истраживања био да се одреде преваленција пародонтитиса код младих одраслих особа са менталним поремећајима и могући фактори ризика за њихово опште пародонтално здравље.

Методе Истраживање је обухватило две групе испитаника са по 81 пацијентом у свакој групи – студијску групу (младе одрасле особе са менталним поремећајима) и контролну групу (ментално здраве младе одрасле особе). Инструменти коришћени у истраживању су били упитник (старост, пол, употреба психоактивних супстанци и одржавање оралне хигијене) и пародонтални индекс заједнице за обе групе, као и подаци о примарној болести испитаника студијске групе (дијагностичка категорија, трајање менталног поремећаја, број хоспитализација и психотропни лекови).

Резултати У погледу коришћења психоактивних супстанци и одржавања оралне хигијене уочене су статистички значајне разлике између испитаника у свим независним варијаблама. Младе одрасле особе са менталним поремећајима показале су високу преваленцију пародонтитиса у поређењу са ментално здравим младим одраслим особама. Такође, пол, пушачке навике и коришћење антипсихотика показали су се као могући фактори ризика који доприносе постојећем стању пародонталног здравља младих одраслих особа са менталним поремећајима.

Закључак Ово истраживање указује на потребу за даљим истраживањима пародонталног здравља особа са менталним поремећајима и одређивањем могућих модела за његово побољшање.

Кључне речи: пародонтитис; преваленција; младе одрасле особе; ментални поремећаји

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Effects of metformin and its combinations with other repurposed drugs on fibrosarcoma in hamsters

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SUMMARY

Introduction/Objective Many drugs registered for various other indications can act selectively on tumor receptors, signaling pathways, metabolic processes, bioenergetic factors, enzymes, proteins and genes that regulate tumor proliferation, apoptosis, and neoangiogenesis without affecting these activities in healthy cells. Introduction of new drugs is a very long, complex, and expensive process of research. Detecting an anticancer effect in drugs already registered for other indications and forming their combinations may directly reduce the time and cost of such research.

Methods Anticancer efficacy of metformin and its combinations with caffeine, itraconazole and nitroglycerin was tested on fibrosarcoma experimentally induced by BHK21/C13 cells in Syrian golden hamsters (six animals per group, randomly allocated to control and experimental groups, doses equivalent to usual human doses). After animal sacrifice, tumors were excised and their size, biophysical characteristics, histology, and immunohistochemistry were assessed. Blood samples were collected for hematological and biochemical analyses and the main organs were toxicologically analyzed. Statistical significance was determined by one-way ANOVA followed by the Student–Newman–Keuls post hoc test.

Results Two-drug combinations of metformin with caffeine or itraconazole or nitroglycerin showed significant antitumor effects on hamster fibrosarcoma compared to control, regarding all tested tumor parameters ($p < 0.05$) without toxicity.

Conclusion Administration of metformin in combination with caffeine or itraconazole or nitroglycerin might be an effective and safe approach in novel nontoxic adjuvant anticancer treatment.

Keywords: metformin; caffeine; itraconazole; nitroglycerin; hamsters; fibrosarcoma

INTRODUCTION

Metformin activates 5´AMP-activated protein kinase (AMPK), which reduces mammalian target of rapamycin (mTOR) complex 1 signaling, inhibits nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), protein synthesis, and cancer cell proliferation [1]. Metformin inhibits glycolytic capacity and mitochondrial respiration in lymphocytic leukemia cells *in vitro* [2]. AMPK activation and glucose metabolism reduction negatively regulate Warburg effect (aerobic glycolysis – tumor cells preferentially use glucose rather than oxidation for energy production) and inhibit tumor progression [2]. Also, suppression of the Warburg effect in cancer cells by metformin decreases aerobic glycolysis and promotes oxidative phosphorylation, making cancer cells vulnerable to chemotherapy. Metformin interacts with respiratory electron transport chain in mitochondria to cause reactive oxygen species (ROS) production and oxidative stress [1]. Metformin therapy is also connected with both cyanocobalamin and folic acid deficiencies in patients with diabetes [3].

Caffeine induces apoptosis in many human tumor cells (lung, pancreatic, leukemia) *in vitro* [4]. Caffeine enhances tumor cells susceptibility to antineoplastic drugs and radiotherapy [5]. An important finding was that caffeine increased antifolate activity of pemetrexed in the various mesothelioma cell lines [6].

Itraconazole exhibits significant anticancer effects in different cancer tissues *in vitro* via suppression of the following: AMPK/mTOR pathway, neoangiogenesis, folic acid activity and autophagy [7, 8], Hedgehog signaling [9], P-glycoprotein (P-gp), and cholesterol transportation [7]. Itraconazole also induces chemosensitization [7]. Itraconazole, as ergosterol biosynthesis inhibitor, showed synergy with antifolates [8]. In addition to antifolate activity, the published studies have shown that itraconazole, as metformin, activates AMPK and thus downstream inhibits mTOR, protein synthesis, cell growth, proliferation, and stimulates apoptosis [9].

Nitroglycerin acts through the liberation of nitric oxide (NO) in the tissues. NO may modify cancer tissue metabolism by modulating the Warburg effect in oncological treatment [10]. NO donors are especially useful as chemotherapeutic and radio-therapeutic sensitizing preparations and increase cancer hemodynamics, amplifying the effects of cancer treatment [10]. NO can produce nitrosative stress, showing effects likewise to oxidative stress (elevation

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of ROS) [10]. As NO from nitroglycerin results in cyanocobalamin and folic acid deficiency [11, 12] similar to metformin [3], it can be expected that the mixture of nitroglycerin and metformin has synergistic antitumor effects via cyanocobalamin and folic acid deficiency. Nitroglycerin inhibits NF-κB and downstream P-gp [13].

In order to contribute to anticancer treatments, we conducted this study aiming to define the new efficacious, non-toxic and low-cost pleiotropic drug combinations, that can be immediately used in oncology.

METHODS

Three two-drug combinations – metformin and caffeine, metformin and itraconazole, metformin and nitroglycerin – were investigated in three separate independent experiments with three different control groups and simultaneously with appropriate investigated single and combined drug treatments.

Hamster model

Experiments were performed on *Mesocricetus auratus* (six male Syrian golden hamsters per group; 12–15 weeks old; body mass ~90 g). The hamsters were kept under default housing conditions: diurnal light cycle 12 hours of light / 12 hours of dark, at temperatures 25°C ± 2°C and humidity 60% ± 2%. The hamsters had access to food and water *ad libitum*.

The experiments for this study were performed in accordance with national regulations for the handling of laboratory animals: Law on Animal Welfare of the Republic of Serbia dated June 10, 2009 and the University of Novi Sad Rules for Work with Experimental Animals, dated June 11, 2020. All the animals were met with protocols approved by the University of Novi Sad Animal Ethics Committee (Novi Sad, Serbia): No. 04-81/25-5 dated July 22, 2020, Doc. No. EK: П-Е-2020-07; No. 04-150/15 dated March 14, 2022, Doc. No. EK: I-2022-01; No. 04-150/15 dated March 14, 2022, Doc. No. EK: I-2022-02; and approved by the Ministry of Agriculture, Forestry and Water Management – Veterinary Directorate (Belgrade, Serbia): No. 323-07-09359/2020-05 dated September 2, 2020; No. 323-07-03995/2022-05 dated March 28, 2022; No. 323-07-03996/2022-05 dated March 28, 2022; No. 323-07-03997/2022-05 dated March 28, 2022.

Treatment with metformin, caffeine, itraconazole, and nitroglycerin (all Galenika a.d., Belgrade, Serbia) and their co-administration to animals started after the hypodermic injection of 1 ml of BHK-21/C13 cell suspension (2×10^6 cells/ml) into the backside for the hypodermal fibrosarcoma growth. The following criteria for the humane termination of an animal's life were defined: serious body mass loss (20%), diminished activity/responsiveness with loss of body mass, poor posture, incapability to eat, urinate, or defecate, largest cancer dimension > 3.5 cm, cancer burden > 10% body mass, or cancer ulceration. The following characteristics were observed: general condition; general clinical characteristics (breathing disorders, diarrhea, neurological signs); behavior; body weight (measured daily); tumor diameter, location and ulceration; appearance of multiple tumors.

Each of three experiments included four groups of hamsters which received different daily therapies via a gastric probe after fibrosarcoma cell inoculation.

The first experiment: peroral application of 1) water (control group with inoculated tumor); 2) 500 mg/kg metformin; 3) 100 mg/kg caffeine; or 4) combination of 500 mg/kg metformin and 100 mg/kg caffeine.

The second experiment: peroral application of 1) water (control group with inoculated tumor); 2) 250 mg/kg metformin; 3) 250 mg/kg itraconazole; or 4) combination of 250 mg/kg metformin and 250 mg/kg itraconazole.

The third experiment: peroral application of 1) water (control group with inoculated tumor); 2) 1000 mg/kg metformin; 3) 50 mg/kg nitroglycerin; or 4) combination of 500 mg/kg metformin and 25 mg/kg nitroglycerin.

The hamsters were sacrificed 19 days after fibrosarcoma cell inoculation. Before animal sacrification, intraperitoneal dose of 90 mg/kg pentobarbital was applied. The hamsters were evaluated for sleep into coma at 5 minutes by combined methods, such as a toe pinch, lack of respiration and lack of reaction on palpation. Immediately after confirmation of loss of consciousness, total cardiac exsanguination was performed. Depending on animal weight, the volume of blood extracted was 3–5.5 ml. Two to three milliliters of the blood obtained was subjected to biochemical and hematological analyses. After exsanguination and life deprivation, main organs (brain, heart, lungs, kidneys, liver, stomach, intestine) were excised for pathological, histological, and toxicological examination. At the time of sacrifice, the weights of the animals were documented. All hamsters were in a good state during experiments, and none of the animals were euthanized before the end of the examination. During the experiment, the fibrosarcoma diameters and the tumor burdens were measured daily using calipers. The next formula for ellipsoid volume was used: volume = $4\pi abc/3$, where a, b, and c are ellipsoid half-diameters. After animal life deprivation, the cancers were excised, weighed and tumor diameters were exactly determined. The exact cancer volume was obtained by determination of the water level in a graduated cylinder before and after the submergence of the tumor (commonly used water volume displacement method).

In all the experiments, the drugs were dissolved in water and daily administered to animals in 1 ml/100 g body mass doses. The doses were < 50% of oral median lethal LD_{50} for hamsters and equivalent to human doses (by normalization to surface area).

The relative tumor weight (tumor burden) was calculated as tumor mass and animal body weight ratio. The tumor density was determined as density=mass/volume. The tumor surface area (S) was determined using the formula from three ellipsoid half diameters (a, b, and c): $S = 4\pi\{[(ab)^{1.6} + (ac)^{1.6} + (bc)^{1.6}]/3\}^{1/1.6}$.

Tumor slices (4 μm) were analyzed pathohistologically and immunohistochemically for the determination

of tumor development, tissue infiltration, necrosis and hemorrhagic zones expansion, proliferation, angiogenesis, apoptosis, glucose, and NO-metabolism.

Immunohistochemical examinations

In addition to the primary hematoxylin and eosin staining, immunohistochemical Ki-67, PCNA, CD34, CD31, COX4, cytochrome C, GLUT1 and iNOS staining (Thermo Fisher Scientific, Inc., Waltham, MA, USA; Abcam, Cambridge, UK) was performed according to already published methodology [14], to analyze cancer cell mitosis (Ki-67, PCNA), angiogenesis (CD34, CD31), apoptotic activity (COX4, cytochrome C), glucose turnover intensity (GLUT1), and nitric oxide expression (iNOS). The stained fibrosarcoma slices were analyzed under microscope (Leica DMLB 100T, Leica Microsystems GmbH, Wetzlar, Germany) with 400× magnification. Images were taken by a Leica MC190 HD camera (Leica Microsystems GmbH). The Ki-67 and PCNA staining images were analyzed using the UTHSCSA Image Tool for Windows Version 3.00. Individual Ki-67 or PCNA-positive cells were counted in each sample image. The mean numbers of Ki-67 and PCNA-positive cells in 20 cancer images from each hamster were compared among the experimental groups. Immunoexpression level was assessed by measuring part of stained surface area (stained/whole surface ratio) in the fibrosarcoma slices (mean of 20 measurements) by software UTHSCSA Image Tools for Windows Version 3.00.

Blood biochemical tests and hematological analyses

Blood was collected for standard laboratory analyses: glucose, serum proteins, albumins hemoglobin, sedimentation, leucocytes, granulocytes, lymphocytes, monocytes, platelets, erythrocytes, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, hematocrit, in all three experiments.

Statistical evaluation

Means and standard deviations were determined for the experimental data. The differences among the groups in all measured parameters were calculated using one-way ANOVA followed by a Student–Newman–Keuls *post hoc* test. A probability p-value less than 0.05 was considered to be statistically significant. Data analysis was performed using TIBCO Statistica 13.3.1 software (TIBCO Software Inc., Palo Alto, CA, USA) in all experiments.

RESULTS

The subcutaneous application of BHK-21/C13 cell culture caused fibrosarcoma production in all animals. Experimental animals had separated, well-delimited solid tumors without side effects on the overall state and welfare. The largest tumor diameters after animal life deprivation, were < 3.5 cm in all experiments. The maximal tumor burdens after animal life deprivation were much below 10% of the hamster body weight in all experiments. Pathological, histopathological and toxicological analysis following autopsy revealed no marks of toxic influence on main organs (brain, heart, lungs, kidneys, liver, stomach, and intestine), nor ascites or metastases.

The experimental and control groups were parametrically and nonparametrically tested for sedimentation, red and white blood cell counts, platelet number, glucose levels, hematocrit levels, hemoglobin levels, serum proteins, but no statistically important inequalities were detected among the groups in all three experiments ($p > 0.05$).

Treatment with metformin and caffeine

Treatment with combination of metformin and caffeine significantly suppressed cancer development as demonstrated by statistically important reduction of fibrosarcoma weight, volume, and Ki-67 (mean for 20), compared with control (Table 1).

Only the comedication of metformin with caffeine produced statistically important ($p < 0.05$) anticancer effects in comparison to the control. Neither metformin, nor caffeine given alone showed significant antitumor effects compared to the control. The treatments had no statistically important effects on the body weight of the animals during the experiment, in comparison to the control (Table 1).

The results proved the statistically important antitumor influence of the metformin and caffeine combination on experimental fibrosarcoma, without toxic effect.

Treatment with metformin and itraconazole

Treatment with the combination of metformin and itraconazole significantly suppressed cancer development as demonstrated by statistically important reduction of fibrosarcoma weight, length, volume, surface area, relative weight, density, ratio of tumor surface area to volume, compared with the control and single treatments (Table 2, Table 3, Figure 1, Figure 2).

The pathohistological and immunohistochemical analysis showed a decrease in tissue insertion, an extension of necrosis and hemorrhagic areas, statistically important reduction in cancer cell proliferation, as shown by Ki-67, statistically important reduction of the following: glucose metabolism, as demonstrated by GLUT1; NO metabolism, as demonstrated by iNOS staining; tumor vasculature, as demonstrated by CD34; and apoptosis intensity, as demonstrated by COX IV in all examined cancer slices from hamsters treated with the combination of metformin and itraconazole, in comparison with the control group and the single-treatment groups (Table 3, Figure 1, Figure 2).

Only the combined treatment with metformin and itraconazole produced statistically important ($p < 0.05$) anticancer effects in comparison to the control. Neither metformin, nor itraconazole given alone showed significant antitumor effects compared to control (Table 2, Table 3, Figure 1, Figure 2). The treatments had no statistically

Table 1. Characteristics of animals and tumors in control and groups treated with metformin and caffeine, with significance (p-values)

	Hamster			Tumor				
	Weight at start (g)	Weight at end (g)	Serum glucose (mM/l)	Weight (g)	D_{max} (cm)[a]	Volume (cm^3)	Density (mg/mm^3)	Mean[c] No Ki-67-positive cells
Control group with inoculated tumor, without treatment								
Mean	88.7	99	4.4	2.54	1.71	1.84	1.38	19.1
± SD	6.05	8.75	0.75	2.3	0.29	1.67	0.194	2.99
Group treated with metformin (500 mg/kg) daily								
Mean	86.36	88.72	4.2	1.1	1.58	0.82	1.34	14.45
± SD	12.9	13.99	0.99	0.81	0.42	0.56	0.123	6.03
p			> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Group treated with caffeine (100 mg/kg) daily								
Mean	95.11	100.1	5	2.32	1.85	1.96	1.18	16.28
± SD	10.95	12.55	3.02	1.31	0.5	1.11	0.177	4.86
p			> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Group treated with metformin (500 mg/kg) and caffeine (100 mg/kg) daily								
Mean	91.47	98	3.95	0.42	1.12	0.36	1.17	13.27
± SD	12.05	13.75	2.31	0.32	0.22	0.11	0.087	3.39
p			> 0.05	< 0.05[b]	< 0.05[b]	< 0.05[b]	< 0.05[b]	< 0.05[b]

[a]Largest tumor diameter (cm);
[b]p < 0.05 significant difference between treated groups compared to control;
[c]mean of 20 analyses of each tumor

Table 2. Characteristics of animals and tumors in control and groups treated with metformin and itraconazole

	Hamster			Tumor				
	Weight at start (g)	Weight at end (g)	Serum glucose (mM/l)	Weight (g)	D_{max} (cm)[a]	Volume (cm^3)	Density (mg/mm^3)	Mean[b] No Ki-67-positive cells
Control group with inoculated tumor, without treatment								
Mean	115	133.6	5.77	7.99	3.1	7.41	1.09	22
± SD	15	23.2	2.39	5.76	0.7	5.45	0.031	7
Group treated with metformin (250 mg/kg) daily								
Mean	88.7	95.4	4.95	4.59	2.5	4.33	1.075	18.2
± SD	14.3	9.8	3.11	2.14	0.45	1.95	0.04	4.8
Group treated with itraconazole (250 mg/kg) daily								
Mean	102.7	107.2	4.82	6.57	3.12	6.32	1.074	19
± SD	19.9	9.3	3.74	0.98	0.11	0.98	0.042	4.1
Group treated with metformin (250 mg/kg) and itraconazole (250 mg/kg) daily								
Mean	97.2	102.5	5.57	1.97	1.93	1.89	1.032	11.2
± SD	7.3	8.1	2.81	0.71	0.44	0.64	0.012	5.9

[a]Largest tumor diameter (cm);
[b]mean of 20 analyses of each tumor

Table 3. Statistical evaluation of tumor characteristics following treatment with metformin and itraconazole

Group comparison	Tumor (p-values)							
	Weight	Relative weight	Volume	Length	Surface area	Density	Surface/ volume	Mean Ki-67
C/M	0.200	0.840	0.122	0.137	0.349	0.490	0.045[a]	0.615
C/I	0.609	0.891	0.553	0.675	0.648	0.470	0.047[a]	0.769
C/M+I	0.034[a]	0.047[a]	0.037[a]	0.043[a]	0.045[a]	0.002[a]	0.048[a]	0.040[a]
M/I	0.052	0.217	0.047[a]	0.011[a]	0.021[a]	0.967	0.721	0.763
M/M+I	0.019[a]	0.014[a]	0.017[a]	0.061	0.009[a]	0.030[a]	0.457	0.048[a]
I/M+I	0.003[a]	0.003[a]	0.004[a]	0.006[a]	0.001[a]	0.040[a]	0.561	0.024[a]

C – control group; M – group treated with metformin (250 mg/kg); I – group treated with itraconazole (250 mg/kg);
M+I – group treated with the combination of metformin (250 mg/kg) and itraconazole (250 mg/kg);
[a]p < 0.05

important effects on the body weight of the animals during the experiment, in comparison to the control (Table 2).

The results proved the statistically important antitumor influence of the metformin and itraconazole combination on experimental fibrosarcoma, without toxic effect.

Treatment with metformin and nitroglycerin

Treatment with the combination of metformin and nitroglycerin significantly suppressed cancer development as demonstrated by statistically important reduction of fibrosarcoma weight, length, volume, density, compared with the control (Table 4).

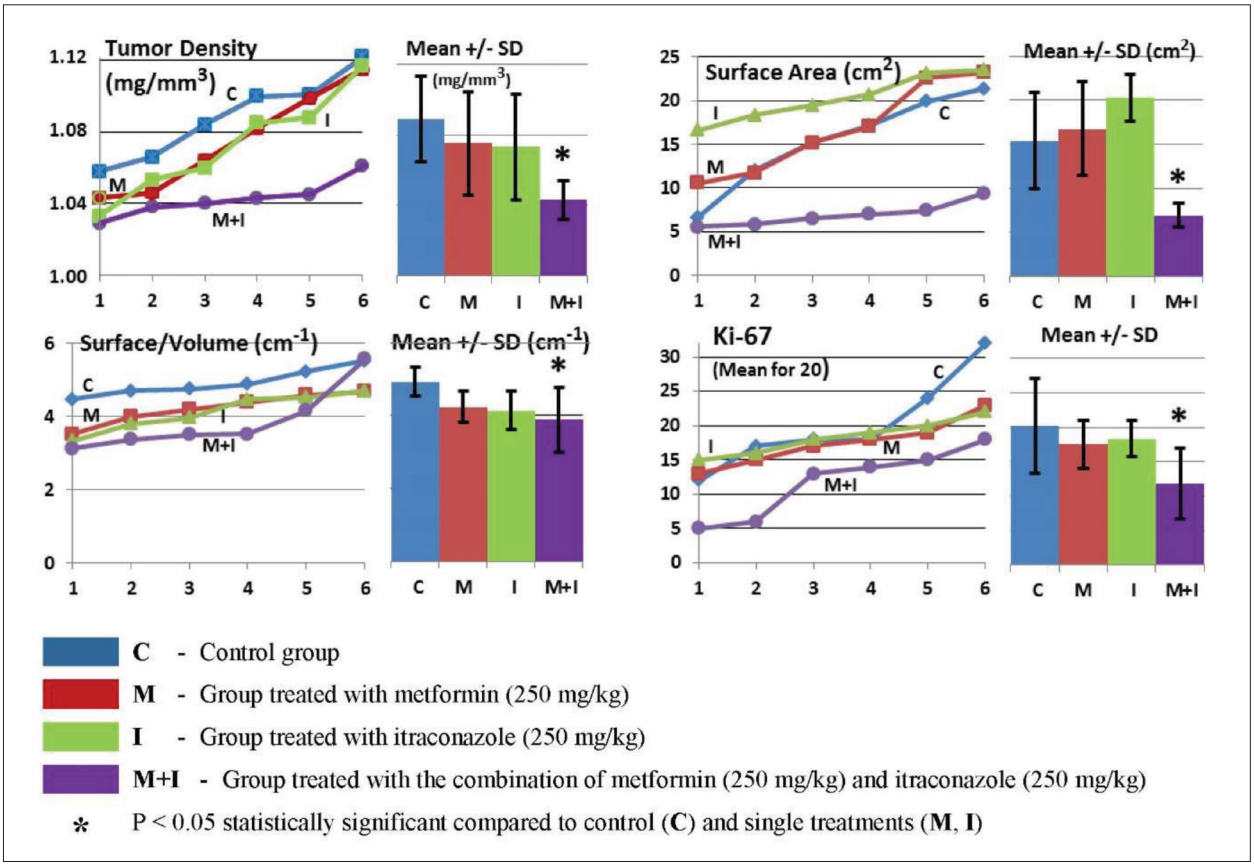

Figure 1. Biophysical and immunohistochemical characteristics of the excised tumors: tumor density, surface area, surface/volume ratio and Ki-67 positivity among the groups of animals treated with metformin and itraconazole;

*p < 0.05, as indicated

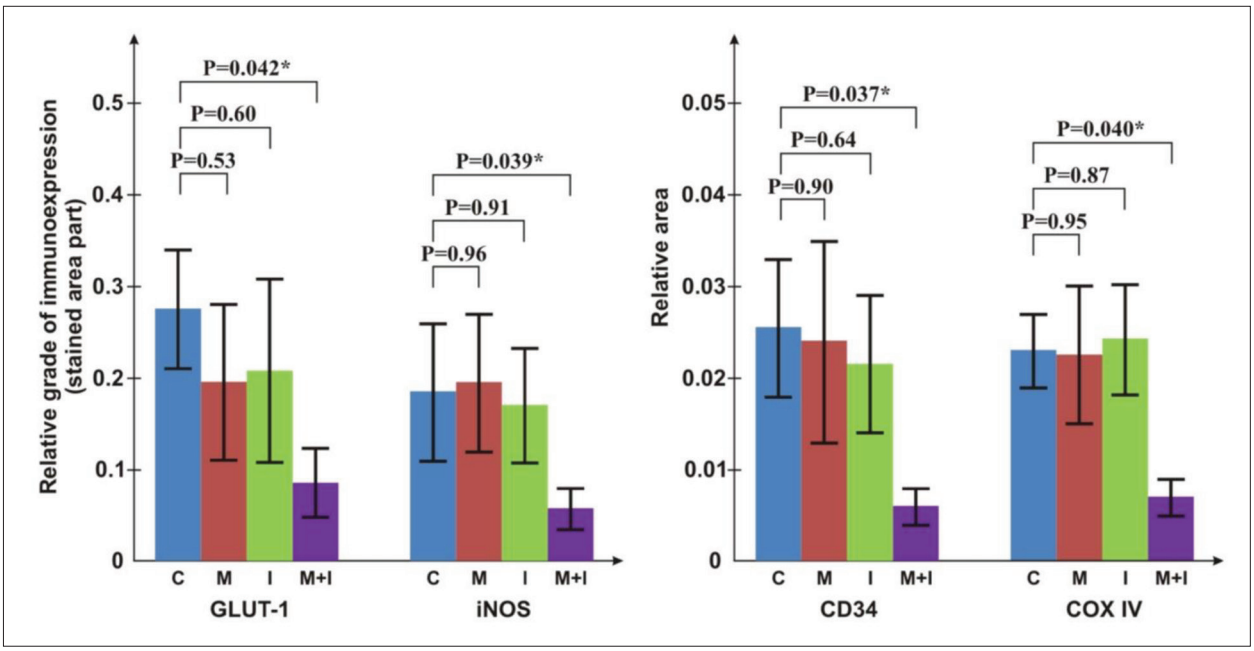

Figure 2. Immunohistochemical characteristics of the excised tumors (Mean ± SD);

GLUT-1, iNOS, CD 34, COX IV, in the second experiment;
C – control group; M – group treated with metformin (250 mg/kg); I – group treated with itraconazole (250 mg/kg); M+I – group treated with the combination of metformin (250 mg/kg) and itraconazole (250 mg/kg);
*p < 0.05, as indicated

Table 4. Comparison of fibrosarcoma growth between hamsters treated with metformin and nitroglycerin, with significance (p-values)

	Hamster			Tumor				
	Weight at start (g)	Weight at end (g)	Serum glucose (mM/l)	Weight (g)	D_{max} (cm)[a]	Volume (cm^3)	Density (mg/mm^3)	Mean[c] No Ki-67 positive cells
Control group (C)								
Mean	65.89	87.2	6.77	3.7	3.13	3.11	1.21	19.5
± SD	8.32	6.44	2.41	0.89	0.39	0.95	0.09	5.8
Group treated with metformin (1000 mg/kg) daily (M)								
Mean	62.73	82.83	5.55	3.63	3.01	3.07	1.24	19
± SD	7.34	7.02	1.31	0.88	0.51	0.87	0.095	7.06
p (MN/M)				0.011[b]	0.010[b]	0.020[b]	0.020[b]	0.010[b]
Group treated with nitroglycerin (50 mg/kg) daily (N)								
Mean	67.35	85.74	6.21	3.38	2.93	2.83	1.22	18.5
± SD	4.21	4.03	1.91	0.87	0.58	0.81	0.03	7.72
P(MN/N)				0.029[b]	0.037[b]	0.048[b]	0.010[b]	0.017[b]
Group co-treated with metformin (500 mg/kg) and nitroglycerin (25 mg/kg) daily (MN)								
Mean	70.04	87.72	6.27	2.33	2.32	2.11	1.12	10.2
± SD	6.03	6.32	1.92	0.69	0.43	0.50	0.09	3.79
P(MN/C)				0.010[b]	0.002[b]	0.030[b]	0.037[b]	0.003[b]

C – control group; M – metformin; N – nitroglycerin; MN – combination of metformin and nitroglycerin;
[a]largest tumor diameter (cm).
[b]$p < 0.05$ significant difference between treatments;
[c]mean of 20 analyses of each tumor

Table 5. Statistical evaluation of immunohistochemical tumor characteristics (p-values)

Group comparison	Tumor (p-values)							
	Ki-67	PCNA	CD 34	CD 31	GLUT-1	iNOS	COX 4	Cytochr. C
C/M	0.8895	0.9705	0.5870	0.4110	0.4610	0.4190	0.1126	0.9870
C/N	0.6790	0.6404	0.4130	0.2910	0.2590	0.3090	0.2370	0.4019
C/M + N	0.0032[a]	0.0082[a]	0.0153[a]	0.0157[a]	0.0091[a]	0.0081[a]	0.0085[a]	0.0137[a]
M/N	0.9670	0.7105	0.9120	0.6062	0.6010	0.7890	0.2470	0.0461[a]
M/M + N	0.0097[a]	0.0089[a]	0.0487[a]	0.0909	0.0077[a]	0.0087[a]	0.0094[a]	0.0179[a]
N/M + N	0.0167[a]	0.0091[a]	0.0801	0.2029	0.0074[a]	0.0085[a]	0.0413[a]	0.0109[a]

C – control group; M – group treated with metformin; N – group treated with nitroglycerin;
M + N – group treated with combination of metformin and nitroglycerin;
[a]$p < 0.05$

The pathohistological and immunohistochemical analysis showed a decrease in tissue insertion, an extension of necrosis and hemorrhagic areas, statistically important reduction in cancer cell proliferation, as shown by Ki-67 and PCNA, statistically important reduction of: glucose metabolism, as demonstrated by GLUT1; NO metabolism, as demonstrated by iNOS staining; tumor vasculature, as demonstrated by CD34 and CD31; and statistically important reduction in apoptosis intensity, as demonstrated by COX IV and cytochrome C, in all examined cancer slices from hamsters treated with the combination of metformin and nitroglycerin, in comparison with the control group and the single-treatment groups (Table 4, Figure 3, Table 5).

Only the combined treatment with metformin and nitroglycerin produced statistically important ($p < 0.05$) anticancer effects in comparison with the control. Neither metformin, nor nitroglycerin given alone showed significant antitumor effects compared to the control (Table 4, Table 5, Figure 3). The treatments had no statistically important effects on the body weight of the animals during the experiment, in comparison to the control (Table 4).

The results proved the statistically important antitumor influence of the metformin and nitroglycerin combination on experimental fibrosarcoma, without toxic effect.

DISCUSSION

Our three experiments suggested that dual therapy by using two repositioned drugs with anticancer and NF-κB activity (such as metformin combinations with caffeine, itraconazole, and nitroglycerin) shows effectiveness against fibrosarcoma in hamsters, contrary to ineffective monotherapy with each of them.

In the Introduction, many pathways are listed and examples of literature for several relevant *in vitro* experiments are given, yet none of this is shown for BHK21/C13 cancer cell line used in our *in vivo* experiments. For proposing a feasible synergistic influence free from the toxic effects, the reasonable approach would be a double-hit one targeting distinct pathways or different targets within the same pathway. In agreement with our effective combined treatment experiments with two NF-κB inhibitors, it can be supposed that combined treatment affects different targets within NF-κB pathway and that NF-κB is one of signaling pathways underlying anticancer mechanism of our three effective two-drug combinations with metformin.

Three weeks peroral administration of metformin, efavirenz and fluoxetine combination resulted in drastic decrease of cancer weight and volume in human colon cancer

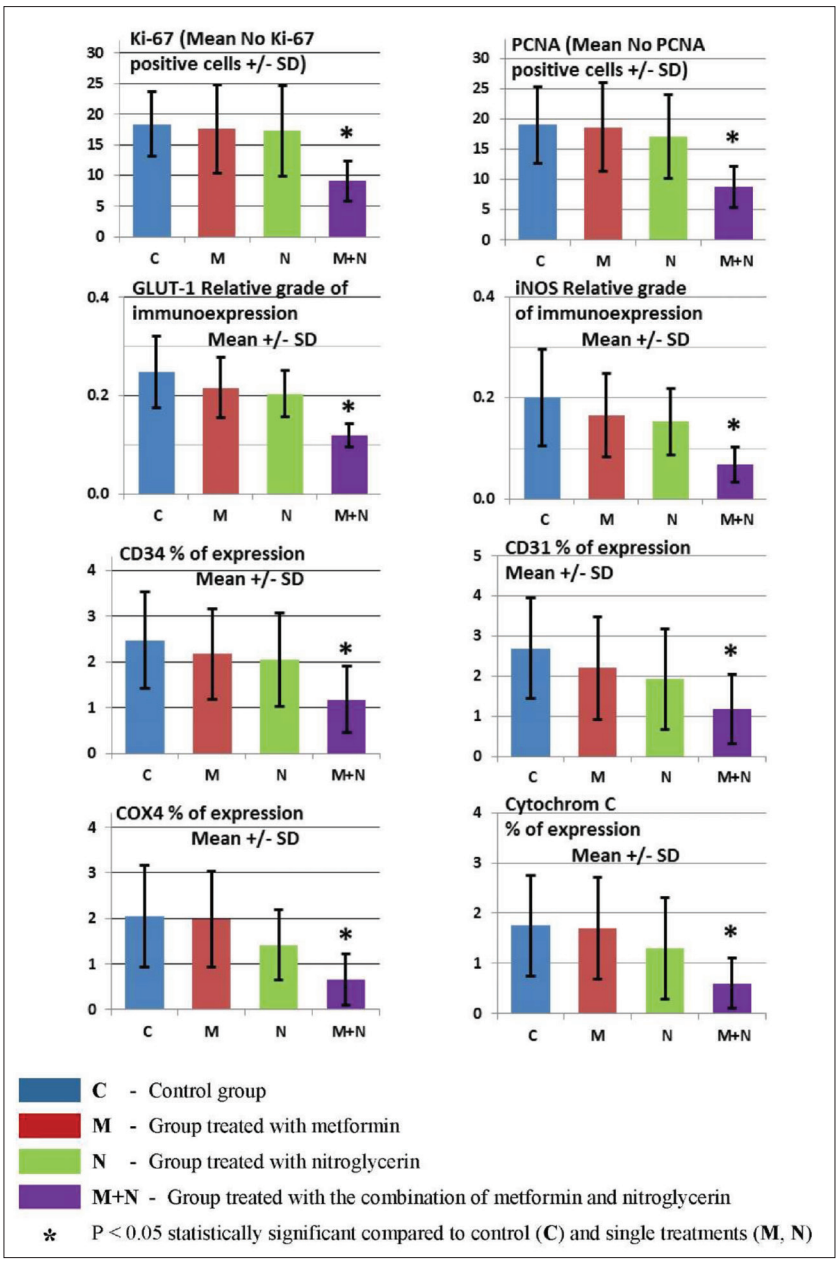

Figure 3. Means and standard deviations (SD) of immunohistochemical-histopathological characteristics of the excised tumors in the third experiment: Ki-67, PCNA, GLUT-1, iNOS, CD34, CD31, COX4, cytochrom C;

*p < 0.05, as indicated

xenografts of mice compared with untreated controls [15]. Profound anticancer activities *in vitro* and *in vivo* of the drug combination used in that study were explained by ROS amplification, which caused DNA damage, apoptosis, autophagy, and necroptosis [15].

The results of a recent meta-analysis showed favorable clinical signal on the response rate after adding metformin to chemotherapy in the breast cancer treatment [16]. Breast cancer patients receiving chemotherapy have been shown to experience better therapeutic responses with the use of metformin [16].

Comedication of metformin with various oncological drugs showed significant synergism in different cancer types [17]. Metformin activated AMPK, causing inhibition of mTOR and also reduced protein kinase B (PKB = Akt), causing inhibition of the phosphatidylinositol 3 (PI3)/Akt/mTOR and various pathways (RAS/RAF/MAPK/ERK), reducing transcription, protein synthesis, and proliferation [17].

Disclosure of anticancer effects in the tested non-oncological marketed drug combinations may be the first step to finding effective, cheap and immediately applicable treatment for tumors.

CONCLUSION

The results of our three experiments proved significant anticancer effects of the metformin co-treatments with caffeine, or itraconazole, or nitroglycerin on hamster fibrosarcoma, without toxicity. Opposite to the examined drug combinations, monotherapies did not show anticancer effects. Anticancer properties of the three examined two-drug combinations (metformin and caffeine, metformin and itraconazole, metformin and nitroglycerin) in hamsters, with used doses equivalent to standard human doses, suggest that effective nontoxic oncological therapies in humans and cancer relapse prevention using these drug combinations may be attainable. Treatment with metformin in combination with caffeine, or itraconazole, or nitroglycerin may be a promising efficacious nontoxic new adjuvant anticancer therapy and invites further clinical investigation.

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Conflict of interest: None declared.

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Ефекти метформина и његових комбинација са другим пренамењеним лековима на фибросарком код хрчака

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САЖЕТАК

Увод/Циљ Многи лекови регистровани за различите друге индикације могу селективно деловати на туморске рецепторе, сигналне путеве, метаболичке процесе, биоенергетске факторе, ензиме, протеине и гене који регулишу пролиферацију, апоптозу и неоангиогенезу тумора без утицаја на ове активности у здравим ћелијама. Увођење нових лекова је веома дуг, сложен и скуп процес истраживања. Откривање антиканцерског ефекта код лекова који су већ регистровани за друге индикације и формирање њихових комбинација могу директно смањити време и цену таквог истраживања.

Методе Антиканцерска ефикасност метформина и његових комбинација са кофеином, итраконазолом и нитроглицерином тестирана је на фибросаркому експериментално изазваном ћелијама *BHK21/C13* код сиријских златних хрчака (шест животиња по групи, насумично распоређених у контролне и експерименталне групе, дозе једнаке уобичајеним дозама за људе). После жртвовања животиња, тумори су ексцидирани и одређене су њихове величине, биофизичке карактеристике, хистологија и имунохистохемија. Узети су узорци крви за хематолошке и биохемијске анализе, а главни органи су токсиколошки анализирани. Статистичка значајност је одређена једносмерним *ANOVA* тестом, који је пратио *Student–Newman–Keuls post hoc* тест.

Резултати Комбинације два лека, метформина са кофеином, или итраконазолом, или нитроглицерином, показале су значајне антитуморске ефекте на фибросарком хрчка у поређењу са контролом, у односу на све тестиране параметре тумора ($p < 0,05$), без токсичности.

Закључак Примена метформина у комбинацији са кофеином, или итраконазолом, или нитроглицерином може бити ефикасан и безбедан приступ новој нетоксичној адјувантној антиканцерској терапији.

Кључне речи: метформин; кофеин; итраконазол; нитроглицерин; хрчци; фибросарком

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Experimental evaluation of the effects of anticancer modulation therapy on MAPK/PI3K/AKT/mTOR /NF-κB signaling with non-toxic drugs

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SUMMARY

Introduction/Objective Large diversity in molecular mechanisms of cancer regulation allows some marketed pleiotropic non-oncological non-toxic pharmaceuticals to be used in oncology, which reduces duration and cost of novel anticancer treatment research. To date, there are no published *in vivo* results on anticancer effects of certain combinations of non-oncological pleiotropic drugs (disulfiram, metformin, deoxycholic acid, mebendazole) that influence MAPK/PI3K/AKT/mTOR/NF-κB signaling.

Methods The anticancer effects of certain aforementioned repurposed drugs combinations, < 50 % LD_{50} (equivalent to the usual human dose) were assessed by fibrosarcoma growth kinetics (measured daily *in vivo* by calipers) and tumor proliferation (Ki-67, PCNA), neoangiogenesis (CD34, CD31), glucose metabolism (GLUT1), NO metabolism (iNOS) and apoptosis (COX4, cytochrome C) in hamsters, randomly allocated to control and experimental groups (six animals per group). The animals were sacrificed 19 days after BHK-21/C13 tumor inoculation. The tumors were excised, measured, and blood was collected. Biophysical, pathohistological, toxicological, hematological, and biochemical analyses were performed.

Results Disulfiram with metformin, disulfiram with deoxycholic acid and deoxycholic acid with metformin are the combinations that have shown significant antitumor effects on the fibrosarcoma growth kinetics and tumor immunohistochemical markers in hamsters ($p < 0.05$). All used drugs in efficacious combinations can inhibit MAPK/PI3K/AKT/mTOR/NF-κB signaling. The addition of NF-κB stimulator mebendazole to effective two-drug combinations rescued cancer growth, indicating that these pathways may be responsible for antitumor action.

Conclusion Combinations of non-oncological drugs: disulfiram with metformin, disulfiram with deoxycholic acid and deoxycholic acid with metformin have the potential to be used as effective non-toxic adjuvant anticancer therapy in oncology.

Keywords: disulfiram; deoxycholic acid; metformin; hamsters; BHK-21/C13; fibrosarcoma

INTRODUCTION

Activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling has been found in many types of tumors, including breast, colon, prostate, skin, lymphoid tumors [1]. NF-κB is an antiapoptotic factor responsible for cancer occurrence, development, and resistance to chemo- and radio-therapy. Hence, therapeutic blockade of NF-B or upstream signals of the cascade MAPK/PI3K/AKT/mTOR/NF-κB (mitogen-activated protein kinase / phosphatidylinositol 3-kinase / protein kinase B – PKB / mammalian target of rapamycin / NF-κB) in cancer cells provides an attractive strategy for the development of anticancer drugs.

For our *in vivo* analysis we selected: registered, non-oncological, low-toxic, pleiotropic drugs with common anticancer mechanisms already established *in vitro*, e.g. via NF-κB modulation.

Antialcoholic drug disulfiram inhibits the NF-κB signaling pathway, and hence inhibits proliferation and induces apoptosis of various cancer cell lines [2, 3].

Antidiabetic drug metformin inhibits MAPK, AKT, mTOR, and NF-κB in various cancer cells, resulting in inhibition of proliferation and stimulation of apoptosis *in vitro* and in mouse xenograft models *in vivo* [4]. Metformin inhibited proliferation by suppression of NF-κB in the lung, ovarian, gastric, and prostate human cancer cells [5].

Deoxycholic acid, used for liver cirrhosis and for serum cholesterol lowering, can produce oxidative stress [6]. The early phase of oxidative stress is associated with temporary activation of the NF-κB pathway, but sustained oxidative stress decreases NF-κB activity [7]. Deoxycholic acid induces programmed cell death via MAPK/PI3K/AKT/mTOR/NF-κB signaling [8]. It has been shown that deoxycholic acid inhibits NF-κB activity, limits cancer cell proliferation, invasion and induces apoptosis *in vitro* in human: pancreatic, gastric, lung, prostate, breast, colon, and hepatic carcinoma cells [9, 10].

In this study we have applied pleiotropic non-toxic drugs, modulators of NF-κB, with *in vitro* approved anticancer characteristics:

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disulfiram, metformin, deoxycholic acid, mebendazole, and their combinations.

If stimulation of the NF-κB can block or eliminate the anticancer effect, i.e. can "rescue" the cancer, than anticancer treatment targets NF-κB. To test whether NF-κB inhibition underlies the anticancer mechanism of the examined drug therapy, we co-medicated a NF-κB stimulator mebendazole for tumor rescue.

In order to contribute to anticancer treatments and underlying mechanisms, we conducted this study aiming to define the new efficacious, non-toxic, and inexpensive pleiotropic drug combinations that can be immediately used in oncology.

METHODS

Single and combined anticancer treatments with repurposed drugs are as follows: I. disulfiram and metformin; II. disulfiram and deoxycholic acid; III. deoxycholic acid and metformin were analyzed simultaneously in three separate independent experiments with three different control groups.

For our rescue treatments we used antihelmintic drug mebendazole, which strongly depolymerizes microtubules and thus activates NF-κB [11, 12].

Animal model

We conducted experiments on Syrian golden hamsters (six males per group; weight, ~70 g; age, ~13 weeks).

Our study followed internationally recognized guidelines on animal welfare, as well as local and national regulations (ARRIVE guidelines; Law on animal welfare of the Republic of Serbia; University Of Novi Sad Rules For Work With Experimental Animals).

All animals were subjected to protocols approved by the University of Novi Sad Animal Ethics Committee (Novi Sad, Serbia): Doc. No. EK: II-E-2020-07; Doc. No. EK: I-2022-01; No. 04-150/15; Doc. No. EK: I-2022-02; and approved by the Ministry of Agriculture, Forestry and Water Management – Veterinary Directorate (Belgrade, Serbia): No. 323-07-09359/2020-05; No. 323-07-03995/2022-05; No. 323-07-03996/2022-05; No. 323-07-03997/2022-05.

Treatments with disulfiram, metformin, deoxycholic acid, mebendazole (all Galenika a.d., Belgrade, Serbia) and their combinations in experimental groups were initiated after the subcutaneous inoculation of 1 ml of BHK-21/C13 cell suspension (2×10^6 cells/ml) into the animals' back for fibrosarcoma development. BHK-21/C13 cells were produced at the Department of Histology and Embryology, Faculty of Medicine, University of Novi Sad, Serbia. The humane endpoints were the following: significant body weight loss (20%), decreased activity/responsiveness with loss of body weight, impaired posture, inability to eat, urinate or defecate, tumor diameter > 3.5 cm, tumor burden > 10% of body weight, or tumor ulceration. General condition, behavior, general clinical signs (diarrhea, breathing disorders, neurological signs), tumor location and ulceration, appearance of multiple tumors were monitored on the daily basis as were tumor diameter and body weight.

Each treatment was administered via a gastric probe daily after cancer cell inoculation.

I. Disulfiram and metformin experiment: peroral treatment with 1) water (control group with inoculated tumor); 2) disulfiram 50 mg/kg; 3) metformin 500 mg/kg; 4) combination of disulfiram 50 mg/kg and metformin 500 mg/kg; 5) disulfiram double dose 100 mg/kg (for validation); 6) metformin double dose 1000 mg/kg (for validation); 7) combination of disulfiram 50 mg/kg, metformin 500 mg/kg, and mebendazole 460 mg/kg (for tumor rescue); 8) mebendazole 460 mg/kg. Two single drug treatments were applied with doubled doses of disulfiram alone and metformin alone (maximum tolerated < 50% LD_{50}) on two groups of animals for validation of the synergistic combinatory two-drug effect.

II. Disulfiram and deoxycholic acid experiment: peroral treatment with 1) water (control group with inoculated tumor); 2) disulfiram 50 mg/kg; 3) deoxycholic acid 100 mg/kg; 4) combination of disulfiram 50 mg/kg and deoxycholic acid 100 mg/kg; 5) combination of disulfiram 50 mg/kg, deoxycholic acid 100 mg/kg, and mebendazole 460 mg/kg (for tumor rescue); 6) mebendazole 460 mg/kg.

III. Deoxycholic acid and metformin experiment: peroral treatment with 1) water (control group with inoculated tumor); 2) deoxycholic acid 100 mg/kg; 3) metformin 500 mg/kg; 4) combination of deoxycholic acid 100 mg/kg and metformin 500 mg/kg; 5) combination of deoxycholic acid 100 mg/kg, metformin 500 mg/kg and mebendazole 460 mg/kg (for tumor rescue); 6)mebendazole 460 mg/kg.

The animals were sacrificed 19 days post-inoculation. Ninety mg/kg of pentobarbital was administered intraperitoneally to each animal before sacrification. For animal blood biochemical and hematological analyses, 2–3 ml of the total collected blood was used. After exsanguination, vital organs were removed for pathological, histological and toxicological examinations. Toxicity was analyzed based on the gross and microscopic standard pathological examination of main organs, tissues, and whole bodies, influence on body weight, determination of organ-weight to body-weight ratios of brain, heart, lungs, stomach, liver intestine and kidneys in treated hamsters versus control. Biochemical and hematological blood tests were also performed. Body mass of the animals was measured before the sacrification. All animals were in good condition during the study. Humane endpoints were not reached and none of the hamsters were euthanized prior to the end of the experiment. The tumor diameters and the tumor burdens were evaluated daily using calipers and the following ellipsoid volume formula: volume = $4\pi abc/3$, where a, b, and c are half-diameters. After sacrifice, the tumors were excised and weighed, their diameters were exactly measured using calipers, and the exact tumor volume was determined using the standard water volume displacement method. The relative tumor weight (tumor burden) was determined as the weight ratio of tumor/animal.

In all experiments, drugs were dissolved in water and administered to hamsters daily in 1 ml/100 g animal weight. Doses were < 50% of oral median lethal LD_{50} for

hamsters and equivalent to human doses (by normalization to surface area).

The tumor density was calculated as density = mass/volume; the tumor surface area (S) was calculated using the standard ellipsoid surface formula from three half-diameters (a, b, and c): $S = 4\pi\{[(ab)^{1.6} + (ac)^{1.6} + (bc)^{1.6}]/3\}^{1/1.6}$. The ratio of tumor surface area to volume, relative weight, surface/maximal length ratio, surface/tumor weight ratio, surface/density ratio, and maximum length/density ratio among the treated groups of animals were also calculated.

For the verification of tumor growth, tissue penetration, expansion of necrosis and hemorrhagic areas, proliferation, angiogenesis, apoptosis, glucose, and NO-metabolism, tumor slices (4 μm) were assessed pathohistologically and immunohistochemically.

Immunohistochemistry

In addition to the principal hematoxylin and eosin (HE) staining, immunohistochemical staining was performed to assess tumor proliferation (Ki-67, PCNA), neoangiogenesis (CD34, CD31), glucose metabolism (GLUT1), NO metabolism (iNOS) and apoptosis (COX4, cytochrome C) (Table 1).

Table 1. Antibody information

Antibodies	Manufacturer	Cat. No.	Dilution ratio
Ki-67	Thermo Fisher Scientific, Inc.	RB-9043-P0	1:300
PCNA	Thermo Fisher Scientific, Inc.	RB-9055-P	1:300
CD34	Abcam	ab81289	1:200
CD31	Abcam	ab28364	1:200
GLUT1	Thermo Fisher Scientific, Inc.	RB-9052-P0	1:200
iNOS	Thermo Fisher Scientific, Inc.	RB-9242-P0	1:100
Cytochrome C	Abeam	ab133504	1:200

Tissue sections preparation, staining, and analyzing

Tumor sections (4 μm) were deparaffinized in xylene (100%) and rehydrated in descending ethanol series (100% twice for three minutes: 95% for three minutes, and 70% for three minutes). For antigen retrieval, the sections were microwaved (850 W; ~98°C) for 20 minutes in Tris-EDTA buffer [10 mM Tris Base, 1 mM EDTA solution, 0.05% Tween 20 (pH 9.0)], washed twice for five minutes with tris-buffered saline (TBS) plus 0.025% Triton X-100 (with agitation) and blocked by immersion in 10% goat serum (cat. no. G6767; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) in TBS with 1% BSA (cat. no. T6789; Sigma-Aldrich; Merck KGaA) for two hours at room temperature. Primary antibodies dissolved in TBS with 1% BSA were incubated at 4°C overnight. The sections were washed twice for five minutes with TBS plus 0.025% Triton X-100 (with agitation) and incubated with 0.3% H_2O_2 in TBS for 15 minutes at room temperature. Horseradish peroxidase-conjugated goat polyclonal rabbit immunoglobulin G secondary antibody (cat. no. ab6721; Abeam) dissolved in TBS with 1% BSA was added to the sections for two hours at room temperature. The sections were washed three times for five minutes with TBS. For visualization, the chromogen 3,3-diaminobenzidine tetrahydrochloride (cat. no. K3468; Liquid DAB + Substrate – Chromogen System; Dako; Agilent Technologies, Inc., Santa Clara, CA, USA) was added and incubated for 10 minutes at room temperature. The sections were washed with water for five min. and were stained with Mayer's hematoxylin for five min. at room temperature.

The stained tumor slices were assessed using Leica DMLB 100T (Leica Microsystems GmbH) microscope at 400× magnification. Images were captured using a Leica MC190 HD camera (Leica Microsystems GmbH). Immunoexpression was evaluated based on the positive cells counts (stained/total number of cells) or on the stained portions of surface area (stained surface / whole surface) in the tumor sections (mean of 10 measurements) using UTHSCSA Image Tools for Windows version 3.00.

Blood biochemical tests and hematological analyses

Standard laboratory blood analyses were performed: glucose, serum proteins, albumins hemoglobin, sedimentation, erythrocytes, leucocytes, lymphocytes, monocytes, granulocytes, platelets, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, in all experiments.

Statistical analysis

Means, standard deviations (SD), or standard errors (SEM) were calculated for the experimental data. The differences among the groups in all measured parameters were determined using one-way ANOVA followed by a Student–Newman–Keuls post-hoc test. Statistically significant difference was indicated at $p < 0.05$. The two-sided Mann–Whitney U tests were additionally performed to check significances obtained by parametric testing. Data analysis was conducted using TIBCO Statistica 13.3.1 software (TIBCO Software, Inc., Palo Alto, CA, USA) in all experiments.

RESULTS

After inoculation of BHK-21/C13 cells, fibrosarcoma developed in all hamsters. Animals had isolated, well-demarcated solid tumors without adverse effects on general health and well-being. The maximum tumor diameters after sacrifice were < 3.5 cm in all the experiments. The maximum tumor burdens after sacrifice were much below 10% of the animal body weight in all the experiments. Pathological, histopathological, and toxicological analysis following autopsy revealed no signs of toxicity on main organs (heart, lungs, stomach, intestine, liver, kidneys, and brain), nor metastases or ascites.

The experimental and control groups were statistically compared for glucose levels, hemoglobin levels, hematocrit levels, serum proteins, sedimentation, red and white blood cell counts, platelet number, but no significant differences

Table 2. Characteristics of animals and quantitative pathological characteristics of tumors in control and treated groups (the disulfiram and metformin experiment)

	Hamster		Tumor		
	Weight at start (g)	Weight at end (g)	Weight (g)	D_{max} (cm)	Volume (cm^3)
C – Control group					
Mean	67.5	94.5	4.42	3.37	3.61
± SD	5.55	5.11	0.48	0.32	0.55
D - Group treated with disulfiram (50 mg/kg/day)					
Mean	68.3	90.2	4.3	3.22	3.55
± SD	6.23	6.88	0.79	0.56	0.82
M – Group treated with metformin (500 mg/kg/day)					
Mean	69.1	91	4.12	3.3	3.33
± SD	4.32	5.73	0.68	0.64	0.57
*DM – Group treated with the combination of disulfiram (50 mg/kg/day) and metformin (500 mg/kg/day)					
Mean	68.5	96.2	0.63	1.25	0.59
± SD	5.72	4.92	0.25	0.43	0.25
2D – Group treated with disulfiram double dose (100 mg/kg/day)					
Mean	70.2	97.2	4.13	3.11	3.4
± SD	5.72	5.76	0.66	0.4	0.65
2M – Group treated with metformin double dose (1000 mg/kg/day)					
Mean	69.5	91.5	3.92	2.7	3.27
± SD	5.21	5.41	0.6	0.53	0.72
DMMb – Group treated with the combination of disulfiram (50 mg/kg/day), metformin (500 mg/kg/day) and mebendazole (460 mg/kg/day)					
Mean	70	94.9	4.41	3.34	3.63
± SD	6.22	6.26	0.39	0.26	0.45
Mb – Group treated with mebendazole (460 mg/kg/day)					
Mean	68.8	94.8	4.22	3.05	3.35
± SD	4.29	4.39	0.81	0.61	0.75

D_{max} = largest tumor diameter (cm);
*p < 0.05

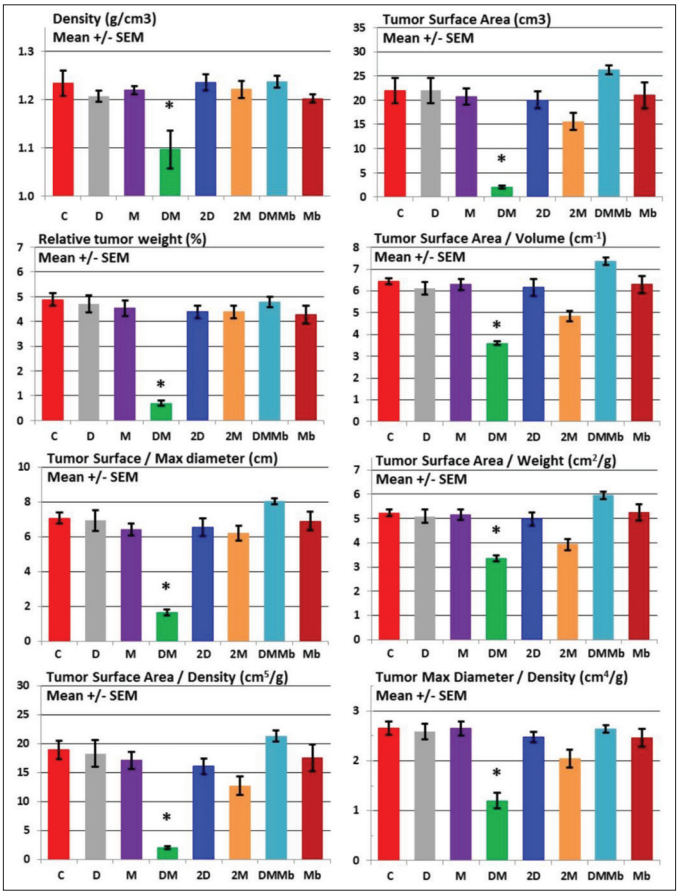

Figure 2. Means and standard errors of the mean (SEM) of quantitative pathological and physicochemical characteristics of the excised tumors in the I. disulfiram and metformin experiment;
DM Mb – group treated with the combination of disulfiram, metformin and mebendazole; Mb – group treated with mebendazole;
*p < 0.05

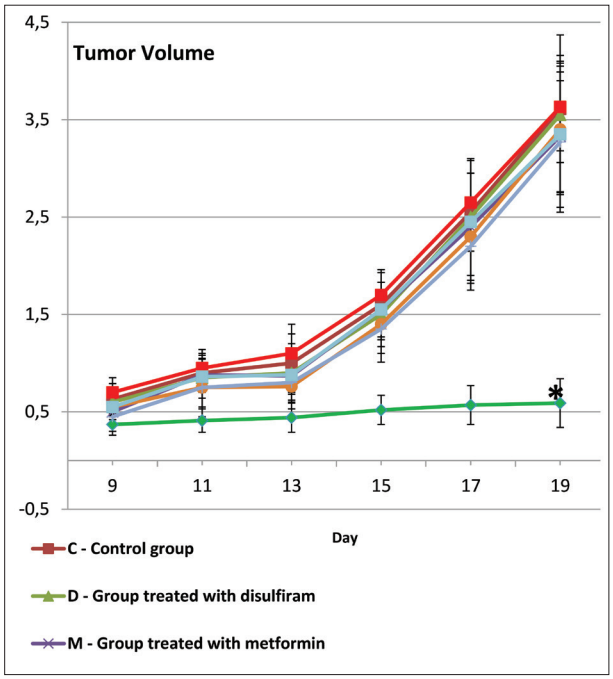

Figure 1. Tumor volume growth during course of the I. disulfiram and metformin experiment: interpolated line chart between the average values and SD values;
*p < 0.05

were observed among the groups in all three experiments (p > 0.05).

In all experiments, peroral co-treatment with examined dual drug combination significantly inhibited tumor growth as indicated by significant decreases in tumor weight, volume, maximum diameter, density, tumor surface area, relative tumor weight, tumor surface area/volume ratio, tumor surface / maximum diameter ratio, tumor surface area / weight ratio, tumor surface area / density ratio, tumor maximum diameter / density ratio, compared with control, as shown for the I. disulfiram and metformin experiment in Table 2, Figures 1 and 2; for the II. disulfiram and deoxycholic acid experiment in Table 3, Figures 3 and 4, and for the III. deoxycholic acid and metformin experiment in Table 4, Figures 5 and 6.

In all experiments, the pathohistological and immunohistochemical evaluation revealed a decrease in tissue penetration, an expansion of necrosis and hemorrhagic areas, significantly decreased proliferation status of tumor cells, as demonstrated by Ki-67 (and additionally by PCNA for the I. disulfiram and metformin combination), significant inhibition of glucose metabolism, as demonstrated by GLUT1, significant inhibition of NO metabolism, as demonstrated by iNOS staining, significant inhibition of tumor vasculature, as demonstrated by CD31 (and additionally by

Table 3. Characteristics of animals and quantitative pathological characteristics of tumors in control and treated groups (*the* disulfiram and deoxycholic acid *experiment*)

	Hamster		Tumor		
	Weight at start (g)	Weight at end (g)	Weight (g)	D_{max} (cm)	Volume (cm^3)
Control group					
Mean	68.1	95.1	3.22	2.92	2.62
± SD	1.85	2.01	0.2	0.1	0.19
Group treated with disulfiram (50 mg/kg/day)					
Mean	67.5	92.3	3.06	2.88	2.55
± SD	2.44	2.49	0.19	0.16	0.17
Group treated with deoxycholic acid (100 mg/kg/day)					
Mean	70.1	93.4	1.83	2.74	1.49
± SD	2.09	2.14	0.11	0.18	0.11
*Group treated with the combination of disulfiram (50 mg/kg/day) and deoxycholic acid (100 mg/kg/day)					
Mean	69.3	95.2	0.25	0.94	0.23
± SD	2.18	1.96	0.07	0.09	0.06
Group treated with the combination of disulfiram (50 mg/kg/day), deoxycholic acid (100 mg/kg/day) and mebendazole (460 mg/kg/day)					
Mean	69.9	93.8	4.16	3.25	3.4
± SD	2.42	2.43	0.17	0.13	0.15
Group treated with mebendazole (460 mg/kg/day)					
Mean	68.3	93.9	3.21	2.85	2.69
± SD	1.99	2.09	0.18	0.19	0.16

D_{max} = largest tumor diameter (cm);
*p < 0.05

Table 4. Characteristics of animals and quantitative pathological characteristics of tumors in control and treated groups (the deoxycholic acid and metformin experiment)

	Hamster		Tumor		
	Weight at start (g)	Weight at end (g)	Weight (g)	D_{max} (cm)	Volume (cm^3)
Control group					
Mean	69.1	96.2	3.1	2.97	2.5
± SD	1.77	2.07	0.1	0.21	0.09
Group treated with deoxycholic acid (100 mg/kg)					
Mean	70.3	94.3	2.2	2.96	1.76
± SD	2.19	2.11	0.05	0.11	0.05
Group treated with metformin (500 mg/kg/day)					
Mean	70.1	92.2	2.65	2.71	2.1
± SD	1.75	2.27	0.09	0.15	0.08
*Group treated with the combination of deoxycholic acid (100 mg/kg/day) and metformin (500 mg/kg/day)					
Mean	68.9	95.2	1.45	0.98	1.4
± SD	2.32	2.04	0.03	0.07	0.03
Group treated with the combination of deoxycholic acid (100 mg/kg/day), metformin (500 mg/kg/day) and mebendazole (460 mg/kg/day)					
Mean	70.9	94.7	2.71	2.96	2.2
± SD	2.39	2.41	0.12	0.11	0.1
Group treated with mebendazole (460 mg/kg/day)					
Mean	69.5	95.7	2.29	2.88	1.83
± SD	2.01	1.99	0.07	0.12	0.06

D_{max} = largest tumor diameter (cm);
*p < 0.05

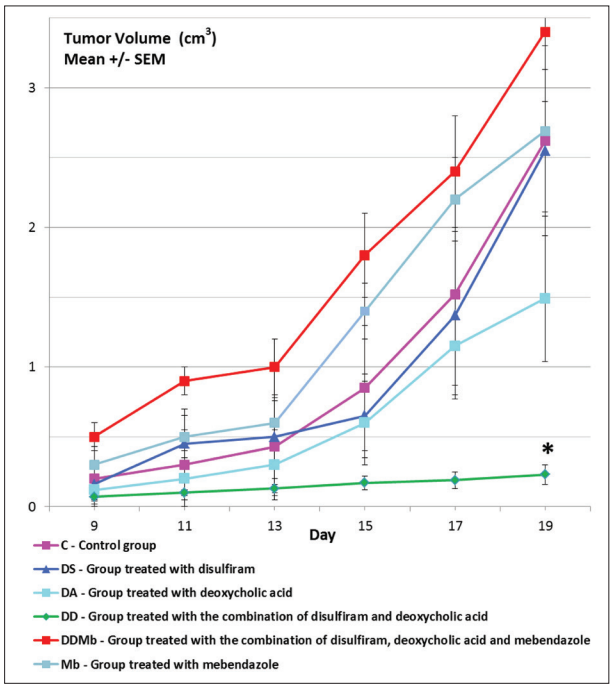

Figure 3. Tumor volume growth during course of the II. disulfiram and deoxycholic acid experiment: interpolated line chart between the average values and standard errors of the mean (SEM) values;
*p < 0.05

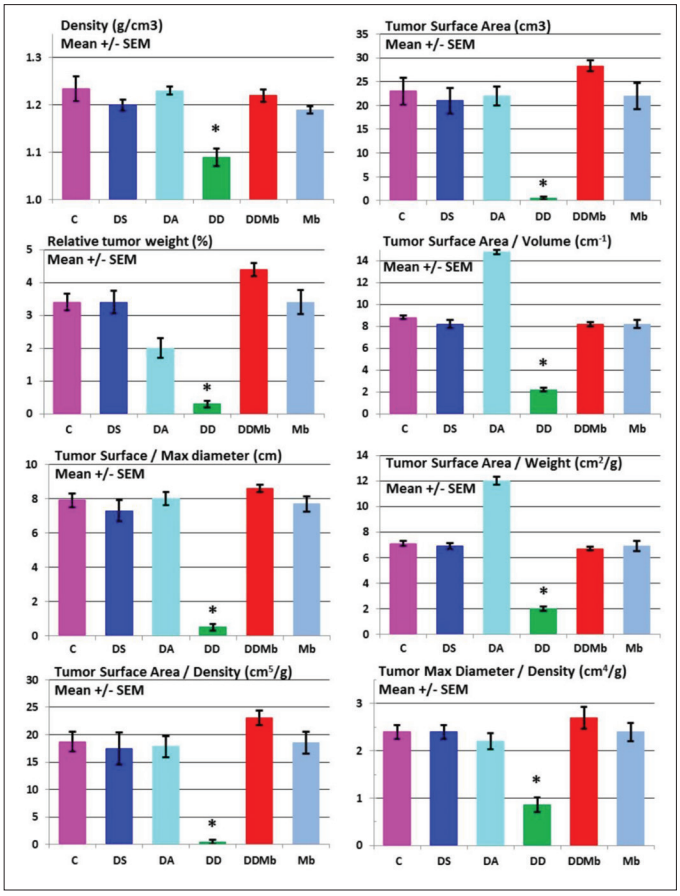

Figure 4. Means and standard errors of the mean (SEM) of quantitative pathological and physicochemical characteristics of the excised tumors in the II. disulfiram and deoxycholic acid experiment;
C – control group; DS – group treated with disulfiram; DA – group treated with deoxycholic acid; DD – group treated with the combination of disulfiram and deoxycholic acid; DDMb – group treated with the combination of disulfiram, deoxycholic acid and mebendazole; Mb – group treated with mebendazole;
*p < 0.05

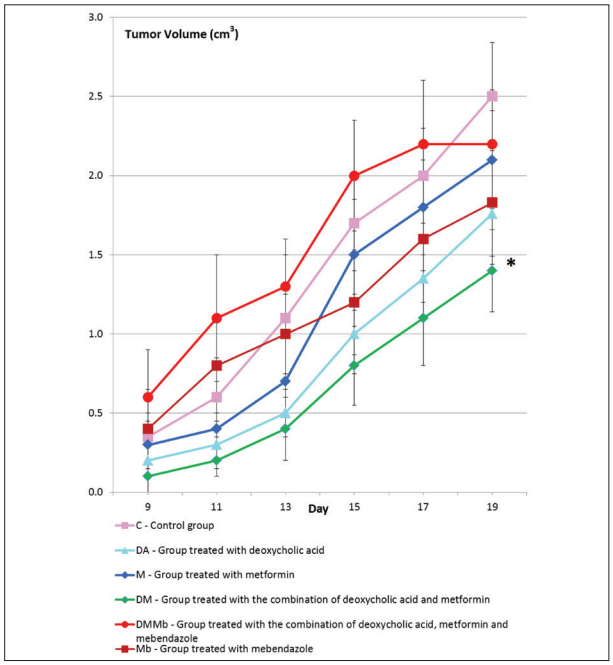

Figure 5. Tumor volume growth during course of the III. deoxycholic acid and metformin experiment: interpolated line chart between average values and standard error of the mean values;
*p < 0.05

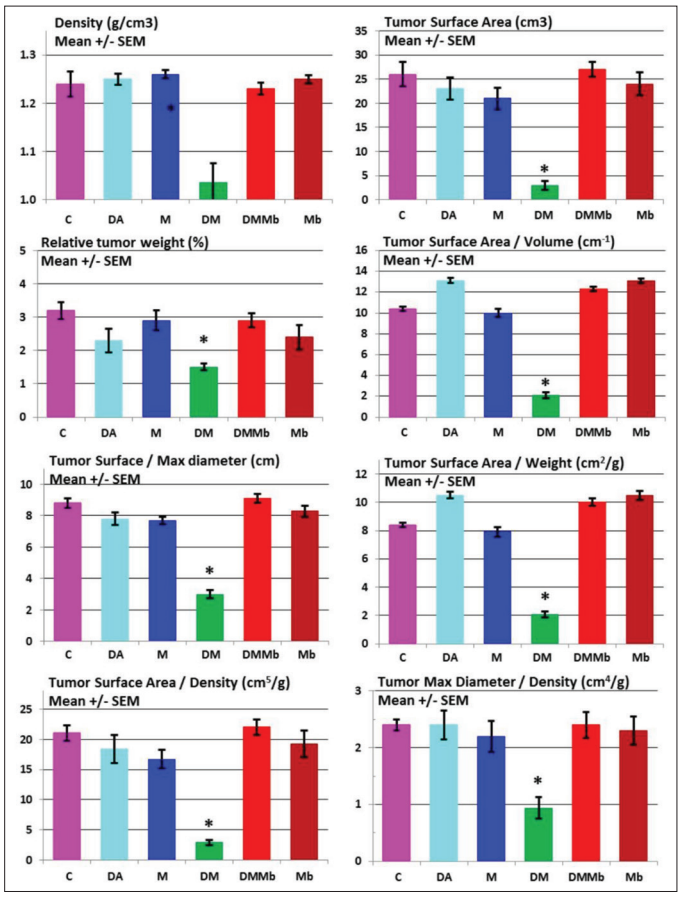

Figure 6. Means and standard errors of the mean (SEM) of quantitative pathological and physicochemical characteristics of the excised tumors in the III. deoxycholic acid and metformin experiment.
C – control group; DA – group treated with deoxycholic acid; M – group treated with metformin; DM – group treated with the combination of deoxycholic acid and metformin; DM Mb – group treated with the combination of deoxycholic acid, metformin and mebendazole; Mb – Group treated with mebendazole;
*p < 0.05

CD34 for the I. disulfiram and metformin combination), and significant difference in apoptosis intensity, as demonstrated by COX4 and cytochrome C, in all analyzed slices of tumors from animals treated with the examined dual drug combination, compared with the control group and the single-treatment groups, as shown for the I. disulfiram and metformin experiment in Figure 7; for the II. disulfiram and deoxycholic acid experiment in Figure 8, and for the III. deoxycholic acid and metformin experiment in Figure 9. Results gained with HE staining and different antibodies are illustrated by Figure 10.

In all experiments, only the examined dual drug combination resulted in a statistically significant ($p < 0.05$) antitumor effects compared with control (Tables 2, 3, and 4, Figures 1–9). In the I. disulfiram and metformin experiment, neither disulfiram, nor metformin single treatments, even in double doses, exhibited significant anticancer effect in comparison to control (Table 2, Figure 1, Figure 2, Figure 3).

In all three experiments (I. disulfiram and metformin; II. disulfiram and deoxycholic acid; III. deoxycholic acid and metformin), co-treatment with NF-κB stimulator mebendazole inhibited anticancer activity of the examined dual drug combination. Mebendazole rescued tumor progression suppressed by each examined dual drug combination of the two NF-κB inhibitors. This indicates that synergistic antitumor effects of each examined dual drug combination (I. disulfiram and metformin; II. disulfiram and deoxycholic acid; III. deoxycholic acid and metformin) may be caused by NF-κB suppression.

During the course of all experiments, the treatments had no significant effect on the body weight of the hamsters (compared with the control), as shown for the I. disulfiram and metformin experiment in Table 2; for the II. disulfiram and deoxycholic acid experiment in Table 3 and for the III. deoxycholic acid and metformin experiment in Table 4.

The results of all three experiments confirmed the significant synergistic anticancer effects of each examined dual drug co-treatment (I. disulfiram and metformin; II. disulfiram and deoxycholic acid; III. deoxycholic acid and metformin) on hamster fibrosarcoma, without toxicity.

DISCUSSION

In our experiments, disulfiram doses were 50 and 100 mg/kg, i.e. ~10% and ~20% of hamster oral LD_{50}, respectively (oral LD_{50} rat: 500 mg/kg, oral LD_{50} mouse: 1013 mg/kg). Dose of 50 mg/kg corresponds to the usual human dose of 4 mg/kg by normalization to body surface.

Metformin doses of ~25% and ~50% of the oral LD_{50} for hamsters were selected in our experiments. Since oral metformin LD_{50} is about 2000 mg/kg (2400 mg/kg in mice, 1770 mg/kg in rats), 500 mg/kg and 1000 mg/kg were used in this study. The daily

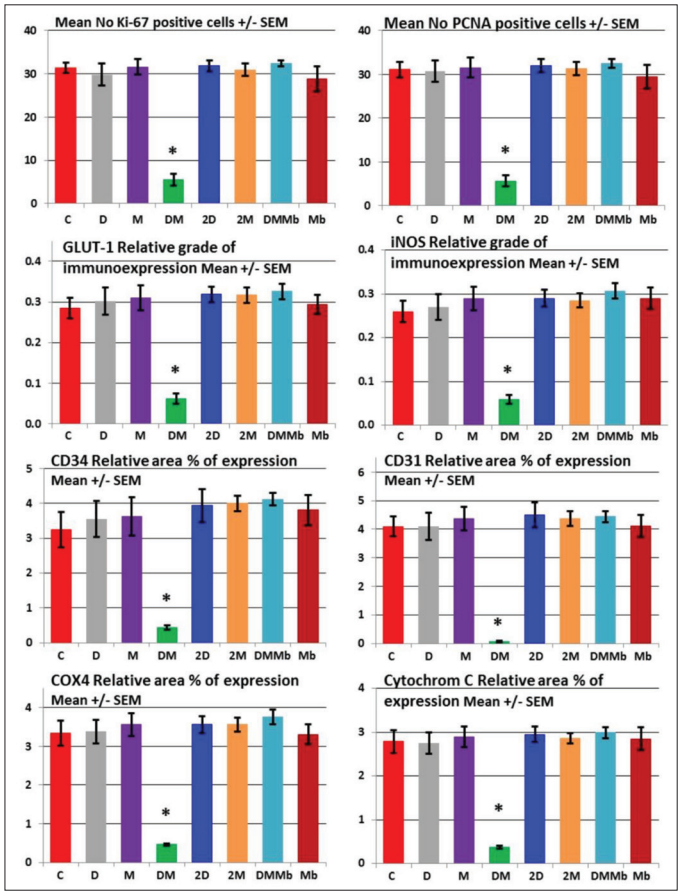

Figure 7. Means and standard errors of the mean (SEM) of histopathological-immunohistochemical characteristics of the excised tumors in the I. disulfiram and metformin experiment;
C – control group; D – group treated with disulfiram; M – group treated with metformin; DM – group treated with the combination of disulfiram and metformin; 2D – group treated with disulfiram doubled dose; 2M – group treated with metformin doubled dose; DM Mb – group treated with the combination of disulfiram, metformin and mebendazole; Mb – group treated with mebendazole; *p < 0.05

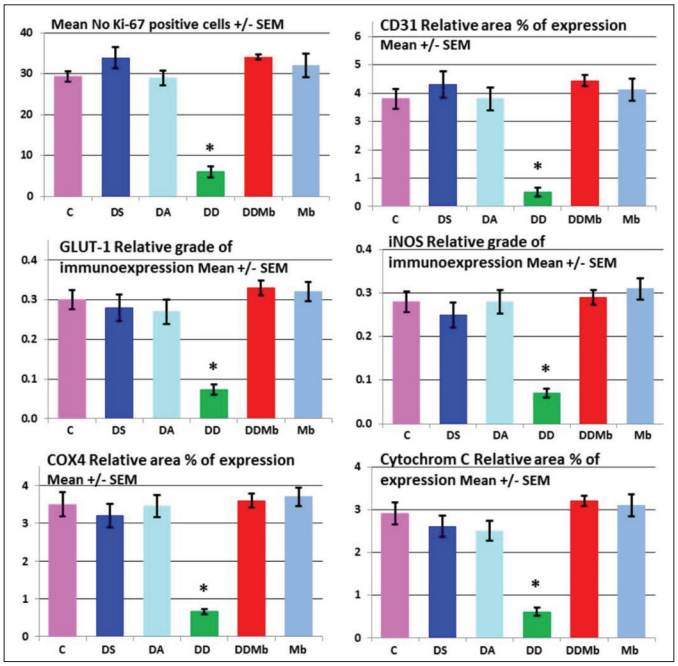

Figure 8. Means and standard errors of the mean (SEM) of histopathological-immunohistochemical characteristics of the excised tumors in the II. disulfiram and deoxycholic acid experiment;
C – control group; DS – group treated with disulfiram; DA – group treated with deoxycholic acid; DD – group treated with the combination of disulfiram and deoxycholic acid; DDMb – group treated with the combination of disulfiram, deoxycholic acid and mebendazole; Mb – group treated with mebendazole; *p < 0.05

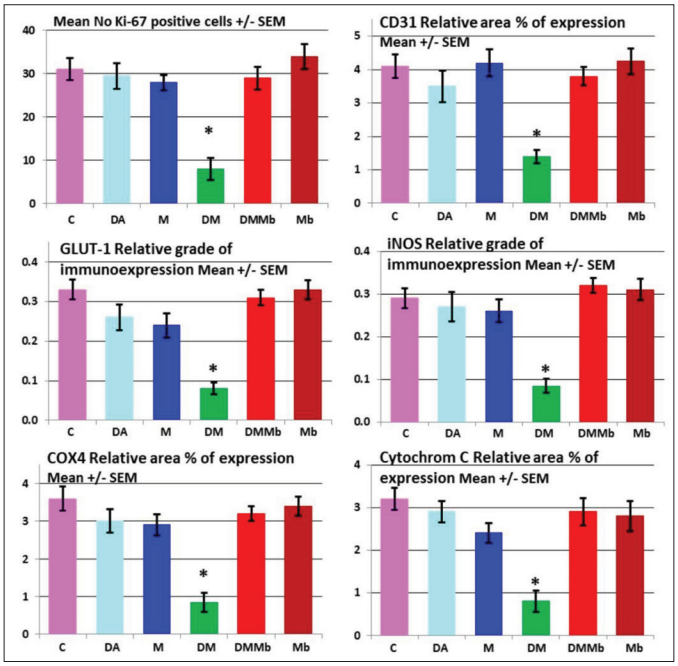

Figure 9. Means and standard errors of the mean (SEM) of histopathological-immunohistochemical characteristics of the excised tumors in the III. deoxycholic acid and metformin experiment;
C – control group; DA – group treated with deoxycholic acid; M – group treated with metformin; DM – group treated with the combination of deoxycholic acid and metformin; DM Mb – group treated with the combination of deoxycholic acid, metformin and mebendazole; Mb – group treated with mebendazole; *p < 0.05

dose of 500 mg/kg metformin in hamsters corresponds to the maximum daily dose of 40 mg/kg in patients with diabetes normalized to body surface.

Deoxycholic acid lowered the serum cholesterol after the administration of 750 mg/day for 3–4 weeks. The effect of deoxycholic acid ingestion 750 mg/day on bile acid kinetics was studied in healthy volunteers [13]. Equivalent dose for hamsters based on body surface for human dose of 750 mg/day is 100 mg/kg (750 mg/day = 12 mg/kg/day × 7.4 ≈ 100 mg/kg/day, where 7.4 is biometric conversion factor for hamsters based on body surface) [14]. This dose for hamsters (100 mg/kg/day), equivalent to oral human dose, is significantly below 25% hamster LD_{50} (oral LD_{50} in mouse 1000 mg/kg, oral LD_{50} in rat 1370 mg/kg). This is the underlying rationale for using deoxycholic acid dose of 100 mg/kg for hamsters in our study.

In our experiments, mebendazole was administered to hamsters orally at a dose of 460 mg/kg daily (~50% of oral LD_{50} for hamsters), equivalent to an oral human dose of 62 mg/kg/day normalized to body surface area (biometric conversion factor 7.4 [14]), which is comparable to human daily dose of 50 mg/kg/day for the 1–6 months of treatment of echinococcosis.

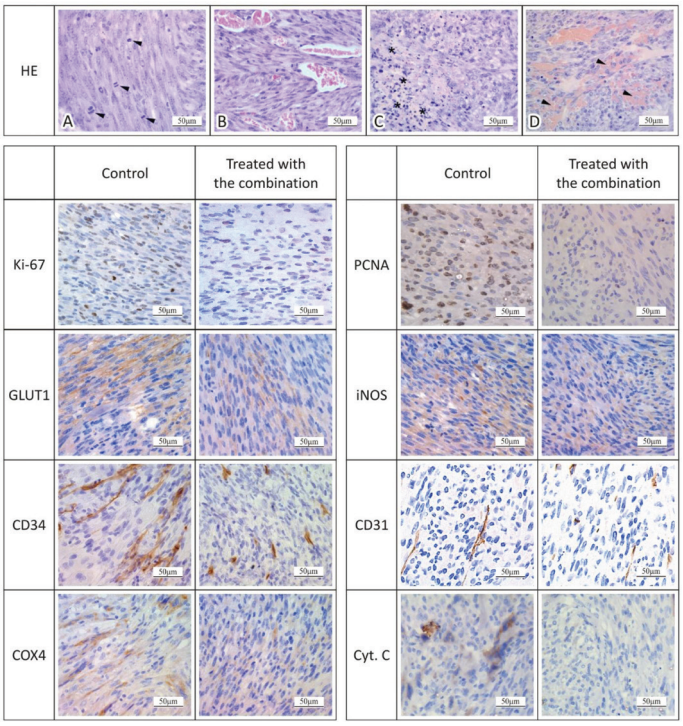

Figure 10. Illustration of results obtained by used staining methods; HE staining images of BHK fibrosarcoma sections (the first row): A – numerous mitotic figures within the tissue of an experimental BHK sarcoma (arrow); B – tumor angiogenesis; C – multiple areas of tumor necrosis (*); D – frequent areas of fresh bleeding in the tumor tissue (arrow);
immunohistochemical staining images of hamster fibrosarcoma sections: Ki-67, PCNA, GLUT1, iNOS, CD34, CD31, COX4, and cytochrome C, examples from the control group and the group treated with the examined combination

Since in our study, doses for disulfiram, metformin, deoxycholic acid and mebendazole for hamsters were equivalent to used human doses (based on surface body area biometric conversion factor of 7.4), it follows that anticancer effects seen in our study can be achievable with the usual human doses in oncology [14].

Previous research has found that disulfiram suppressed proliferation of various malignant cell types also via the reactive oxygen species (ROS) activation, as well as the simultaneous NF-κB inhibition. Simultaneous induction of ROS and inhibition of NF-κB by disulfiram induces cell cycle arrest and apoptosis [2, 3].

Studies published so far show that metformin inhibits complex I of the mitochondrial electron transport chain, which leads to membrane depolarization and the release of ROS with the chemical damage of cell components and apoptosis [5]. ROS, elevated by metformin, activates AMPK, inhibits mTOR signaling and expression of NF-κB [4, 5], activates tumor proliferation suppressor p53, arresting the cell cycle.

Deoxycholic acid has been shown to cause damage to mitochondrial membrane. Also, it was shown that induction of oxidative stress by deoxycholic acid leads to impaired NF-κB transcriptional activity, which facilitates apoptosis [6–10].

As can be seen from cited publications, efficacious anticancer combinations of repositioned drugs in our experiments (I. disulfiram and metformin, II. disulfiram and deoxycholic acid, III. deoxycholic acid and metformin) encompass agents with NF-κB inhibitory effect. Since results of our study show that well known NF-κB stimulator mebendazole annulled anticancer effects of examined two-drug combinations (I. disulfiram and metformin, II. disulfiram and deoxycholic acid, III. deoxycholic acid and metformin) and rescued tumor growth in all three experiments, it can be supposed that NF-κB inhibition is an important underlying mechanism of observed anticancer effects.

Our findings confirmed the significant anticancer effects of non-oncological drug combinations: I. disulfiram with metformin, II. disulfiram with deoxycholic acid and III. deoxycholic acid with metformin on hamster fibrosarcoma, without toxicity. The single treatments did not exhibit significant antisarcoma effects. Rescue treatments with co-medicated mebendazole to combined two-drug therapies in all three experiments indicate important underlying role of NF-κB in observed anticancer effects.

CONCLUSION

The anticancer properties of the three examined two-drug combinations (I. disulfiram and metformin, II. disulfiram and deoxycholic acid, III. deoxycholic acid and metformin) in hamsters, with used doses equivalent to standard human doses, suggest that effective nontoxic oncological therapies in humans and prevention of cancer relapse using these drug combinations may be achievable and that their administration may be an effective and safe approach in novel nontoxic adjuvant anticancer treatment.

ACKNOWLEDGMENT

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Conflict of interest: None declared.

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Експериментална евалуација ефеката антиканцерске модулационе терапије на сигнализацију *MAPK/PI3K/AKT/mTOR/NF-κB* нетоксичним лековима

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САЖЕТАК

Увод/Циљ Велика разноликост у молекуларним механизмима регулације канцера омогућава да се неки плејотропни неонколошки нетоксични лекови, који су већ на тржишту, користе у онкологији, што смањује трајање и цену нових истраживања антиканцерских третмана. До данас, не постоје објављени резултати *in vivo* о антиканцерским ефектима одређених комбинација неонколошких плејотропних лекова (дисулфирам, метформин, деоксихолна киселина, мебендазол) који утичу на сигнализацију *MAPK/PI3K/AKT/mTOR/NF-κB*.

Методе Антиканцерски ефекти одређених комбинација наведених пренамењених лекова, дозе < 50% LD_{50} (еквивалентно уобичајеној дози за људе), процењени су кинетиком раста фибросаркома (мерено свакодневно *in vivo* помоћу калипера) и маркерима туморске пролиферације (*Ki*-67, *PCNA*), неоангиогенезе (*CD*34, *CD*31), метаболизма глукозе (*GLUT*1), метаболизма *NO* (*iNOS*) и апоптозе (*COX*4, цитохром *C*) код хрчака, који су насумично распоређени у контролне и експерименталне групе (шест животиња по групи). Животиње су жртноване 19 дана након инокулације тумора *BHK*-21/*C*13. Тумори су изрезани, измерени и прикупљена је крв. Урађене су биофизичке, патохистолошке, токсиколошке, хематолошке и биохемијске анализе.

Резултати Дисулфирам са метформином, дисулфирам са деоксихолном киселином и деоксихолна киселина са метформином су комбинације које су показале значајне антитуморске ефекте на кинетику раста фибросаркома и имунохистохемијске маркере тумора код хрчака ($p < 0,05$). Сви коришћени лекови у ефикасним комбинацијама могу инхибирати туморску сигнализацију *MAPK/PI3K/AKT/mTOR/NF-κB*. Додавање *NF-κB* стимулатора мебендазола ефикасним комбинацијама два лека сачувало је раст канцера, што указује да ови путеви могу бити одговорни за антитуморско деловање.

Закључак Комбинације неонколошких лекова: дисулфирам са метформином, дисулфирам са деоксихолном киселином и деоксихолна киселина са метформином имају потенцијал да се користе као ефикасна нетоксична помоћна антиканцерска терапија у онкологији.

Кључне речи: дисулфирам; деоксихолна киселина; метформин; хрчци; *BHK*-21/*C*13; фибросарком

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Acute kidney injury and necessity of renal replacement therapy in acutely intoxicated patients with rhabdomyolysis

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SUMMARY

Introduction/Objective This study aimed to analyse the characteristics of the selective parameters related to the development of acute kidney injury and the necessity of renal replacement therapy in patients with rhabdomyolysis due to acute intoxication with psychotropic and chemical substances in the first 24 hours.

Methods In a clinically controlled prospective study, 140 patients with rhabdomyolysis were divided into two groups depending on the intoxicating substance, i.e., psychotropic or chemical. Patients were selected according to predetermined inclusion and exclusion criteria.

Results Acute kidney injury occurred in 15% of 140 patients with rhabdomyolysis of whom 14 (66.7%) had psychotropic intoxication and seven (33.3%) had chemical intoxication. Statistical analysis showed significantly increased prevalence in the psychotropic group compared to those with chemical intoxication (p 0.0002). Creatine kinase values for median interquartile range in patients without/with renal replacement therapy were in psychotropic – 753 (446–753) *vs*. 42,670 (22,357–42,670) U/L; and chemical – 478.3 (321.5–1111.9) *vs*. 648.6 (495.6–2065) U/L. In psychotropic intoxications this difference was significant (p = 0.00002), while in the chemical ones it was insignificant (p = 0.2885). The renal replacement therapy was applied in 13 (9.3%) patients with rhabdomyolysis, nine of which (69.2%) were with psychotropic intoxication and four (30.8%) were with chemical intoxication.

Conclusion The prevalence of acute kidney injury and necessity for necessity for renal replacement therapy was significantly higher in psychotropic intoxication compared to chemical intoxication. The level of creatine kinase and myoglobin on the first day in the group with psychotropic substances, and high-sensitivity troponin I in both groups –psychotropic and chemical substances – are significantly higher in patients who need renal replacement therapy compared to those who do not need this therapy.

Keywords: toxicity; creatine kinase; myoglobin

INTRODUCTION

Rhabdomyolysis (RML) is a clinical syndrome resulting from the destruction of muscle fibers and the consequent release of intracellular constituents, such as myoglobin, creatine kinase (CK), and lactate dehydrogenase (LDH) into the bloodstream, which have the potential to cause local and systemic complications [1]. Common causes include crush injuries, heat injuries, toxins, and overexertion [2].

The most common life-threatening complication of RML is acute kidney injury (AKI). Some possible causes are direct tubular toxicity of myoglobin, vasoconstriction, formation of intra-tubular casts, and renal ischemia caused by low blood volume [3]. Myoglobin released from damaged muscles is a major renal injury factor deposited in renal tubules [4]. During muscle breakdown, excessive amounts of myoglobin are released, exceeding the renal threshold, leading to myoglobinuria and renal damage [4]. As an iron-containing protein, it has the ability to bind molecular oxygen, which may produce a hydroxyl radical in the oxidation of ferrous oxide (Fe_2+) to ferric oxide (Fe_3+) [5]. Nephrotoxic effects of myoglobin through free radical production and lipid peroxidation leading to renal vasoconstriction and oxidative damage to renal tubules also contribute to the development of AKI [6]. Metabolic acidosis and increased uric acid concentrations potentiate the nephrotoxic properties of myoglobin through its precipitation and interaction with Tamm-Horsfall protein to form casts in tubules [7]. Patients with AKI are classified into three clinical stages based on increase in creatinine and/or decrease in urine output, according to Kidney Disease Improving Global Outcomes (KDIGO) recommendations [8].

This study aimed to analyze the characteristics of the selective parameters related to the development of AKI and the necessity of renal replacement therapy in patients with RML due to acute intoxication with psychotropic and chemical substances in the first 24 hours.

METHODS

This was a prospective clinical study conducted during 2019 at the University Clinic for

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Toxicology in Skopje. The study included patients with RML divided into two groups, depending on the toxic substance consumed by them (psychotropic or chemical). RML was defined as a creatinine kinase (CK) > 250 U/L according to the Poisoning Severity Score. We included adult patients aged 18 years and older with RML. They had been acutely intoxicated with either psychotropic or chemical substances within the 48 hours prior to admission to the hospital. We excluded patients with pre-existing renal disease, chronic renal disease, muscle trauma as a result of a traumatic accident and patients with myocardial infarction. According to KDIGO criteria, the AKI was categorized as AKI I, II, or III based on the increase in serum creatinine ≥ 26.5 μmol/L or an increase to ≥ 1.5-fold to two-fold from baseline, > twofold to threefold from baseline, and > threefold from baseline or serum creatinine ≥ 354 μmol/L [8]. Individuals who receive renal replacement therapy were considered to have met the criteria of AKI III regardless of their serum creatinine value.

Patients' informed consent was obtained prior to their inclusion in the study.

The study was approved by the Ethics Commission of the Faculty of Medicine, Ss. Cyril and Methodius at the University of Skopje, Republic of Northern Macedonia (Ethics Code: 03-1864/4; dated 19.04.2019).

Statistical analysis

The data obtained in the study were analyzed using IBM SPSS Statistics, Version 22.0 (IBM Corp., Armonk, NY, USA). The quantitative data were analyzed in series, using central tendency (mean and median) and dispersion measures (standard deviation and interquartile range – IQR). Fisher's exact test was used to determine the association among certain features in the group of subjects. Mann–Whitney U test was used to compare the average values, according to distribution. Values of $p < 0.05$ were considered statistically significant. The binary logistic regression was used to identify the predicative parameters for developing AKI.

RESULTS

A total of 1446 patients diagnosed with acute intoxications received treatment during the study period at the University Clinic for Toxicology in Skopje, Republic of North Macedonia. Among them, 140 patients developed RML. Ninety-six (68.6%) patients with RML were poisoned with psychotropic drugs, while the remaining 44 individuals (31.4%) ingested chemical agents. Intoxications involving psychotropic substances were significantly more common than those involving chemical substances.

Among patients with RML, a total of 21 (15%) had AKI, with 14 (66.7%) resulting from psychotropic intoxication and seven (33.3%) from chemical intoxication. The analysis revealed a significantly higher prevalence of AKI in psychotropic intoxications compared to chemical intoxications (difference 33.4% [(15.6–48.2) 95% CI]; $\chi^2 = 13.552$; df = 1; p = 0.0002).

In the group with psychotropic intoxications and AKI, 13 (92.8%) were male and one (7.14%) was female, while in the group with chemical intoxications, the distribution was three (42.86%) male and 4 (57.1%) female. The average age of patients with RML and AKI in the psychotropic intoxication group was 39.9 ± 13.4, with a range of 26–53 years, compared to 57.8 ± 15.1 years, with a range of 41–82 years in the chemical intoxication group. Median IQR age distribution indicated that 50% of patients in the psychotropic intoxication group were under 40 years old [median IQR = 40 (36–47)], while in the chemical intoxication group, 50% were under 54 years old [median IQR = 54 (52–65)]. There was a significantly older patient population in the group with chemical intoxications (Mann–Whitney U Test: Z = -3.0597; p = 0.002221*) (Table 1).

We individually analyzed the etiological factors for AKI, considering the prevalence of psychotropic and chemical parameters (Table 2). In psychotropic intoxications, AKI occurred in 14 (14.6%) patients, with the highest prevalence found in the following: a) heroin three (60%); b) methadone six (40%); c) neuroleptics three (25%); d) anticonvulsants one (17.7%); and e) antidepressants one (8.3%). In chemical intoxications, AKI was reported in seven (15.9%) patients, with the highest prevalence in ethylene glycol (1; 100%) and herbicides (1; 33.3%), followed by insecticides (3; 20%) and corrosives (2; 16.7%).

With stage I acute renal injury there were two (9.53%) patients, stage II had six (28.57%) patients, and stage III consisted of 13 (61.90%) patients in need of renal replacement therapy. A total of 13 (9.3%) patients with RML received renal replacement therapy, of which nine (69.2%) had psychotropic intoxication and four (30.8%) had chemical intoxication. The analysis showed a significantly higher prevalence of renal replacement therapy (RRT) in psychotropics compared to chemical intoxications (difference 38.4% [(20.7–52.7) 95% CI]; $\chi^2 = 18,036$; df = 1; p = 0.0001). Out of 21 patients diagnosed with AKI, 13 (61.9%) received RRT, while eight (38.1%) did not require this therapy.

In the group of psychotropic intoxications, RRT was applied in 9 (9.4%) patients. The prevalence of RRT according to etiological cause was the highest in the following: a) heroin two (40%); and b) methadone four (26.7%); followed by c) neuroleptics three (25%). In chemical intoxications, RRT was applied in four (9.1%) patients. The prevalence of RRT according to etiological cause was highest in ethylene glycol (1; 100%) and herbicides (1; 33.3%), followed by insecticides (1; 6.7%) and corrosives (1; 8.3%).

We analyzed the association of AKI with selected parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), troponin, and myoglobin obtained on the first day of hospitalization of patients with RML (Table 3).

We found that patients with AKI from the whole sample with RML as well as those in the psychotropic intoxications group had significantly higher values for all selected parameters compared to those without AKI. However, the whole sample with RML had insignificantly higher values for Na (p = 0.89) (Table 3). Regarding the group with

Table 1. Demographic characteristics of the study population

Parameter		Total
Type of intoxication		
Psychotropic	n (%)	14 (66.67)
Chemical	n (%)	7 (33.33)
Difference test: Difference 33.34% df 1; p 0.0001*		
Sex		
Psychotropic	Men n (%)	13 (92.86)
	Women n (%)	1 (7.14)
Chemical	Men n (%)	3 (42.86)
	Women n (%)	4 (57.14)
Pearson's χ^2 test 6.4312 df 1; p 0.011213*		
Age		
Psychotropic	$\bar{X} \pm SD$	39.93 ± 13.41
	Min/Max	26/53
	Median (IQR)	40 (36–47)
Chemical	$\bar{X} \pm SD$	57.86 ± 15.18
	Min/Max	41/82
	Median (IQR)	54 (52–65)
Mann–Whitney U Test: Z -3.0597; p 0.002221*		

*Significant for $p < 0.05$

Table 2. Etiological agent of acute kidney injury (AKI) and renal replacement therapy (RRT) in patients with rhabdomyolysis

Etiological agents		Total	AKI		RRT	
		N	N	%	N	%
1	Benzodiazepines	20	0	0	0	0
2	Neuroleptics	12	3	25	3	25
3	Anticonvulsants	6	1	16.7	0	0
4	Antidepressants	12	1	8.3	0	0
5	Antiparkinsonic	2	0	0	0	0
6	Heroin	5	3	60	2	40
7	Methadon	15	6	40	4	26
8	Amfetamines	4	0	0	0	0
9	Cocain	1	0	0	0	0
10	Tramadol	3	0	0	0	0
11	Ethyl alcohol	15	0	0	0	0
12	Canabis	1	0	0	0	0
13	Other	1	0	0	0	0
14	Calcium-channel inhibitor	2	0	0	0	0
15	Herbicides	3	1	33.3	1	33.3
16	CO	7	0	0	0	0
17	Other gases	1	0	0	0	0
18	Gasoline	2	0	0	0	0
19	Ehylen glycol	1	1	100	1	100
20	Insecticides	15	3	20	1	6.67
21	Corrosive agents	12	2	16.7	1	8.3
	Total	140	21	15	13	9.3

chemical intoxications, we found that patients with AKI had insignificantly higher values for the following parameters: Na ($p = 0.311$), K ($p = 0.22$), Ca ($p = 0.25$), AST ($p = 0.3277$), ALT ($p = 0.9616$), and high-sensitivity troponin I ($p = 0.0051$) compared to those without AKI. For the other parameters, the values observed in patients with AKI in this group were insignificantly higher compared to those without AKI for CK ($p = 0.8348$) and myoglobin ($p = 0.1127$) (Table 3).

In the whole sample as well as individually in the groups with psychotropic or chemical intoxication, we found that the level of CK on the first day was higher in patients with RRT compared to those without this therapy (Table 4). CK values for median IQR in patients without/with RRT were as follows: a) whole sample – 634 (339.6–1532) *vs.* 22,357 (3350–42,670) U/L; b) psychotropic – 753 (446–753) *vs.* 42,670 (22,357–42,670) U/L; and c) chemical – 478.3 (321.5–1111.9) *vs.* 648.6 (495.6–2065) U/L. In the whole sample and in psychotropic intoxications, this difference was significant ($p = 0.00004$ *vs.* $p = 0.00002$), while in the chemical ones it was insignificant ($p = 0.2885$).

The value of myoglobin on the first day in the whole sample as well as individually in the groups with psychotropic or chemical intoxications was higher in patients with RRT compared to those without this therapy (Table 4). Myoglobin values for median IQR in patients without/with RRT were as follows: a) whole sample – 155.3 (126.8–425.2) *vs.* 1018.5 (604.3–3741.5); b) psychotropic – 186.2 (12.7–568.4) *vs.* 1308.5 (1018.5–6421.5); and c) chemical – 140.7 (126.8–291.1) *vs.* 454.5 (227.4–604.3). In the whole sample and in psychotropic intoxications group, this difference was significant ($p = 0.00002$ *vs.* $p = 0.00003$), while in the chemical intoxications group it was insignificant ($p \neg 0.1081$). The value of troponin on the first day in the whole sample as well as individually in the groups with psychotropic or chemical intoxications was higher in patients with RRT compared to those without RRT (Table 4). High-sensitivity troponin I values for median IQR in patients without/with RRT were as follows: a) whole sample – 3.1 (1.3–6.9) *vs.* 83.9 (14.1–111.1); b) psychotropic – 2.3 (1.3–6.8) *vs.* 73.4 (19.9–121.3); and c) chemical – 3.7 (2.2–8.2) *vs.* 94.5 (5.2–101). In all, this difference was significant for the consequent $p = 0.00004$ *vs.* $p = 0.00003$ *vs.* $p = 0.0481$.

The results of Fisher's exact test ($p = 0.018$) indicate a significant association between AKI and the used substance in poisoning (Table 5). The adjusted residuals showed a significantly higher presentation of AKI in poisoning with heroin, methadone in the psychotropic group, and ethylene glycol in the chemical group and significantly lower presentation in poisoning with benzodiazepines in the psychotropic group than expected.

The adjusted residuals were used as a parameter to present the significance of the difference between the AKI+ and AKI- groups for each type of poisoning.

A logistic regression was performed to ascertain the effects of age, sex, group of substances, creatinine, and creatine phosphokinase (CPK) on the likelihood that participants have AKI. The logistic regression model was statistically significant, $\chi^2(5) = 83.389$, $p < 0.0001$ (Table 6). The model explained 78% (Nagelkerke R^2) of the variance in AKI and correctly classified (percentage accuracy in classification) 95.7% of cases, with PPV being 94.2% and NPV 95.9%. Only creatinine was a significant predictor of the likelihood that participants had AKI. The increase in creatinine for one unit was 1.05 times more likely to exhibit AKI with 95% CI 1.016–1.083. Increasing CPK, age, use of psychotropic drugs, and male sex insignificantly increased the likelihood of exhibiting AKI.

Table 3. Acute kidney injury and laboratory parameters according to the type of intoxication

Parametar			N	Average (Mean)	Standard deviation	Percentiles			p
						25th	50th (median)	75th	
BUN	Psychoactive								
	AKI	no	82	4.9	3	3.3	4.5	5.5	Z -5.555;
		yes	14	18.1	10.4	9.9	14.1	25.3	p > 0.001*
	Chemical								
	AKI	no	37	5.9	2.5	4.1	5.4	6.8	Z - 2.087;
		yes	7	10.3	5.9	5.9	7.3	16.1	p 0.037*
	Total								
	AKI	no	119	5.2	2.9	3.5	4.7	5.8	Z -5.906;
		yes	21	15.5	9.7	7	12.6	22.7	p > 0.01*
Creatinine	psychoactive								
	AKI	no	82	81	18.9	65.8	78	91.6	Z -5.949;
		yes	14	332.7	255.4	209.8	277.1	359.3	p > 0.001*
	chemical								
	AKI	no	37	86.9	23.4	73.8	83	105.1	Z -2.070;
		yes	7	170.6	103.7	69	145.8	279	p 0.038*
	Total								
	AKI	no	119	82.8	20.5	67	79	96	Z -6.143;
		yes	21	278.6	227.5	143.4	272.9	333.9	p > 0.01*
Na	Psychoactive								
	AKI	no	82	137.5	4.3	136	138	139.6	Z -2.915;
		yes	14	131.1	7.6	125	131.5	139	p 0.004*
	Chemical								
	AKI	no	37	137.8	3.5	136	137	140.1	z -1.012;
		yes	7	141.6	8.5	134.6	140	144	p 0311
	Total								
	AKI	no	119	137.6	4.1	136	138	139.8	Z -1.699;
		yes	21	134.6	9.2	129	134.6	139.6	p 0.89
K	Psychoactive								
	AKI	no	82	4	0.7	3.6	3.9	4.5	Z - 3.630;
		yes	14	5.4	1.2	4.5	6	6.3	p > 0.001*
	Chemical								
	AKI	no	37	4.5	1	3.9	4.1	4.7	Z -1.220;
		yes	7	4.5	0.8	4.2	4.7	5.1	p 0.22
	Total								
	AKI	no	119	4.2	0.8	3.7	4	4.6	Z -3.588;
		yes	21	5.1	1.1	4.2	5	6.1	p > 0.01*
Ca	Psychoactive								
	AKI	no	82	2.3	0.3	2.2	2.2	2.4	Z - 2.760;
		yes	14	2.1	0.2	2	2.1	2.2	p 0.006*
	Chemical								
	AKI	no	37	2.32	0.27	2.150	2.400	2.485	Z -1.141;
		yes	7	2.26	0.17	2.100	2.290	2.390	P 0.25
	Total								
	AKI	no	119	2.3	0.3	2.2	2.3	2.4	Z 4.277;
		yes	21	2.1	0.2	2.1	2.1	2.3	P 0.008*

AST	Psychoactive								
	AKI	no	82	76.9	140.5	24	36.5	59.3	Z 5.6576;
		yes	14	990.9	669.2	733	822	1171	p 0.00001*
	Chemical								
	AKI	no	37	81.9	161.3	25.5	34	64.1	Z 0.9787;
		yes	7	344.5	556.8	29	38.7	804.6	p 0.3277
	Total								
	AKI	no	119	78.4	146.6	25	36	61.8	Z 5.1646;
		yes	21	775.4	694	79	778.6	1052	p 0.00001*
ALT	Psychoactive								
	AKI	no	82	56.3	162.1	17	24.6	38	Z 5.5901;
		yes	14	734.1	952.4	182	353	632	p 0.00001*
	Chemical								
	AKI	no	37	68.6	116.1	21	26	54	Z 0.0481;
		yes	7	813.1	1950.3	15	26	294.1	p 0.9616
	Total								
	AKI	no	119	60.1	149	17.4	25	41.6	Z 4.6861;
		yes	21	760.5	1316.1	99	294.1	533.3	p 0.00001*
CK	Psychoactive								
	AKI	no	82	1850.1	3186.8	338.9	709.5	1701	Z 5.6368;
		yes	14	38522.1	34806.9	15146.5	34227.2	42670	p 0.00001*
	Chemical								
	AKI	no	37	2782.7	8541.1	339.6	491.1	1119.5	Z 0.2086;
		yes	7	891.6	1105	465.5	517.2	780	p 0.8348
	Total								
	AKI	no	119	2140.1	5423.6	338.9	633	1492	Z 4.2747;
		yes	21	25978.6	33440.5	780	15146.5	42670	p 0.00002*
hs-troponin I	Psychoactive								
	AKI	no	78	15.3	59.2	1.3	2.2	5.8	Z 4.3535;
		yes	14	121.2	165.1	8.3	62.5	121.3	p 0.00001*
	Chemical								
	AKI	no	35	12.4	26.1	1.4	3.6	7.2	Z 2.8014;
		yes	5	65.6	39.8	46.4	81	94.5	p 0.0051*
	Total								
	AKI	no	113	14.4	51.2	1.3	2.7	6.3	Z 5.1180;
		yes	19	106.6	143.7	8.3	67	101	p 0.00001*
MyogJobin	Psychoactive								
	AKI	no	80	754.2	1565.4	123	160.5	539.9	Z 4.3098;
		yes	13	2461	2549.3	1003	1336.9	1972	p 0.00002*
	Chemical								
	AKI	no	35	257.3	280	126.8	138.9	291.1	Z 1.5826;
		yes	7	323.5	208.1	142.4	314	586.6	p 0.1127
	Total								
	AKI	no	115	603	1332	123.3	154	376.6	Z 4.2768;
		yes	20	1712.9	2283.1	318.3	1001.5	1408	p 0.00002*

AKI – acute kidney injury; AST – aspartate aminotransferase; ALT – alanine aminotransferase; CK – creatine kinase; hs-troponin I – high-sensitivity troponin I; BUN – blood urea nitrogen;
Mann–Whitney U test Z;
*significant for $p < 0.05$

DISCUSSION

The most serious complication of RML is AKI, which in our analysis is present in 15% of patients with acute intoxication. This is in accordance with a previously published study which indicates a prevalence of AKI of 5–30% in patients with RML [9].

In a study by Mousavi et al. [10], the prevalence of AKI was 15% of 114 patients acutely intoxicated with RML, which is compatible with our results. The prevalence of AKI in acute intoxication with RML was 37.1% in the retrospective study by Rogliano et al. [11]. In patients with RML acutely intoxicated, the prevalence of AKI was 16.8%, according to a group of authors [12]. Possible explanations for this discrepancy are methodological differences.

The prevalence of AKI is significantly higher in psychotropic compared to chemical intoxications. AKI in patients intoxicated with psychotropic substances is registered in overdose with heroin – 60%, methadone – 40%, followed by antipsychotics – 25%, anticonvulsants – 17.7%, and antidepressants – 8.3%. In chemical intoxications, AKI is registered in 15.9% of patients. The prevalence of AKI is the

Table 4. Renal replacement therapy and selected parameters by type of intoxication

Type of intoxication			Renal replacement therapy		p
			No – N (%)	Yes – N (%)	
Psychotropic		N (%)	87 (90.63)	9 (9.38)	Fisher's exact test: p 0.9571
Chemical		N (%)	40 (90.91)	4 (9.09)	
Total		N (%)	127 (90.91)	13 (9.29)	
CK	Psychotropic	$\bar{X} \pm SD$	3413 ± 10726	43786 ± 34398.2	Z -4.7325; p 0.00002*
		Min/Max	51/93950	10776/22357	
		Median (IQR)	753 (446–753)	42670 (22357–42670)	
	Chemical	$\bar{X} \pm SD$	2602.1 ± 8231.4	1280.3 ± 1386.4	Z -1.0614; p 0.2885
		Min/Max	65.4/45404	474/3350	
		Median (IQR)	478.3 (321.5–1111.9)	648.6 (495.6–2065)	
	Total	$\bar{X} \pm SD$	3157.7 ± 9981.9	30707.3 ± 34731	Z -4.1176; p 0.00004*
		Min/Max	51/9395	474/129077	
		Median (IQR)	634 (339.6–1532)	22357 (3350–42670)	
Myoglobin	Psychotropic	$\bar{X} \pm SD$	778.9 ± 1527.7	3265.9 ± 2996.8	Z -3.6583; p 0.0002*
		Min/Max	54.3/7213	954/7676	
		Median (IQR)	186.2 (12.7–568.4)	1308.5 (1018.5–421.5)	
	Chemical	$\bar{X} \pm SD$	252.8 ± 269.8	415.8 ± 231.5	Z -1,6068; p 0,1081
		Min/Max	82.7/1467	132.2/622	
		Median (IQR)	140.7 (126.8–291.1)	454.5 (227.4–604.3)	
	Total	$\bar{X} \pm SD$	616.3 ± 1299.5	2315.9 ± 2774.7	Z -3.8078; p 0.0001*
		Min/Max	54.3/7213	132.2/7676	
		Median (IQR)	155.3 (126.8–425.2)	1018.5 (604.3–3741.5)	
hs-troponin I	Psychotropic	$\bar{X} \pm SD$	21.7 ± 79.5	122.4 ± 139.8	Z -3.614; p 0.0003*
		Min/Max	0.3/515	1.5/299.1	
		Median (IQR)	2.3 (1.3–6.8)	73.4 (19.9– 121.3)	
	Chemical	$\bar{X} \pm SD$	15.2 ± 28.3	66.9 ± 53.5	Z -1.977; p 0.0481*
		Min/Max	0.6/138.4	5.2/101	
		Median (IQR)	3.7 (2.2–8.2)	94.5 (5.2–101)	
	Total	$\bar{X} \pm SD$	19.6 ± 67.9	108.5 ± 123.9	Z -4.1003; p 0.00004*
		Min/Max	0.3/515	1.5/399.1	
		Median (IQR)	3.1 (1.3–6.9)	83.9 (14.1–111.1)	

hs-troponin I – high-sensitivity troponin I;
Mann–Whitney U test Z;
*significant for $p < 0.05$

highest in ethylene glycol (100%) and herbicides (33.3%), followed by insecticides (20%), and corrosives (16.7%).

The most common cause of AKI in patients with RML acutely intoxicated is opioid overdose, according to one study by a group of authors [12]. These observations are in line with ours. We found significantly higher presentation of AKI in poisoning with heroin, methadone, in the psychotropic group. According to a group of authors, AKI is associated with a higher rate of opioid and cocaine use in patients with RML [13]. Rogliano et al. [11] reported that overdoses with beta-blockers, calcium-channel inhibitors, acetaminophen, colchicine, lithium, angiotensin-converting enzyme inhibitors / angiotensin II-receptor-blockers were significantly associated with an increased risk of AKI in poisoned patients with RML.

RML is not the only cause of AKI in acutely intoxicated patients, unlike RML resulting from trauma. According to our analysis, patients intoxicated with chemicals who developed AKI were in the group with mild to moderate RML depending on the CK value. AKI in intoxications with ethylene glycol, insecticides, and concentrated acetic acid is due to their nephrotoxic action. Metabolites in ethylene glycol poisoning such as oxalic acid are responsible for the associated end-organ injury, nephrotoxicity. Oxalic acid deposits in renal tubules as insoluble calcium oxalate monohydrate, leading to proximal tubular necrosis. The exact mechanism in organophosphate poisoning is unknown but it may be multifactorial, including direct renal toxicity, or secondary to dehydration/hemodynamic instability causing renal hypoperfusion, or seizure and muscular fasciculation-related RML [14]. Coma, shock, hemolysis, and anuric kidney injury have been reported with poisoning with acetic acid [15].

The results indicate that certain toxic agents in acutely intoxicated patients with RML may play an important role in the development of AKI. We found that patients with AKI acutely intoxicated with RML as well as those intoxicated with psychotropic substances had significantly higher values for creatine, blood urea nitrogen, Na, K, Ca, CK, AST, ALT, troponin, and myoglobin compared with those without AKI. Compatible findings of our analysis are found in the study by Mousavi et al. [10], for a significant positive correlation between serum creatinine values and CK values. Regarding the increased risk of developing

Table 5. Etiological agents in poisoned patients who developed rhabdomyolysis with vs. without acute kidney injury (AKI)

Agents	AKI No		AKI Yes		Adjusted residuals
	N	%	N	%	
Benzodiazepine	20	16.81	0	0	-2*
Neuroleptics	9	7.56	3	14.29	1
Anticonvulsants	5	4.2	1	4.76	0.1
Antidepressants	11	9.24	1	4.76	-0.7
Antiparkinsonic	2	1.68	0	0	-0.6
Heroin	2	1.68	3	14.29	2.9*
Methadone	9	7.56	6	28.57	2.9*
Amphetamine	4	3.36	0	0	-0.9
Cocaine	1	0.84	0	0	-0.4
Tramadol	3	2.52	0	0	-0.7
Ethyl alcohol	15	12.61	0	0	-1.7
Cannabis	1	0.84	0	0	-0.4
Other	1	0.84	0	0	-0.4
Calcium-channel inhibitor	2	1.68	0	0	-0.6
herbicides	2	1.68	1	4.76	0.9
CO	7	5.88	0	0	-1.1
Other gases	1	0.84	0	0	-0.4
Gasoline	2	1.68	0	0	-0.6
Ethylene glycol	0	0	1	4.76	2.4*
Insecticides	12	10.08	3	14.29	0.6
Corrosive agents	10	8.4	2	9.52	0.2
Total	119	100	21	100	-

Table 6. Predictive parameters for developing acute kidney injury in patients with rhabdomyolysis

Parameters	B	S.E.	Sig.	OR	95% CI for OR	
					Lower	Upper
Creatinine	0.048	0.016	0.003	1.049	1.016	1.083
CPK	0.000	0.000	0.180	1.000	1.000	1.000
Sex	-0.993	1.196	0.406	0.371	0.036	3.859
Age	0.046	0.038	0.230	1.047	0.971	1.128
Substance (P/Ch)	-1.043	0.970	0.282	0.352	0.053	2.360
Constant	-8.669	2.983	0.004	0.000		

CPK – creatine phosphokinase

complications such as AKI, similar findings were found in the study by Pajoum et al. [16]. Eizadi-Mood et al. [17] in the prospective study indicated that a CK value > 10,000 IU/L was associated with a higher complication rate and could be an acceptable predictor of outcome in intoxicated patients. In a retrospective study by Nielsen et al. [18], on patients with RML, elevated initial CK values were associated with an increased risk of AKI. In trauma patients, admission myoglobin better predicted AKI than admission CK [19]. Regarding the group with chemical intoxications, we found that patients with AKI have insignificantly higher values for AST, ALT, and troponin compared to those without AKI. CK and myoglobin values in this group were insignificantly higher in patients with AKI compared to those without AKI. We found that serum creatinine on admission in both groups is a predictor of AKI. According to the study by Rogliano et al. [11], serum creatinine ≥ 125 µmol/L on admission was the highest predictive variable for AKI in poisonings.

Recommendations to lower the risk of AKI in patients with RML include fluids to correct hypovolemia, achieve adequate diuresis with a goal urine output of 300 mL/h, and even dilute the released toxic endogenous metabolites, despite their relatively low level of evidence [2]. Bicarbonate, mannitol, and loop diuretics are not strong evidence for improved outcomes [2].

The need for RRT in our analysis in patients acutely intoxicated with RML is 9.3%. Intermittent RRT was used in our patients with RML due to acute intoxication with psychotropic and chemical substances. RRT was initiated two or three days after admission and our patients required one hemodialysis session.

According to the literature, the need for RRT in patients with RML ranges 4–20% [20]. The prevalence of RRT is significantly higher in psychotropic compared to chemical intoxications. In acutely intoxicated patients with RML and in the group with psychotropic intoxications, the level of CK on the first day is significantly higher in patients who need RRT compared to those who do not need this therapy, and in the group of chemical intoxications, this difference is insignificant. Eizadi-Mood et al. [17] in their study reported that in acutely intoxicated patients with coma increased CK values were associated with an increased need for dialysis. According to a study by Dadpour et al. [21], approximately 80% of patients with serum CK levels < 10,000 IU/L required dialysis. In contrast to our results, the study by Pajoum et al. [16] presents that there is no significant correlation between CK levels and the need for dialysis. Stopping RRT depends on multiple factors: resolution of the underlying cause, creatine level and the option of being managed effectively using other therapies (e.g., furosemide for fluid balance) [22]. In most patients with RML, renal function is restored within a few months [23, 24].

The limitation of the study is the small number of patients with RML as a result of acute intoxication. The small number of patients with AKI in most of the different types of poisoning limited the possibility of performing standard *post hoc* test for each of the used substances for poisoning. Urine output, which is an important clinical parameter, is missing.

CONCLUSION

The prevalence of AKI and the necessity of RRT was significantly higher in psychotropic intoxication compared to chemical intoxication. Certain toxic agents in acutely intoxicated patients with RML may have an important role in the development of AKI. Serum creatinine on admission in acute intoxication is a predictor of AKI. In the group of psychotropic intoxications, RRT was used in overdoses with heroin, methadone, and antipsychotics, while in the chemical group it was used in those intoxicated with ethylene glycol, herbicides, insecticides, and corrosive agents. The level of high-sensitivity troponin I in both psychotropic and chemical group are significantly higher in patients who need RRT compared to those who do not need this therapy. Larger cohorts are needed to improve our findings.

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Акутно оштећење бубрега и неопходност терапије замене функције бубрега код акутно интоксираних болесника са рабдомиолизом

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САЖЕТАК

Увод/Циљ Ово истраживање је имало за циљ да анализира карактеристике селективних параметара повезаних са настанком акутног оштећења бубрега и неопходношћу терапије замене функције бубрега код болесника са рабдомиолизом услед акутне интоксикације психотропним и хемијским супстанцама у прва 24 часа.

Методе У клинички контролисаној проспективној студији, 140 болесника са рабдомиолизом подељено је у две групе у зависности од супстанце која је изазвала интоксикације – психотропне или хемијске. Болесници су одабрани према унапред одређеним критеријумима за укључивање и искључивање.

Резултати Акутно оштећење бубрега јавило се код 15% од 140 болесника са рабдомиолизом, од којих је 14 (66,7%) имало психотропну интоксикацију, а седам (33,3%) хемијску интоксикацију. Статистичка анализа је показала значајно већу преваленцију у групи са психотропном интоксикацијом у односу на ону са хемијском интоксикацијом ($p = 0,0002$). Вредности креатин киназе за средњу вредност интерквартилног распона код болесника без терапије и са терапијом замене функције бубрега биле су у случају психотропних супстанци 753 (446–753) наспрам 42.670 (22.357–42.670) *U/L*, док су за хемијске супстанце износиле 478,3 (321,5–1111,9) наспрам 648,6 (495,6–2065) *U/L*. Код психотропне интоксикације ова разлика је била значајна ($p = 0,00002$), док је код хемијске незнатна ($p = 0,288$). Терапија замене функције бубрега је спроведена код 13 (9,3%) болесника са рабдомиолизом, од којих је девет (69,2%) било са психотропном интоксикацијом, а четири (30,8%) болесника са хемијском интоксикацијом.

Закључак Преваленција акутног оштећења бубрега и потреба за терапијом замене функције бубрега биле су значајно веће код психотропне интоксизације у поређењу са хемијском интоксикацијом. Ниво креатин киназе и миоглобина првог дана код болесника са психотропном интоксикацијом, и високо осетљиви тропонин I у обе групе болесника – са психотропном и хемијском интоксикацијом – значајно су већи код болесника којима је неопходна терапија замене функције бубрега у односу на оне којима ова терапија није потребна.

Кључне речи: токсичност; креатин киназа; миоглобин

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Relationship between serum AGE precursor levels, oxidative stress, and quality of life in patients receiving hemodialysis

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SUMMARY

Introduction/Objective The aim of this study was to determine the relationship between serum advanced glycation end product (AGE) precursors, oxidative stress levels, and quality of life in hemodialysis patients.

Methods A descriptive form and the Kidney Disease Quality of Life Form (KDQOL-36) were used in the study. Serum levels of AGE precursors [methylglyoxal (MGO) and glyoxal (GO)] and oxidative stress [malondialdehyde (MDA)] were determined in blood samples taken from the patients.

Results The KDQOL-36 subscale scores were 71.65 ± 17.76 for the symptoms/problems list, 66.35 ± 19.06 for the effect of kidney disease, 40.6 ± 24.01 for the kidney disease burden, 41.6 ± 9.83 SF-12 for physical health, and 37.83 ± 9.69 for SF-12 mental health. The serum levels were 3.96 ± 1.01 μmol/L for MDA, 1029.87 ± 314.43 ng/mL for GO, and 115.2 ± 75.54 ng/mL for MGO. A positive and significant correlation was detected between serum MGO and GO ($r = .285$, $p < 0.01$) and MDA ($r = 0.284$, $p < 0.01$). A positive correlation was noted between serum MDA and GO ($r = 1{,}000$, $p < 0.05$) and a negative correlation with kidney disease burden ($r = -0.205$, $p < 0.05$). A negative and significant correlation was detected between GO and kidney disease burden ($r = -0.204$, $p < 0.05$).

Conclusion Serum MGO, GO, and MDA levels were high in patients undergoing hemodialysis. High serum MDA levels are associated with high serum GO and MGO levels. High serum levels of MDA and GO had a negative impact on the quality of life of hemodialysis patients.

Keywords: hemodialysis; oxidative stress; serum advanced glycation end product precursor; quality of life

INTRODUCTION

Hemodialysis (HD) is a medical procedure used to treat end-stage renal disease (ESRD) by removing excess waste products, electrolytes, and fluids from the blood. This helps to maintain the electrolyte balance and acid-base status in the body and can also assist in regulating blood pressure. HD is one of the primary forms of renal replacement therapy for individuals with ESRD, along with peritoneal dialysis and kidney transplantation [1]. One consequence of renal insufficiency is a gradual increase in the levels of advanced glycation end-products (AGE), and renal functions decrease in proportion to this increase. The kidneys have an important role in AGE metabolism. As AGE levels increase in the plasma, the glomerular filtration rate decreases, thereby exacerbating the increase in AGE. The precursors of the most reactive AGE are glyoxal (GO) and methylglyoxal (MGO) [2].

Patients with HD are subjected to dietary recommendations (fruit and vegetable restrictions) to prevent the risk of hyperkalemia and the high prevalence of malnutrition. However, the recommended diet for HD patients impairs antioxidant defense mechanisms and increases oxidative stress by disrupting the rate of production and destruction of reactive oxygen species (ROS). In addition, every HD session causes more losses in antioxidant molecules (e.g., vitamins and trace elements), thereby suppressing the removal of ROS [3]. At excessive levels, ROS interact with many biomolecules, such as proteins, lipids, and nucleic acids, and may cause cellular damage, leading to negative effects on tissue function and structure [4]. The HD procedure itself is also known to trigger pro-oxidant mechanisms [5]. The presence of several interconnected factors, including oxidative stress, loss of important antioxidants, and chronic inflammation, are all critical factors that collectively contribute to a higher risk of cardiovascular disease and mortality in HD patients [6]. Consequently, AGE and oxidative stress levels, which cause many other known diseases, also have particularly adverse effects in HD patients [6].

HD treatment is a long-term therapy that can affect the quality of life of patients with ESRD [7]. The HD treatment guidelines highlight the importance of quality of life as a key outcome and recommend assessing quality of life via repetitive measures as a parameter for monitoring the quality of care given to HD

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patients [8]. Determination of the quality of life can help healthcare professionals assess the well-being of their patients and make decisions about the health care for those patients (e.g., new treatments and interventions) [7, 8]. In the current study, the relationships between serum AGE precursors, oxidative stress levels (i.e., MDA levels), and quality of life were investigated in HD patients.

METHODS

Study type

In this study, the aim was to assess whether a correlation exists between the serum levels of AGE precursors and MDA, as markers of oxidative stress and inflammation, and quality of life among individuals undergoing HD.

Location and time of study

The study was conducted in April 2021 with patients who were treated at two HD centers in Istanbul after obtaining the approval of the University Ethics Committee.

Population and sample of the participants in the study

A total of 170 patients who were treated at HD centers at the time of the study constituted the study population. Data were collected from 117 (68% participation) patients who met the following inclusion criteria: absence of communication problems (hearing, language, understanding, etc.), willingness to participate in the study, being 18 years old and over, and possessing the cognitive ability to answer the questions in the data collection tools. The G-Power 3.1.9.4 (Axel Buchner, Edgar Erdfelder, Franz Faul, Albert-Georg Lang; Heinrich Heine University Düsseldorf, Düsseldorf, Germany) program was used for *post hoc* power analysis to confirm that the sample size was sufficient (the effect size was 0.3 and the power was 0.9 at the 95% confidence interval, at a significance level of 0.05).

Data collection tools

Personal data form

Questions about sex, age, marital status, educational status, occupation, income level, and disease (duration of the disease, duration of HD, etc.) were prepared by the researcher to determine the socio-demographic and disease-related characteristics of the participating patients.

Kidney disease Quality of Life Scale (KDQOL-36)

This tool was developed to measure the quality of life of individuals with chronic kidney disease (CKD) who receive dialysis treatment. It provides an overall measure of the health status and outcomes of patients receiving dialysis treatment from their own perspectives. The KDQOL-36 questionnaire consists of 36 statements with five subscales. Two of the subscales measure the overall quality of life, while the other three measure the quality of life specific to patients with kidney disease. The general quality of life was measured using a short form of the general quality of life scale known as the SF-12. The subscales of the quality of life specific to kidney disease estimate the burden of kidney disease, the symptoms/problems of kidney disease, and the effects of kidney disease. Each dimension was scored according to the answers given to the related statements. Scores can be a minimum of 0 and a maximum of 100. A total score of 0 indicates the worst quality of life, while a total score of 100 indicates the best quality of life.

Measurement of serum AGE precursors

Blood samples were taken from the patients, and the levels of the AGE precursors MGO and GO were measured. Venous blood was collected in yellow-cap gel tubes and EDTA test tubes, and the plasma was separated by centrifugation at 3000 rpm for 30 minutes at 4°C. The plasma was aliquoted into polypropylene tubes and stored in a -80°C freezer until analysis.

Chemicals for AGE precursor analysis, including tetraethoxypropane, trichloroacetic acid (TCA), thiobarbituric acid (TBA), ethanol, GO (40%), MGO (40%), methanol, sodium acetate, 4-nitro-1,2-phenlenediamine, and acetonitrile, were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Previously described extraction and high-performance liquid chromatography (HPLC) methods were used, with small modifications, for GO and MGO analysis in plasma samples. In brief, 0.5 mL of plasma sample was placed in a 10 mL glass tube, and 2 mL of TCA (10%) solution was added, followed by centrifugation at 8000 rpm for 5 minutes. A 1 mL aliquot of the supernatant was then removed and combined with 1 mL sodium acetate buffer (0.1 M, pH: 3) and 0.5 mL derivatization solution (4-nitro-1,2-phenlenediamine in 1% methanol). This mixture was incubated in a water bath for 20 minutes at 70°C and then passed through a cellulose acetate filter (0.45 μm) for injection into the HPLC.

The HPLC system consisted of a Shimadzu LC20AT pump and a Shimadzu SPD-20A UV/VIS detector (Shimadzu Corporation, Kyoto, Japan). The mobile phase consisted of a mixture of methanol, water, and acetonitrile (42:56:2, v/v/v). The detector wavelength was set at 254 nm. An Inertsil ODS-3 (250 × 4.6 mm, 5 μm) column was used for the separation of GO and MGO in the plasma samples. The flow rate was 1 mL/minute and the column oven temperature was 25°C.

Measurement of oxidative stress levels

The plasma samples of the patients were evaluated using MDA as a marker of oxidative stress.

A previous method for the extraction and HPLC analysis of MDA in plasma samples was used with small modifications. In brief, a 0.5 mL tetraethoxypropane standard

was placed in a 100 mL flask and made to 100 mL with ethanol. A 0.1 mL volume of this standard was placed in a 100 mL flask and made to 100 mL with TCA (10%). For MDA derivatization, a 0.5 mL plasma sample was placed in a 10 mL glass tube, 2 mL of TCA (10%) solution was added, and the tube was centrifuged at 8000 rpm for 5 minutes. A 1 mL sample of the supernatant was removed and combined with 1 mL TBA solution (0.67%) and incubated in a 90°C water bath for 30 minutes. The solution was then filtered through a 0.45-micron cellulose acetate filter into an amber vial for injection into the HPLC.

The HPLC system consisted of a Shimadzu Nexera-i pump and a Shimadzu RF-20A fluorescence detector (Shimadzu Corporation). The mobile phase was a mixture with 0.05M KH_2PO_4 buffer, methanol, and acetonitrile (72:17:11, v/v/v). The excitation and emission wavelengths of the fluorescence detector were set at 530 nm and 550 nm, respectively. MDA separation was achieved using an Inertsil ODS-3 (4.6 × 150 mm) column at a flow rate of 1 mL/minute and a column temperature of 25°C.

Data analysis

The IBM SPSS Statistics, Version 25.0 (IBM Corp., Armonk, NY, USA) package program was used for the statistical analysis of the results of this study. The descriptive statistics of the variables are presented as percentages, numbers, arithmetic standard deviations, and means.

The Mann–Whitney U test was used for the analysis of binary independent variables that did not have a normal distribution (between -2 and +2), as determined by the skewness and kurtosis values. Spearman's correlation analysis was used to determine the relationships between the variables. A p value < 0.05 was considered statistically significant.

Ethical considerations

Participation in this research was voluntary. The research aims were explained to the participants both verbally and in written form. Patient anonymity, confidentiality, and privacy of data were also explained in both verbal and written forms, and all data were guaranteed to be used only for research purposes. The study was performed according to the tenets of the Declaration of Helsinki. The study was approved by the Ethical Committee of the University (Nr 2020/11, 27.11.2020). Informed consent was obtained from all subjects involved in the study.

RESULTS

The average age of the patients was 60.32 ± 12.51 years, the duration of chronic kidney failure disease was 9.42 ± 8.12 years, and the duration of HD therapy was 6.53 ± 7.28 years. Overall, 31.6% of the patients were female, 66.7% were married, 46.2% had graduated from primary school, 66.7% were employed, 53.8% were retired, 35.9% had equal income–expense status, 76.9% did not smoke, 94.9% did not consume alcohol, and 44.4% exercised regularly (Table 1). All patients had a conventional HD regimen consisting of four-hour sessions three times weekly.

Table 1. Distribution of socio-demographic and health characteristics of the patients (n = 117)

Characteristics		Mean ± SD	Min–Max (median)
Age		60.32 ± 12.51	25–83 (62)
Disease Period of Chronic Renal Failure (Years)		9.42 ± 8.12	0.5–44 (8)
Application Period of Hemodialysis (Years)		6.53 ± 7.28	0.5–40 (4)
		n	%
Sex	Female	37	31.6
	Male	80	68.4
Marital status	Married	78	66.7
	Single	39	33.3
Educational status	Illiterate	8	6.8
	Literate	4	3.4
	Primary School	54	46.2
	Secondary school	31	26.5
	High school	15	12.8
	University and higher	5	4.3
Employment status	Yes	78	66.7
	No	39	33.3
Occupational status	Housewife	28	23.9
	Tradesman	9	7.7
	Worker	4	3.4
	Officer	5	4.3
	Retired	63	53.8
	Unemployed	8	6.8
Income status	Income exceeds expenses	39	33.3
	Income is equal to expenses	42	35.9
	Income is less than expenses	36	30.8
Smoking	Yes	27	23.1
	No	90	76.9
Alcohol use	Yes	6	5.1
	No	111	94.9
Regular exercising	Yes	52	44.4
	No	65	55.6

The mean CRP was 21.94 ± 45.02 mg/L, the mean HbA1C was 7.13 ± 1.62%, the mean KT/V ratio was 1.65 ± 0.3, the mean serum urea reduction ratio was 70.14 ± 11.07, the mean serum MDA level was 3.96 ± 1.01 μmol/L, the mean serum GO level was 1029.87 ± 314.43 ng/mL, and the mean serum MGO level was 115.2 ± 75.54 ng/mL (Table 2).

The KDQOL-36 sub-scale scores were as follows: the mean symptoms/problems list score was 71.65 ± 17.76, the mean effect of kidney disease score was 66.35 ± 19.06, the mean kidney disease burden score was 40.6 ± 24.01, the mean SF-12 physical health score was 41.6 ± 9.83, and the mean SF-12 mental health score was 37.83 ± 9.69 (Table 3).

The symptoms/problems list, effect of the kidney disease, kidney disease burden, and SF-12 physical health scores were higher in the non-diabetic group than in the diabetic group, and the differences were statistically significant (p < 0.05). The serum GO value was lower in the non-diabetic group than in the diabetic individuals (p = 0.001;

Table 2. Distribution of clinical and biochemical parameters of patients (n = 117)

Parameters	Mean ± SD	Min–Max (median)
Serum calcium (mg/dl)	13.05 ± 17.3	7.48–90 (9.2)
Serum phosphorus (mg/dl)	6.14 ± 7.05	2.1–49 (5.2)
Serum total protein (g/dl)	61.63 ± 102.14	5.1–660 (62.8)
Albumin (g/dl)	27.46 ± 18.44	3.6–49.6 (38.7)
Uric acid (mg/dl)	6.36 ± 1.22	1.8–8.5 (6.4)
C-reactive protein (mg/l)	21.94 ± 45.02	0.54–281.1 (11.4)
HbA1C (%)	7.13 ± 1.62	5.26–12.7 (6.8)
Total cholesterol (mg/dl)	169.11 ± 45.02	62.7–269.47 (159.7)
HDL (mg/dl)	44.01 ± 23.6	21.33–132 (36.8)
LDL (mg/dl)	93.34 ± 31.79	22.82–170 (91)
Triglyceride (mg/dl)	181.71 ± 116.94	45–565.8 (153.57)
Hemoglobin (mg/dl)	10.68 ± 1.55	6.8–13.1 (10.9)
Hematocrit (%)	31.87 ± 4.87	20.1–39.4 (32.6)
Serum iron (ug/dL)	61.93 ± 34.42	25–231 (54)
Total iron binding capacity (ug/dL)	207.54 ± 42.7	23.2–284 (202)
Ferritin (ng/mL)	570.48 ± 591.22	87.3–4130 (438)
KT/V	1.65 ± 0.3	0.93–2.32 (1.68)
URR	70.14 ± 11.07	18.5–85 (72)
Malondialdehyde (µmol/l)	3.96 ± 1.01	2.29–10.17 (3.74)
Glyoxal (ng/mL)	1029.87 ± 314.43	373–2019 (983)
Methylglyoxal (ng/mL)	115.2 ± 75.54	15–534 (97)

HDL – high-density lipoprotein; LDL – low-density lipoprotein; URR – urea reduction ratio

Table 3. Patients' mean scores for KDQOL-36 sub-scales (n = 117)

Sub-scales of KDQOL-36	Mean ± SD
Symptoms/problems list	71.65 ± 17.76
Effect of the kidney disease	66.35 ± 19.06
Kidney disease burden	40.6 ± 24.01
SF-12 physical health	41.6 ± 9.83
SF-12 mental health	37.83 ± 9.69

KDQOL-36 – kidney disease quality of life scale

Table 4. Comparison of KDQOL-36 sub-scales, serum AGE and oxidative stress levels measurements according to the presence of diabetes in patients (n = 117)

Sub-scales	Diabetes presence	n	Ort ± Ss	p
Symptoms/problems list	Non-diabetic individuals	77	75.36 ± 13.95	0.008*
	Diabetic individuals	40	63.63 ± 22.18	
Effect of the kidney disease	Non-diabetic individuals	77	68.95 ± 17.9	0.044*
	Diabetic individuals	40	60.73 ± 20.49	
Kidney disease burden	Non-diabetic individuals	77	44.61 ± 25.11	0.005*
	Diabetic individuals	40	31.93 ± 18.97	
SF-12 physical health	Non-diabetic individuals	77	42.86 ± 10.5	0.024*
	Diabetic individuals	40	38.88 ± 7.63	
SF-12 mental health	Non-diabetic individuals	77	38.48 ± 9.89	0.515
	Diabetic individuals	40	36.44 ± 9.21	
Glyoxal (ng/mL)	Non-diabetic individuals	77	963.59 ± 261.19	0.005*
	Diabetic individuals	40	1152.73 ± 367.4	
Methylglyoxal (ng/mL)	Non-diabetic individuals	77	105.32 ± 61.93	0.128
	Diabetic individuals	40	133.51 ± 93.95	
Malondialdehyde (µmol/l)	Non-diabetic individuals	77	4 ± 1.16	0.936
	Diabetic individuals	40	3.86 ± 0.68	

KDQOL-36 – kidney disease quality of life scale; Mann–Whitney U Test;
*p < 0.05

p < 0.05). No statistically significant differences in MDA and MGO values were observed according to diabetes status (p > 0.05) (Table 4).

A positive and significant correlation was found between the serum MGO and GO (r = 0.285, p < 0.01) levels and the serum MDA level (r = 0.284, p < 0.01) in the HD patients. A positive correlation was noted between MDA and GO (r = 1.000, p < 0.05), and a negative correlation was detected between MDA levels and kidney disease burden (r = -0.205, p < 0.05). A negative and significant correlation was detected between GO and kidney disease burden (r = -0.204, p < 0.05) (Table 5).

DISCUSSION

The aim of this study was to examine the relationship between serum AGE precursors, MDA serum levels, and quality of life in HD patients. The quality of life of the HD patients examined here was moderate in terms of the symptoms experienced and the effect of the disease, and their quality of life was low in terms of physical and mental health and disease burden. The reference ranges for MGO, GO, and MDA in healthy individuals were 6.5 ± 3.6 ng g^{-1}, 4.4 ± 2.9 ng g^{-1} [9], and 0.7 (0.69–0.72) µmol/L [10], respectively. The serum MGO, GO, and MDA levels were high. High serum MDA levels were associated with high GO and MGO levels, and our HD patients showed significant negative correlations between MDA and GO levels and renal disease burden. In addition, significant correlations were detected for the serum MDA and GO levels in diabetic and non-diabetic patients.

AGE are heterogenous compounds produced endogenously from the non-enzymatic glycation of proteins, lipids, and nucleic acids [11], and they play a role in the development of various chronic diseases, including diabetes-related complications, cardiovascular diseases, renal diseases, and neurodegenerative diseases [12]. Reactive carbonyl compounds are reported to accumulate during HD due to the interaction of ESRD and blood with the dialysis membrane, and both contribute to the formation of AGE [6]. Because the kidneys are the most important organ for AGE excretion, the relationship between CKD and AGEs is like a vicious cycle. As AGE increase, the glomerular filtration rate (GFR) decreases, and this exacerbates the AGE increase [13]. Increased plasma AGE levels also cause the activation of receptor of advanced glycation end-products (RAGE), and this generates inflammatory cascades and ROS production [14]. Renal and vascular AGE accumulation triggers AGE accumulation in all systems at levels that cause complications, largely due to increased oxidative stress [15].

Oxidative stress is defined as the imbalance that occurs in the rates of ROS production and destruction. Excess ROS interact with important biomolecules, such as proteins, lipids, and nucleic acids, and may cause cellular damage, with negative

Table 5. Correlation between patients' serum AGE and oxidative stress levels and KDQOL-36 sub-scales (n = 117)

Sub-scales		MDA	GO	MGO
Malondialdehyde (μmol/l)	r	1	1.000	0.284
	p		0.000*	0.002*
Glyoxal (ng/mL)	r	1.000	1	0.285
	p	0.000*		0.002*
Methylglyoxal (ng/mL)	r	0.284	0.285	1
	p	0.002*	0.002*	
Symptoms/Problems List	r	-0.077	-0.077	0.001
	p	0.411	0.411	0.989
Effect of the kidney disease	r	-0.089	-0.088	0.096
	p	0.342	0.348	0.304
Kidney disease burden	r	-0.205	-0.204	-0.137
	p	0.027*	0.027*	0.140
SF-12 physical health	r	-0.12	-0.119	-0.092
	p	0.197	0.202	0.326
SF-12 mental health	r	0.018	0.018	0.103
	p	0.849	0.848	0.268

MDA – malondialdehyde; MGO – methylglyoxal; GO – glyoxal;
*p < 0.05, Spearman's

effects on tissue function and structure [16]. In HD patients, comorbidities such as dyslipidemia, hypertension, metabolic syndrome, diabetes, senility, and atherosclerosis trigger pro-oxidant activity [3]. The resulting imbalance between the pro-oxidant and antioxidant systems in patients receiving HD causes an increase in oxidative stress related to both the pathophysiological mechanism underlying their ESRD and the physical stresses of HD itself. The end product of ROS interactions with polyunsaturated fatty acids is MDA, which can interact with proteins and nucleic acids to induce the pathogenesis of various disorders, including atherosclerosis [17]. In addition, uremic toxin accumulation can simultaneously activate the prooxidant system and inhibit the antioxidant system [18].

In the present study, the serum MDA levels in our HD patients were higher than those reported in an earlier study conducted on healthy individuals [12]. Miyagawa and Tateishi [19] reported that dialysis sessions increased oxidative stress and decreased antioxidant potential. Similarly, Coşkun et al. [20] detected that oxidative stress levels were higher in HD patients than in a control group. In addition, the oxidative stress levels in the patients increased more after the HD procedure [20]. The plasma levels of MDA, a biomarker of lipid peroxidation, were also significantly higher in HD patients after HD than before the HD treatment [5].

In the present study, the GO level was higher than the level previously reported in another study [17]. The MGO level was also higher than that stated in another study [18]. Luketin et al. [6] reported that the AGE levels were significantly higher in their HD group than in their control group. In addition, the AGE levels in the study population indicated a greater number of patients with high cardiovascular disease risk in the HD group than in the control group [6]. Another study that followed patients with CKD for 39 months reported a negative correlation between the estimated GFR (eGFR) and AGE levels, and high AGE levels were independently associated with all-cause mortality [21]. In patients with impaired renal function, serum AGE levels are higher than in patients with normal kidney function [15]. The MGO levels are also higher in patients with CKD than in the general population [22].

In this study, high MDA serum levels were associated with high GO and MGO levels. Studies have shown that AGE initiate intracellular oxidative stress by increasing ROS production [23]. AGE levels increase by the classical glycation pathway in CKD, but increases also occur by enhancements of oxidative stress and carbonyl stress. The clearance of reactive carbonyl compounds is reduced in CKD, leading to carbonyl stress, and a subsequent increase in serum AGE levels was also observed. In patients with a reduced capacity for renal activity, the plasma AGE levels are higher than in patients with normal kidney functions [15]. Increased plasma AGEs also cause the activation of RAGE, which then activates the production of inflammatory cascades and ROS [24]. AGEs cross-link to cell surface receptors or body proteins, and they cause oxidative stress and inflammation because they change the receptor or protein structure and function after binding. Glycated proteins, in turn, create an inflammatory response through their interaction with receptors, causing gene activation and various inflammatory diseases as a result of this activation [25].

In this study, high serum MDA levels had a negative impact on the quality of life of our HD patients. Silva et al. [26] examined the correlation between functional capacity and oxidative stress and inflammation biomarkers in patients receiving HD treatment and reported that oxidative stress and inflammation reduced patient functional capacity, and the negative effects on functional capacity caused limitations in daily activities and decreased patient quality of life. Oxidative stress has been shown to increase in the advanced stages of CKD, and it becomes more severe in HD patients [27]. Oxidative stress plays a role in the development of renal damage and uremic symptoms [28], while it also causes amyloidosis, immunologic disorders, coagulopathy, cataract, endothelial dysfunction, atherosclerosis, and cardiovascular complications in HD patients [19]. Inflammation may also be associated with an increase in resting energy expenditure while also negatively affecting the nutrition status of individuals. Anemia and bone-mineral disorder can also occur in patients due to inflammation [25, 26, 28]. As comorbidities increase, the level of dependency of individuals increases, and the quality of life is adversely affected [29].

Quality of life was also negatively affected by high GO levels in the present study, as high MGO levels reduced cognitive functions in our HD patients. A reduction in cognitive functions in these patients can decrease their quality of life and increase mortality [22]. In HD patients, AGE also function as uremic toxins, while vascular calcification of AGE causes endothelial dysfunction, myocardial changes, dysregulation of the immune system, and progression of atherosclerosis [30]. These changes can lead to further decreases in functional capacity and a worsening quality of life.

Limitations of the study

One limitation of the present study is that its results are valid only for the patients included in the study and, therefore, cannot be generalized to all patients. A second limitation is that the reliability of the data is also limited by the accuracy of the answers given by the patients in the study. Finally, since the duration of HD treatment, comorbidities, nutritional status, and many other parameters affect both oxidative stress and quality of life, examining only the correlation between quality-of-life parameters and a biomarker of oxidative stress may be insufficient and may not reflect the true picture of the relationship.

CONCLUSION

The quality of life of HD patients was moderate in terms of the symptoms experienced and the effects of the disease, but their quality of life was low in terms of physical and mental health and disease burden. The serum levels of MGO, GO, and MDA were high, and the high serum MDA levels were associated with high serum GO and MGO levels. A significant negative correlation was detected between the levels of MDA, GO levels, and renal disease burden. In addition, significant correlations were detected in the serum MDA and GO levels of diabetic and non-diabetic patients. Regular evaluations of symptom severity, quality of life, serum MDA levels, and serum AGE levels are recommended for HD patients, as are the development of strategies to increase the quality of life and reduce oxidative stress and the production of AGE in these patients.

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Однос између нивоа прекурсора *AGE* у серуму, оксидатног стреса и квалитета живота болесника који примају дијализу

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САЖЕТАК

Увод/Циљ Студија има за циљ да утврди везу између прекурсора крајњих продуката напредне гликације у серуму (*AGE*), нивоа оксидативног стреса и квалитета живота болесника на хемодијализи.

Методе У студији су коришћени дескриптивни облик и Упитник о квалитету живота са бубрежном болешћу (*KDQOL*-36). Нивои прекурсора *AGE* [метилглиоксала (МГО), глиоксала (ГО)] и оксидативног стреса [малондиалдехид (МДА)] болесника одређивани су у узорцима крви.

Резултати Утврђено је следеће: резултати подскале *KDQOL*-36 били су 71,65 ± 17,76 за листу симптома, 66,35 ± 19,06 за утицај болести бубрега, 40,6 ± 24,01 за оптерећење болешћу бубрега, 41,6 ± 9,83 за физичко здравствено стање према *SF*-12, 37,83 ± 9,69 за ментално здравствено стање према *SF*-12.

Утврђено је да МДА износи 3,96 ± 1,01 *μmol/l*, ГО 1029,87 ± 314,43 *ng/mL*, а МГО 115,2 ± 75,54 *ng/mL*. Уочена је позитивна и значајна корелација између МГО и ГО (r = 0,285, $p < 0,01$) и МДА ($r = 0,284$, $p < 0,01$) и између МДА и ГО ($r = 1,000$, $p < 0,05$), а негативна корелација са оптерећењем болешћу бубрега ($r = -0,205$, $p < 0,05$). Утврђено је да постоји негативна и значајна корелација између ГО и оптерећења болешћу бубрега ($r = -0,204$, $p < 0,05$).

Закључак Утврђено је да су нивои МГО, ГО и МДА били високи код болесника који су били на хемодијализи. Високи нивои МДА повезани су са високим нивоима ГО и МГО. Утврђено је да високи нивои МДА и ГО имају негативан утицај на квалитет живота болесника.

Кључне речи: хемодијализа; оксидативни стрес; прекурсори крајњих продуката узнапредовале гликације у серуму; квалитет живота

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Predictors of renal function non-recovery in critically ill patients with acute kidney injury treated with continuous renal replacement therapy

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SUMMARY

Introduction/Objective Acute kidney injury (AKI) is a highly prevalent complication among the critically ill individuals who are admitted to the intensive care unit (ICU). This study deals with identifying the frequency and predictors of the lack of renal function recovery in non-renal functions among critically ill patients requiring dialysis for AKI (AKI-D).

Methods The study included 440 ICU patients from the University Clinical Center of Vojvodina in the period from 2014 to 2018. The patients required Continuous Renal Replacement Therapy (CRRT). In this study, we analyzed various factors including demographic features, clinical characteristics, laboratory parameters, comorbidities, as well as the need for vasopressor therapy and mechanical ventilation on the day when AKI was confirmed. Additionally, we examined the different modalities of CRRT, which were used.

Results A retrospective analysis of the results included discovered that out of 440 patients with AKI-D, 242 (55%), average age 63.14, did not recover renal function. Significant predictors of renal function non-recovery in critically ill patients with AKI-D were: the patients age over 65 ($p = 0.044$), starting time of CRRT ($p = 0.043$), mechanical ventilation ($p = 0.044$) and previous kidney disease ($p = 0.005$). Significant predictors of renal function non-recovery in critically ill septic patients with AKI-D were: the patients age over 65 ($p = 0.002$), diabetes mellitus ($p = 0.023$), previous kidney disease ($p = 0.045$), CRP values < 100 mg/l ($p = 0.033$) and procalcitonin ($p = 0.010$), while in non-septic patients, the significant predictors of renal function non-recovery includes previous kidney disease ($p = 0.035$).

Conclusion Out of all examined predictors, both in septic and non-septic patients, previous kidney damage presents the greatest risk for renal function non-recovery in critically ill patients with AKI-D.

Keywords: acute kidney injury; recovery of renal function; critically ill; sepsis

INTRODUCTION

The development of acute kidney injury (AKI) is associated with various adverse outcomes such as prolonged intensive care unit (ICU) stay, development of chronic kidney disease (CKD), increased the mortality rate along with the increased treatment costs [1, 2].

The reported prevalence of AKI in ICUs varies from study to study and runs the gamut from 5% to 67% [3]. According to Opgenorth et al. [4] about 5–10% of these patients require renal replacement therapy (RRT).

In patients with AKI requiring RRT, the recovery of renal function occurs in only 20–60% of patients and patients' survival without the need for RRT is considered successful [4, 5]. Only 1–6% recovery of renal function occurs after 90 days of starting RRT [6]. AKI stage and time required for renal function recovery significantly influence morbidity/mortality rates after an acute episode of patients requiring dialysis for AKI (AKI-D) [7].

The results so far have indicated numerous predictors of renal function recovery and include those related to patients (age, CKD, proteinuria, comorbidity, etiology, stage, and duration of AKI, exposure to nephro-toxic drugs and/or diagnostic contrast agents, multi organ dysfunction), as well as potential predictors related to dialysis procedures itself (modalities, membrane, dose intensity, lasting and frequency of procedures) [5, 7, 8].

The aim of this study is to determine the prevalence and predictors of renal function non-recovery in critically ill patients with AKI-D.

METHODS

This retrospective study was conducted between 2014 and 2018 at the University Clinical Center of Vojvodina. The study included 440 adult surgical and non-surgical patients at the ICU and Emergency Room suffering from AKI and AKI on CKD who required continuous RRT (CRRT).

The study analyzed various parameters, including demographic features, comorbidities, laboratory and clinical analyses such as urea, creatinine, C-reactive protein (CRP), procalcitonin (PCT), quick sequential organ failure

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assessment score (qSOFA) score, the need for vasopressor therapy, and mechanical ventilation (MV) on the day when AKI was confirmed.

Different modalities of CRRT were utilized, including continuous veno-venous hemodiafiltration (CVVHDF), continuous veno-venous hemofiltration, continuous veno-venous hemodialysis (CVVHD), and CVVHD combined with CVVHDF. Discontinuation of CRRT was guided by the establishment of diuresis of 1000–1500 ml/day without diuretics. The recovery of renal function after AKI-D was defined independently from CRRT within 90 days of starting RRT. The criteria for early start of CRRT were stages II or III AKI according to Kidney Disease: Improving Global Outcomes (KDIGO) guidelines and/or hypervolemia with poor response to conservative treatment within 24 hours of AKI diagnosis, while manifested clinical complications related to AKI were criteria for i.e., late start of CRRT after 24 hours.

The choice of CRRT modalities was determined by the clinicians, guided by international guidelines. The CRRT prescription included specific details such as treatment modality, circulation, dilution mode, replacement and dialysis fluid flow, patient weight, and heparin anticoagulation.

The study also highlights the reasons for delayed initiation of RRT, such as organizational issues, unavailability of equipment, difficulty of catheter placement, or the need for surgical interventions or radiological tests before starting RRT. Some patients received intermittent dialysis initially based on factors like hemodynamic stability or equipment availability.

Certain exclusion criteria were applied to patients with urgent need for RRT, including specific laboratory abnormalities (urea > 50 mmol/l, K > 6.5 mmol/l, pH < 7.15) and acute pulmonary edema, and those treated with conservative therapy.

Table 1. Demographic and clinical parameters in critically ill patients and its association with renal recovery function

Variables	Renal function non-recovery n (%)	Renal function recovery n (%)	p
	242 (55)	198 (45)	
Sex Male Female	138/69.7 60/30.3%	156/64.5 86/35.5	p = 0.264
Mean age in years	63.14	60.93	
Comorbidities			
Hypertension	86 (43.4)	103 (42.6)	p = 0.923
Chronic pulmonary disease	25 (12.5)	25 (10.3)	p = 0.455
Diabetes mellitus	43 (21.7)	51 (21.1)	p = 0.907
Cerebrovascular disease	15 (7.6)	23 (9.5)	p = 0.500
Digestive disease	26 (13.1)	29 (12.0)	p = 0.773
Previous kidney disease	16 (8.1)	4 (1.7)	**p = 0.002***
MV support	61 (69.3)	65 (64.4)	p = 0.537
Vasopressor support	138 (69.7)	139 (57.4)	**p = 0.010***
Start of CRRT ≤ 24 hours	121 (61.1)	170 (70.2)	**p = 0.050***
qSOFA score			
0	47(23.7)	76 (31.4)	p = 0.296
1	53 (26.8)	64 (26.4)	
2	26 (13.1)	25 (10.3)	
3	72 (36.4)	77 (31.8)	
Types of CRRT modalities			
CVVHDF	81 (40.9)	88 (36.4)	p = 0.282
CVVHD	72 (36.4)	91 (37.6)	
CVVH	8 (4)	14 (5.8)	
CVVHD + CVVHDF	37 (18.7)	49 (20.2)	
Urea (mmol) + (Mean, SD)	26.32 ± 15.306	26.33 ± 13.962	p = 0.995
Creatinine (μmol) (Mean, SD)	401.43 ± 203.56	373.70 ± 191.51	p = 0.145
CRP (mg/L) (Mean, SD)	153.99 ± 121.44	154.62 ± 126.79	p = 0.966
PCT (ng/l) (Mean, SD)	42.80 ± 68.88	42.59 ± 94.32	p = 0.987
Ultrafiltration (ml) (Mean, SD)	3367.88 ± 5066.81	2592.8 ± 2946.46	**p = 0.046***

CRRT – continuous renal replacement therapy; CVVHDF – continuous veno-venous hemodiafiltration; CVVH – continuous veno-venous hemofiltration; CVVHD – continuous veno-venous hemodialysis; CRP – C-reactive protein; PCT – procalcitonin; MV – mechanical ventilation; qSOFA score – quick sequential organ failure assessment score

CRRT was performed on the Multifilter and the Prismaflex System (Baxter, Deerfield, IL, USA); standard high-flux filters and membranes/adsorbers were used in septic patients. The CRRT prescription included: treatment modality, blood flow, dilution mode, replacement and dialysis fluid flow, and the patient's weight and heparin anticoagulation, according to clinical practice guidelines [9].

The study was carried out according to the principles of the Helsinki Declaration and it was approved by the local Ethics Committee, decision number 00-116.

Statistical analysis

SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) was used for statistical data processing. The obtained data were analyzed using the χ^2 test and t test of independent samples (when examining individual underlying possible predictors) and binomial logistic regression analysis (when examining individual continuous potential predictors, as well as when analyzing the association of all possible predictors along with no renal function recovery). The statistical significance of the influence of potential risk factors for the absence of renal function recovery was examined in the course of the study. The Box–Tidwell test was previously applied in order to confirm that the correlation between each continuous predictor and the corresponding logarithm of the odds ratio is linear.

RESULTS

Out of 440 patients with AKI-D, 242 (55%), average age 63.14, did not recover renal function. In patients who did not recover kidney function, earlier kidney diseases (p = 0.002) and vasopressor therapy (p = 0.010) were significantly more prevalent, and those patients also had a significantly higher average ultrafiltration (ml) (p = 0.046) and a significantly lower percentage of patients started earlier CRRT ≤ 24 hours (p = 0.050). There were more male patients in the group who did not recover their renal function in comparison to the patients who did (69.7% *vs.* 64.5%) (Table 1).

Significant predictors such as age (p = 0.044), CRRT start time (p = 0.043), lung MV (p = 0.044), and previous kidney disease (p = 0.005) were singled out in the entire sample of critically ill patients. CRRT start within 24 hours of AKI diagnosis was associated with a 1.353 times higher risk (95% CI 1.009–1.814) for non-recovery of kidney function, age was associated with a 1.437 times higher risk (95% CI 1.010–2.043), MV was associated with a 1.63 times higher risk (95% CI 1.013–2.633) and previous renal disease with a 5.49 times higher risk for renal function non-recovery (95% CI 1.661–18.148) (Table 2).

In the septic patient group, age (p = 0.002), CRP (p = 0.033), PCT (p = 0.010), diabetes mellitus (DM) (p = 0.023), and previous renal disease (p = 0.045) were significant predictors of renal function non-recovery. The patients over 65 years of age were associated with a 1.63 times higher risk of renal function non-recovery (95% CI 1.063–2.504). The patients over 65 years of age were at three times higher risk (95% CI 1.486–5.760), the ones with CRP higher than 100 mg/l were associated with 2.49 times higher risk (95% CI 1.077–5.748), DM with 2.71 times higher risk (95% CI 1.144–6.414), PCT with 2.84 times higher risk (95% CI 1.279–6.308) and previous kidney disease with 7.08 times higher risk of renal function non-recovery (95% CI 1.046–47.884) (Table 3). In the non-septic group of patients, previous kidney disease was associated with a 6.25 times greater risk of renal function non-recovery (p = 0.035, 95% CI 1.14–34.273) (Table 4).

Table 2. Predictors of renal function non-recovery in critically ill patients requiring dialysis for acute kidney injury with demographic, laboratory and clinical parameters

Parameters	Exp(B)	95% CI for EXP(B)		
		Lower	Upper	Sig.
Sex	1.472	0.940	2.304	0.091
Age	1.437	1.010	2.043	**0.044***
Ultrafiltration	1.081	0.767	1.524	0.657
Modalities of CRRT	0.950	0.787	1.147	0.595
Urea at admission	1.079	0.860	1.353	0.512
Creatinine at admission	1.004	0.979	1.031	0.739
CRP	1.424	0.880	2.304	0.150
PCT	0.620	0.371	1.038	0.069
Length of hospitalization	1.070	0.763	1.502	0.694
Oliguria/anuria	0.649	0.414	1.018	0.060
Start of CRRT ≤ 24 hours	1.353	1.009	1.814	**0.043***
Vasopressors support	0.958	0.507	1.808	0.894
MV support	1.633	1.013	2.633	**0.044***
Sepsis	0.247	0.732	0.431	1.242
CVD	0.886	0.560	1.402	0.606
Pulmonary diseases	1.306	0.850	2.007	0.223
Digestive disease	1.191	0.629	2.254	0.592
Diabetes mellitus	1.299	0.766	2.201	0.331
CD	0.753	0.355	1.599	0.460
Previous kidney disease	5.490	1.661	18.148	**0.005***
Other comorbidities	0.901	0.563	1.443	0.664
qSOFA score	1.111	0.699	1.763	0.657
Constant	0.173			0.167

Exp(B) – odds ratio; CRRT – continuous renal replacement therapy; CRP – C-reactive protein; PCT – procalcitonin; MV – mechanical ventilation; CD – cerebrovascular diseases; CVD – cardiovascular disease; qSOFA score – quick sequential organ failure assessment score

DISCUSSION

Although CRRT has been used for many years in critically ill hemodynamically unstable patients with AKI, the prognosis and recovery of renal function are still uncertain.

A retrospective analysis of the results found that out of a total of 440 patients with AKI, 198 (45%) did not recover their kidney function, which corresponds with previous results [4]. Having analyzed the entire sample, we found that age, the starting time of CRRT, MV, and previous kidney disease play a significant predictive role in the outcome of renal function. The subgroup analysis has also shown that these results were consistent after adjustment for multiple variables. Namely, patients over 65 have a 1.63 times greater risk of renal function non-recovery. In a retrospective study by Jiang et al. [10] that included cardiac surgery patients with AKI, multivariate analysis showed that age was an independent prognostic factor for renal function non-recovery during the first week of CRRT treatment. The authors of previous studies investigated outcomes in patients with AKI treated with CRRT and their results, as well as ours, indicate that older age has a significant prediction for renal function non-recovery [11]. It is highly likely that the reason for such results is the higher frequency of comorbidities in the elderly population, but also the structural and functional changes in the kidney that occur with aging [12]. In critically ill patients, AKI is manifested by varying degrees of uremia, volemia, acid-base status disorders, physiological and non-renal

Table 3. Association between non-recovery of renal function in septic critically ill patients and critically ill patients requiring dialysis for acute kidney injury with demographic, laboratory and clinical parameters

Parameters	Exp(B)	95% C.I. for Exp(B)		
		Lower	Upper	Sig.
Sex	1.728	0.833	3.582	0.142
Age	2.551	1.422	4.579	**0.002***
Ultrafiltration	1.128	0.616	2.066	0.696
Modalities of CRRT	0.997	0.701	1.417	0.986
Urea at admission	1.137	0.749	1.726	0.547
Creatinine at admission	1.012	0.965	1.063	0.616
CRP	2.488	1.077	5.748	**0.033***
PCT	2.841	1.279	6.308	**0.010***
Length of hospitalization	1.234	0.760	2.002	0.395
Oliguria/anuria	0.818	0.380	1.759	0.607
Start of CRRT ≤ 24 hours	1.522	0.926	2.501	0.098
Vasopressors support	0.999	0.296	3.374	0.999
MV support	1.572	0.577	4.284	0.377
CVD	1.391	0.626	3.088	0.418
Pulmonary diseases	1.904	0.776	4.670	0.159
Digestive disease	2.604	0.874	7.757	0.086
Diabetes mellitus	2.709	1.144	6.414	**0.023***
CD	1.562	0.371	6.583	0.543
Previous kidney disease	7.077	1.046	47.894	**0.045***
Other comorbidities	1.522	0.699	3.314	0.290
qSOFA score	0.763	0.354	1.645	0.489
Constant				

Exp(B) – odds ratio; CRRT – continuous renal replacement therapy; CRP – C-reactive protein; PCT – procalcitonin; MV – mechanical ventilation; CD – cerebrovascular diseases; CVD – cardiovascular diseases; qSOFA score – quick sequential organ failure assessment score

Table 4. Association between non-recovery of renal function in non-septic critically ill patients requiring dialysis for acute kidney injury with demographic, laboratory and clinical parameters

Parameters	Exp(B)	95% C.I. for Exp(B)		
		Lower	Upper	Sig.
Sex	1.541	0.816	2.911	0.183
Age	0.981	0.604	1.594	0.939
Ultrafiltration	0.852	0.531	1.367	0.507
Modalities of CRRT	0.938	0.735	1.197	0.608
Urea at admission	1.040	0.776	1.394	0.794
Creatinine at admission	1.002	0.969	1.037	0.887
CRP	1.093	0.578	2.070	0.784
Length of hospitalization	0.776	0.420	1.435	0.419
Oliguria/anuria	1.985	1.053	3.743	**0.034***
Start of CRRT ≤ 24 hours	1.321	0.872	2.002	0.189
Vasopressors support	0.814	0.402	1.647	0.567
MV support	1.423	0.749	2.702	0.282
CVD	0.640	0.338	1.211	0.170
Pulmonary diseases	0.756	0.310	1.843	0.538
Digestive disease	0.840	0.344	2.052	0.702
Diabetes mellitus	0.822	0.389	1.737	0.607
CD	0.621	0.238	1.619	0.330
Previous kidney disease	6.251	1.140	34.273	**0.035***
Other comorbidities	0.632	0.322	1.242	0.183
qSOFA score	1.440	0.746	2.780	0.277
Constant	0.524			0.703

Exp(B) – odds ratio; CRRT – continuous renal replacement therapy; CRP – C-reactive protein; MV – mechanical ventilation; CD – cerebrovascular diseases; CVD – cardiovascular diseases; qSOFA score – quick sequential organ failure assessment score

disorders, and often has a variable course. The decision to start RRT in these patients may depend on numerous factors and it represents a very complex process [13]. In relation to that, the start time of RRT has been difficult to study and has shown considerable variations of clinicians and institutions [14]. Due to its changeable clinical course of AKI, constant monitoring of patients is necessary to ensure that RRT starts timely, i.e., before the appearance of uremic and metabolic complications, but when there are signs of general improvement in the clinical condition and spontaneous recovery of renal function. The results of our study indicate that "early" initiation of RRT in critically ill patients with AKI significantly affects the rate of renal function non-recovery. Contrary to our results, Lin et al. [15] have concluded that the "early" start of RRT does not significantly affect the recovery of kidney function. The aforementioned results correspond to the meta-analysis published by Ponce et al. [16], where it was found that "early" initiation of RRT increases the chance of recovery of renal function by 30% compared to the "late" one. Our previous study, which aimed to identify predictors of kidney function recovery, with the very same criteria used both for "early" and "late" CRRT start time just like in this study, did not find any predictors of recovery of renal function in the "early" group, while in the "late" CRRT group, non-diabetic patients were found to have a 3.5 times higher chance of function recovery compared to patients with DM [17]. Completely different results were published in research by Castro et al. [18] and indicated that "early" initiation of RRT has no significant effect on the recovery of renal function. However, the studies included in this analysis were highly prone to be biased due to the study design itself, mixed cases, definition of timing of RRT initiation and variation in outcome determination [18]. MV, while essential in providing respiratory support for critically ill patients, can potentially exacerbate pre-existing lung injury or lead to the development of new lung injury, commonly known as ventilator-associated lung injury [19]. According to the available literature, we have observed that the majority of studies investigated the impact of MV on the development of AKI in critically ill patients, but we have not found any research on whether MV has an impact on the recovery of renal function. In our study, we found that MV was associated with a 1.63 times higher risk for failure to recover renal function. In a randomized clinical study, Yang et al. [20] found that protective MV compared to conventional MV had no significant effect on AKI progression, although AKI progression occurred in both groups of patients. Ostermann and Lumlertgul [21] performed a murine model of ventilator-associated lung injury and concluded that a short period of MV with high tidal volumes (17 mL/kg) causes increased lung permeability and liver and kidney inflammation. As pulmonary MV affects both the onset and progression of AKI, it probably has an effect on the recovery of renal function as well, which needs to be determined in future prospective randomized trials.

In the subgroup of septic patients, DM was linked to a 2.71 times higher risk and previous kidney disease with a 7.08 times higher risk of renal function non-recovery. Various studies indicate that DM potentially increases morbidity and mortality in patients with AKI [22]. In a multicenter study by Chung et al. [23], DM did not increase the risk of developing AKI, but significantly decreased the probability of renal function recovery. Regarding previous kidney conditions and renal function recovery, our results correlate with most studies, one of which was conducted by Vijayan et al. [24], which included patients with previous kidney diseases were significantly less likely to recover renal function after AKI. However, we must take into account that the aforementioned authors followed the long-term effects of comorbidities on the recovery of renal function.

In a cohort analysis of the 131 critically ill patients with AKI who underwent the CRRT, along with the older age, two other determined predictors that we did not examine (coronary artery disease and admission to the ICU) were associated with a lower rate of renal function recovery [11].

While our study provides valuable insights, it is important to acknowledge the limitations associated with it. Firstly, this study was conducted retrospectively in a single center, which may limit the generalizability of the findings to other settings or patient populations.

Additionally, we lacked specific data on the etiology of AKI-D, diuresis, and the use of diuretics. These factors could potentially influence the recovery of renal function and should be considered in future studies for a more comprehensive analysis.

Furthermore, we did not have detailed information on the parameters of the CRRT procedure. Factors such as the specific modality of CRRT, dialysis dose, choice of dialysis membrane, and duration of dialysis could potentially impact the chances of renal function recovery. Although there is no definitive evidence linking these factors to the recovery of renal function, their potential influence cannot be entirely ruled out.

Despite these limitations, our study contributes valuable information on the predictors of renal function non-recovery in critically ill patients with dialysis-dependent AKI. It underscores the importance of individual consideration of potential predictors and highlights the need for further research to address the limitations and expand our knowledge in this area [25. 26, 27].

CONCLUSION

After conducting both group and subgroup analyses, it was found that among all the predictors investigated, previous kidney conditions pose the highest risk for the failure to recover kidney function in critically ill patients with dialysis-dependent AKI. It is important to look at individual and overall predictors so that clinicians can assess the potential for recovery of kidney function and implement appropriate interventions to improve outcomes.

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Предиктори неопоравка функције бубрега код критично оболелих болесника са акутним оштећењем бубрега лечених континуираном дијализом

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САЖЕТАК

Увод/Циљ Акутно оштећење бубрега (АОБ) честа је компликација код критично оболелих болесника хоспитализованих у одељењима интензивног лечења. Циљ ове студије је утврђивање преваленције и предиктора неопоравка функције бубрега код критично оболелих болесника са АОБ зависним од дијализе (АОБ-Д).

Методе Студијом је било обухваћено 440 болесника лечених на одељењу интензивног лечења Универзитетског клиничког центра Војводине у периоду од 2014. до 2018. године који су захтевали континуирану замену функције бубрега. Анализирани су демографски, клинички и лабораторијски параметри, коморбидитети, потреба за вазопресорном терапијом и механичком вентилацијом у дану потврђеног АОБ и модалитети замене функције бубрега.

Резултати Ретроспективном анализом резултата установљено је да од укупно 440 болесника са АОБ-Д, 242 (55%) болесника, просечне старости 63,14 година, нису опоравила функцију бубрега. Значајни предиктори неопоравка функције бубрега код критично оболелих са АОБ-Д били су: старост изнад 65 година ($p = 0,044$), време почетка терапије замене функције бубрега ($p = 0,043$), механичка вентилација ($p = 0,044$) и претходне болести бубрега ($p = 0,005$). Значајни предиктори неопоравка функције бубрега код септичних болесника са АОБ-Д били су: старост изнад 65 година ($p = 0,002$), дијабетес мелитус ($p = 0,023$), претходне болести бубрега ($p = 0,045$), *CRP* вредности < 100 *mg/l* ($p = 0,033$) и прокалцитонин ($p = 0,010$), док су значајан предиктор неопоравка функције бубрега код критично оболелих болесника без сепсе биле претходне болести бубрега ($p = 0,035$).

Закључак Од свих испитиваних предиктора на укупном узорку, и код септичних и код несептичних болесника, претходне бубрежне болести представљају највећи ризик за неопоравак функције бубрега код критично оболелих болесника са АОБ-Д.

Кључне речи: акутно оштећење бубрега; опоравак бубрежне функције; критично оболели; сепса

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Diagnostic role and prognostic impact of positron emission tomography/computed tomography in patients treated for uterine corpus cancer

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SUMMARY
Introduction/Objective The goal of our research was to evaluate diagnostic and prognostic role of positron emission tomography/computed tomography (PET-CT) in patients previously treated for uterine cancer and compare it to conventional imaging methods (CIM).
Methods We analyzed 37 patients examined on PET-CT for follow-up or suspicion of uterine cancer recurrence, and who were previously treated with surgery and/or chemoradiotherapy. All patients underwent CT or magnetic resonance imaging prior to PET-CT, and were followed-up for at least one year.
Results PET-CT showed sensitivity, specificity and diagnostic accuracy in uterine cancer relapse detection of 96.3%, 70%, and 89.2%, while those values for CIM were 92.6%, 40%, and 78.4 %, respectively. Correlation of PET-CT and CIM findings was 78% (29/37). In 13 out of 25 true positive patients on CIM, PET-CT found greater number of active sites missed by conventional imaging. Positive findings on PET-CT were associated with shorter progression free survival (p = 0.023, logrank test).
Conclusion PET-CT constitutes an important diagnostic method in management of recurrent cancer of uterine corpus, demonstrating high sensitivity and accuracy. In comparison to CIM, PET-CT can discover larger number of active tumor sites, and also shows better specificity. PET-CT positive patients have worse prognosis with shorter progression free survival.
Keywords: endometrial cancer; fluorodeoxyglucose; progression free survival; sensitivity; specificity; uterine sarcoma

INTRODUCTION

Uterine cancers represent most common gynecological cancer in developed countries, being the fourth most common malignant tumor and participating with up to 5% in cancer-related death among women [1, 2]. Over 400,000 newly developed cases of uterine cancers were documented in 2020 worldwide [3]. During the last couple of decades, slight increase in uterine cancer incidence has been observed, which could be attributed to population aging, fertility decrease, and increase in prevalence of certain risk factors such as obesity, polycystic ovarian syndrome, lack of physical activity etc. [4]. Uterine malignant tumors can be of epithelial (endometrial carcinoma) and mesenchymal (uterine sarcoma) origin. Histopathological classification of uterine cancers should be performed according to World Health Organization criteria [5].

Primary treatment of uterine cancer consists of hysterectomy with ovariectomy, with or without lymph nodes dissection, and in case of positive risk factors adjuvant therapy (chemotherapy and/or irradiation) should be taken into consideration. Despite optimal surgical and adjuvant treatment, 7–15% women who initially had early stage endometrial carcinoma [stages I and II as defined by The International Federation of Gynecology and Obstetrics (FIGO)] develop recurrent disease, and women with more advanced disease at diagnosis have much higher chances of recurrence [6]. Endometrial cancer relapse can be difficult to treat, particularly in patients who already received radiotherapy, or have oligometastatic disease. In the past couple of years, five-year survival rate of women with relapsed endometrial carcinoma registered three-fold increase, from 25% to 75%, due to better selection for treatment [6]. Therefore, for better patient selection, research of predictive biomarkers and prospective outcome analysis in larger study population is of key importance [6]. In patients with uterine sarcomas, high recurrence rate is present in all disease stages, despite surgery and adjuvant therapy, with the recurrence rate of 53–71%, from which 22% was pelvic, 58% distant, and 20% mixed [7].

Positron emission tomography (PET) with ^{18}F-fluorodeoxyglucose (FDG) represents functional imaging modality which is used in initial staging, therapy evaluation, restaging and

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follow-up of cancer patients. Combined with computed tomography (CT) in the same device, PET-CT represents hybrid imaging which evaluates both, functional metabolic parameters assessing disease activity in loco-regional and distant tumor sites and their morphological characteristics. Another important characteristic of FDG PET-CT imaging is its standard procedure of assessing the whole body, i.e., from head to thighs, with a possibility to discover distant lesions together with loco-regional status evaluation, which could suggest its superiority compared to CT and particularly magnetic resonance imaging (MRI). Furthermore, it was shown that certain FDG PET-CT parameters could have potential role as predictive biomarkers in endometrial carcinoma and uterine sarcoma patients [8, 9]. However, current guidelines recommend conventional imaging methods (CIM), that is CT and/or MRI, as imaging modalities of choice in suspected recurrent uterine cancer, with PET-CT being considered only in selected cases [10]. With increase in number of studies regarding the use of diagnostic imaging methods in oncological patients, it was shown that the data obtained by FDG PET-CT could be beneficial in management of these patients. However, information with regard to the role of FGD PET-CT in gynecological malignancies are still relatively limited, with no agreement in FDG PET diagnostic accuracy in comparison to CIM among recent studies when it comes to women with uterine neoplasms, and further investigations are needed to precisely determine the validity and usefulness of these methods [11].

Based on the previously mentioned, primary aim of our research was FDG PET-CT role evaluation in women who underwent treatment for uterine cancer, with the assessment of its diagnostic performances. Additional goals were to compare performances of FDG PET-CT to morphological imaging methods – CT and MRI, and to determine their potential prognostic role in these patients.

METHODS

Study population

Our research was designed as retrospective cohort study which included women who received treatment for cancer of the uterus. Patients were referred to Center for Nuclear Medicine with PET, University Clinical Center of Serbia for FDG PET-CT examination between January 2015. and December 2019, for following indications: clinical suspicion for disease relapse, new lesions on conventional imaging, or routine follow-up. All patients fulfilled certain criteria, and inclusion criteria were the following:

1) pathohistologically proven malignant tumor of uterine corpus;
2) completed primary therapy (surgery ± chemo/radiotherapy);
3) CT or MRI of abdomen and pelvis no more than three months before PET-CT;
4) minimum patient follow-up time of 12 months after PET-CT.

Exclusion criteria were:

1) previously proven another malignant tumor;
2) less than three months after surgery/irradiation and less than four weeks after chemotherapy until PET-CT imaging.

Approval for this study was obtained from the institutions' Ethics board (number 668/6), while all patients have signed written consent.

FDG PET-CT protocol

Hybrid PET-CT machine Biograph True 64 (Siemens AG, Erlangen, Germany) was used for imaging of all patients. After a minimum of six hours of fasting, mean dose of 5.5 MBq/kg of FDG was intravenously administered to examinees. Blood glucose level had to be less than 11 mmol/l, measured just before FDG administration. Patients subsequently rested for 60–90 minutes post-injection, before the imaging. Imaging protocol consisted of non-contrast low-dose CT (120 kV, 40 mAs, slice thickness 5 mm, pitch 1.5, time of rotation 0.5s), and immediately after that three-dimensional acquisition by PET. Images were acquired from scull base to upper femurs. Syngo Multimodality workstation VE31A (Siemens Medical Solutions, USA, Inc.) was used for image interpretation. Qualitative and semi-quantitative analysis of PET-CT findings was performed. All lesions which demonstrated elevated FDG accumulation on PET, even in the absence of morphological lesions on CT, were considered as positive for relapse, after the benign and physiological causes of FDG uptake were excluded. In semi-quantitative analysis, maximum standardized uptake values (SUVmax) of pathological lesions were calculated, with following formula: activity in the tissue (count/pixel/s) multiplied by calibrating factor, divided by administered radioactive dose (MBq/kg body weight). SUVmax is represented by voxel with greatest activity in designated volumes of interest - pathological areas of elevated fluorodeoxyglucose accumulation on reconstructed PET images. However, semi-quantitative analysis using SUVmax was only used as an additional mean of interpretation, with no threshold value for diagnosing recurrence. Two nuclear medicine specialists analyzed PET-CT findings separately, with no available information from previous conventional imaging results. If no agreement was achieved, expert team reviewed the images and made final decision. PET-CT results were assigned as follows: 1 – positive for recurrence; 2 – normal PET-CT findings. Positive PET-CT results were then divided in three categories: only loco-regional relapse, distant metastasis without locally active tumor, and both local and distant recurrence.

Magnetic resonance imaging and computed tomography imaging

For comparison with hybrid imaging, written reports of conventional imaging modalities (CT or MRI) were acquired. MRI examinations contained of T1 and T2 sequences, post-contrast and diffusion-weighted (DWI)

images, with abdominal and pelvic region included, in all women who underwent MRI. High resolution contrast enhanced CT images, with minimum one portal-venous phase acquisition included, were acquired from remaining patients. All conventional imaging results were also defined as positive or negative for recurrence, according to current interpretation guidelines.

For calculation of conventional and hybrid imaging diagnostic performances final diagnosis (reference standard) was obtained pathohistologically (biopsy or surgery) when possible, while in other cases women were followed-up clinically and by imaging, for minimum six months, in order to confirm/refute diagnosis made by index (imaging) tests.

Patients follow-up

Information about disease progression was acquired from patients' medical records. Follow-up of all patients consisted of anamnesis, clinical examinations, and diagnostic imaging in form of CT/MRI/PET-CT according to clinical indications. Longest follow-up period was 3.5 years (41 months), with median follow-up time of 13 months. Progression of disease was declared in cases of new disease sites or progression in size / number / FDG uptake level detected on imaging during follow-up, and in cases of death due to tumor. Progression free survival time was calculated from the day of PET-CT examination until detection of progression, or until the end of follow-up time if there was no progression.

Statistical analysis

Descriptive and analytical statistical methods were used in data analysis. Sensitivity, specificity and diagnostic accuracy of conventional imaging (CT/MRI) and PET-CT were calculated using standard formula, on patient basis. Survival analysis in settings of positive and negative CIM findings, positive and negative hybrid imaging results, and with different types of PET-CT positive results (loco-regional relapse only/distant lesions only/both local and distant disease) was performed using Kaplan–Meier and logrank tests. IBM SPSS Statistics program, Version 25.0 (IBM Corp., Armonk, NY, USA) was used for all statistical calculations, and p value ≤ 0.05 was considered statistically significant.

RESULTS

Total of 37 women was included in the study, average age 60 ± 16 years. Most of the patients had FIGO I or II initial disease stage, which were present in 25/37 females (68%), whereas the remaining patients were diagnosed with advanced disease (FIGO stages III and IV). Endometrioid type endometrial carcinoma was the most common histological tumor type, proven in 27/37 women, while the remaining examinees had some of the other types: carcinosarcoma in four cases, three patients with endometrial stromal sarcoma, two leiomyosarcoma, and one case of serous endometrial adenocarcinoma. In 28/37 cases we managed to acquire information on histological tumor grade, with even distribution of grades among patients. In Table 1, an overview of patients' clinical data, pathohistological and imaging results is displayed.

Table 1. Patients' characteristics

Characteristic	Value
Age *(years)*	
mean ± sd	60 ± 16
Initial FIGO disease stage, n (%)	
I	14 (38%)
II	11 (30%)
III	8 (21%)
IV	4 (11%)
Tumor histological type, n (%)	
Endometrial carcinoma	32 (86%)
Endometrioid type	27 (73%)
serous adenocarcinoma	1 (3%)
carcinosarcoma	4 (11%)
Uterine sarcoma	5 (14%)
Leiomyosarcoma	2 (5%)
Endometrial stromal tumor	3 (8%)
Tumor grade, n (%)	
Low grade	9 (24.3%)
Intermediate grade	9 (24.3%)
High grade	10 (27%)
Unknown	9 (24.3%)
CT/MRI findings, n (%)	
Positive	31 (84%)
Negative	6 (16%)
PET-CT findings, n (%)	
Positive	29 (78%)
Negative	8 (22%)

FIGO – the International Federation of Gynecology and Obstetrics; CT – computed tomography, MRI – magnetic resonance imaging; PET-CT – positron emission tomography / computed tomography

Results of conventional imaging (CT or MRI) were positive for relapse in 31 women (84%), while six patients (16%) had negative CT/MRI results. Calculated sensitivity and diagnostic accuracy of CT/MRI in detection of uterine cancer recurrence were 92.6% and 78.4%, respectively, with much lower specificity of only 40%. Positive predictive value was 80.6% and NPV was 66.7%.

In eight patients (22%), PET-CT results were negative for uterine cancer recurrence. Out of remaining 29 women with positive PET-CT, nine (24%) had loco-regional lesions only, in 10 patients (27%) only distant metastatic sites were detected, and 10 women (27%) were presented with both local and disseminated active lesions on FDG PET-CT (Figures 1 and 2). FDG PET-CT displayed better diagnostic abilities in comparison to CIM, with sensitivity of 96.3%, specificity of 70%, PPV and NPV 89.7% and 87.5% respectively, and diagnostic accuracy of 89.2% (Table 2). Concordance of hybrid imaging with CIM findings was present in 29/37 patients (78%). With regard to conventional imaging true positive examinees, in 52% (13/25) of cases additional active disease sites were seen on hybrid imaging, missed by CT/MRI.

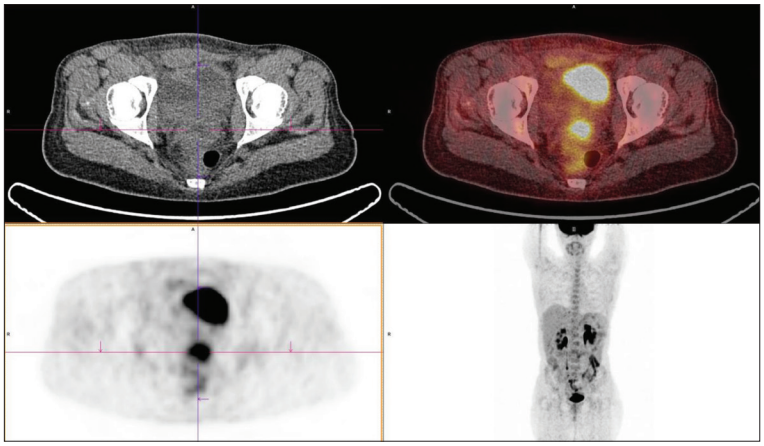

Figure 1. Local relapse after surgery for uterine sarcoma presented as ^{18}F-fluorodeoxyglucose avid lesion on positron emission tomography / computed tomography, without signs of distant disease spread

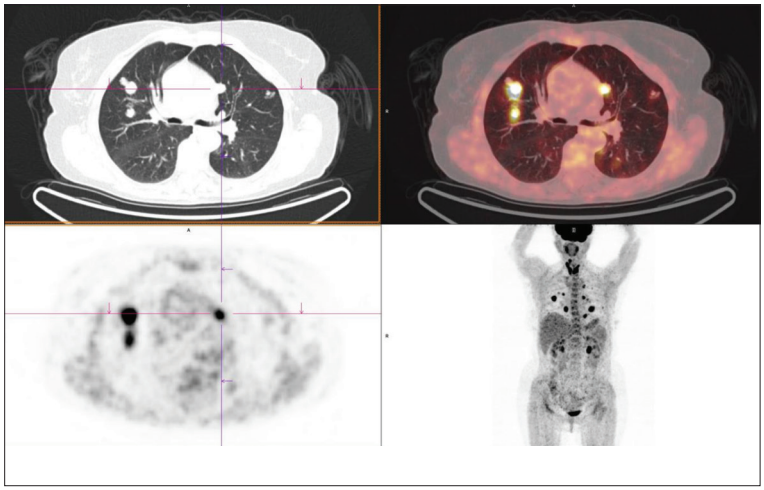

Figure 2. Lung metastasis on ^{18}F-fluorodeoxyglucose positron emission tomography / computed tomography in patient previously treated with surgery and irradiation for endometrial adenocarcinoma

Table 2. Diagnostic performance of CT/MRI and PET-CT

	TP (n)	TN (n)	FP (n)	FN (n)	Sensitivity (%)	Specificity (%)	Accuracy (%)
CT/MRI	25	4	6	2	92.6%	40%	78.4%
PET-CT	26	7	3	1	96.3%	70%	89.2%

CT – computed tomography; MRI – magnetic resonance imaging; PET-CT – positron emission tomography / computed tomography; TP – true positive; TN – true negative; FP – false positive; FN – false negative

In 21/37 cases, there were signs of progressive disease during follow-up. Women with pathological CT/MRI results had mean PFS time of 19.8 ± 2.8 months, while in patients with normal conventional imaging findings mean PFS was 34.3 ± 5.2 months (p = 0.114) (Figure 3). On the other hand, women with positive PET-CT findings had shorter mean PFS of 18.3 ± 2.8 months than PET-CT negative patients, with mean progression free survival time of 31.4 ± 3.4 months (p = 0.023) (Figure 4). Moreover, women who had both loco-regional and distant disease relapse on hybrid imaging had the shortest PFS of 9.4 ± 2.4 months, followed by patients with only distant metastasis (PFS 20.9 ± 4.7 months), and women with loco-regional disease only (PFS 26.3 ± 5.6 months) (p = 0.002) (Figure 5).

DISCUSSION

This study analyzed diagnostic abilities and prognostic role of FDG PET-CT in patients treated for uterine corpus cancer, and compared it to conventional imaging (CT and MRI). Our results suggest high sensitivity and accuracy of FDG PET-CT in detection of uterine cancer relapse, with also better specificity comparing to conventional imaging. Furthermore, PET-CT positive patients show shorter progression free survival.

In our research, obtained values of sensitivity and accuracy of PET-CT in patients treated for uterine cancer were high, reaching 96.3% and 89.2%, respectively. Systematic review and meta-analysis by Bollineni et al. [12], which included eight papers, also reported high sensitivity and diagnostic accuracy of PET-CT in detection of recurrent endometrial cancer, with values of 95% and 93%, respectively, which is in concordance with our results. On the other hand, specificity in our study was only 70%, somewhat lower than in aforementioned meta-analysis where it reached 91%. One of the main drawbacks of FDG PET-CT is its lack of specificity, since both malignant and benign (mostly inflammatory) lesions can show elevated fluorodeoxyglucose uptake, and our results could be explained by high prevalence of post-therapeutic and unspecific inflammation in our patients, combined with relatively low number of true negatives. False positive PET-CT results in our study were caused by granulomatous post-treatment inflammation, reactive inflammatory changes in lymph nodes, and bone osteoporosis. When it comes to uterine sarcomas, literature data show somewhat different results of PET-CT diagnostic abilities in patients after primary treatment. In a paper by Albano et al. [13] that evaluated 41 women treated for uterine sarcoma, obtained values of FDG PET-CT diagnostic capabilities were 88% for sensitivity, 98% for specificity and 93% for accuracy. With regard to low number of uterine sarcoma patients in our research (n = 5), that could explain partial discrepancies with our results.

In women treated for uterine neoplasms in our research, PET-CT showed somewhat better sensitivity than conventional imaging (96.3% for PET-CT *vs*. 92.6% for CT/MRI). However, the main advantage of hybrid imaging comparing to conventional methods in our study was considerably higher specificity (70% for PET-CT *vs*. 40% for CT/MRI)

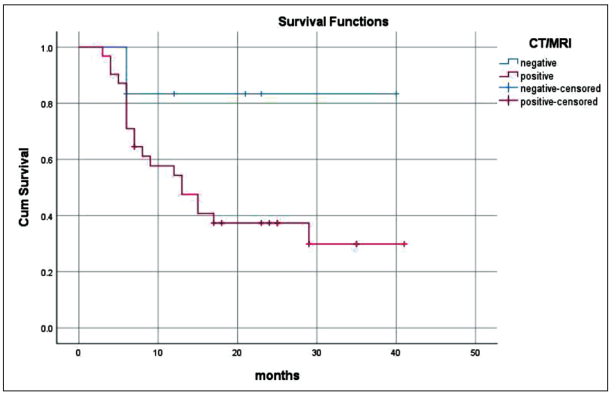

Figure 3. Kaplan–Meier survival curves showing progression free survival in patients with negative and positive computed tomography / magnetic resonance imaging (CT/MRI) findings; logrank, p = 0.114

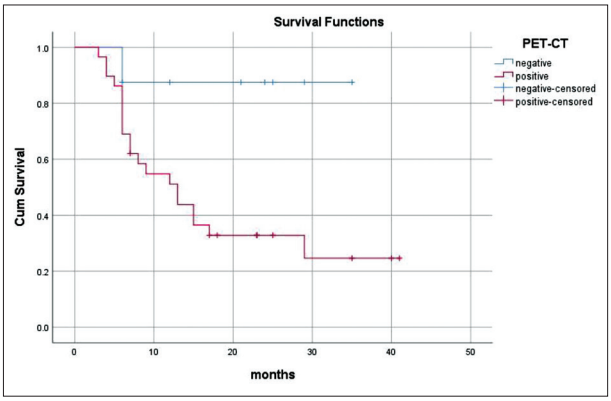

Figure 4. Kaplan–Meier survival curves showing progression-free survival in positron emission tomography / computed tomography (PET-CT) negative and positive patients; logrank, p = 0.023

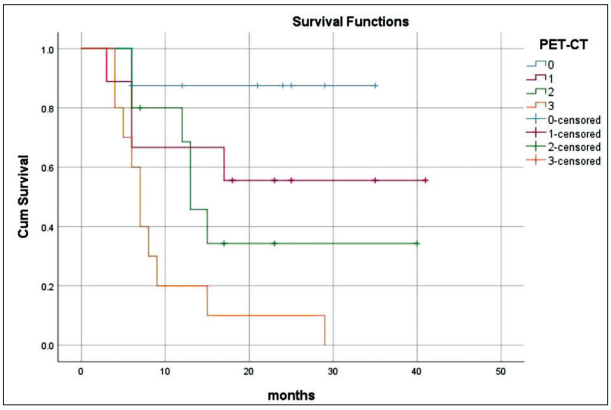

Figure 5. Kaplan–Meier survival curves showing progression free survival depending on type of positron emission tomography / computed tomography (PET-CT) findings: 0 – negative positron emission tomography / computed tomography, 1 – loco-regional lesions only, 2 – distant metastasis only, 3 – both loco-regional and distant active disease; logrank, p = 0.002

and subsequently higher overall accuracy (89.2% for PET-CT *vs*. 78.4% for CT/MRI). Higher specificity of PET-CT comparing to CIM could be the result of morphologically still present lesions (post-therapy sequels) on conventional imaging, but without metabolic activity. Ozcan Kara et al. [14] also evaluated post-treatment PET-CT in 31 patients with endometrial carcinoma, showing its superiority over conventional imaging, but with main difference in sensitivity values (100% PET-CT *vs*. 46% CIM), while both PET-CT and CIM had relatively high specificity. However, overall accuracy of both PET-CT and CIM (97% and 74%) in their research was similar to ours. One of the possible explanations of only partial agreement with our results was relatively small sample size in both studies, with significantly higher fraction of negative vs. positive imaging results in the work of Ozcan Kara et al. [14] comparing to our sample. On the other hand, Albano et al. [15] did an analysis on 157 women with suspicion of relapsed endometrial cancer, where they obtained high values of sensitivity, specificity and accuracy for FDG PET-CT (96%, 99%, 97%, respectively), with inferior results of conventional imaging, particularly concerning specificity (sensitivity 97%, specificity 62%, accuracy 80%), which is in agreement with our results. Similar results were attained in a study by Sharma et al. [16], where PET-CT showed higher specificity and accuracy (96.4% and 92.1%, respectively) in detecting recurrent endometrial cancer than CIM (62% and 76.3%, respectively), with comparable sensitivity (85.1% PET-CT *vs*. 89.5% CIM), thus being in concordance with our research. With regard to imaging of uterine sarcoma after primary treatment, Sharma et al. [17] also demonstrated superior sensitivity, specificity and accuracy of hybrid imaging over CT/MRI (85.7%, 100% and 93.3% *vs*. 57.4%, 87.5% and 73.3%, respectively) in their analysis on 12 patients, with higher fraction of negative *vs*. positive imaging findings than in our sample, which can explain partial discrepancies with our results.

Besides aforementioned, PET-CT showed another advantage over CIM in uterine cancer, since it detected additional metabolically active recurrent lesions in 13 out of 25 CT/MRI true positive women, which can be of great importance in further patient management and change treatment approach, e.g., by choosing systemic over local treatment. Accordingly, it was previously shown by Panagiotidis et al. [18] that in patients with intra-abdominal malignancies (including uterine cancer patients) and with negative CIM results, PET-CT can often detect missed peritoneal and other active lesions. In a paper by Ferioli et al. [19] it was demonstrated that postoperative PET-CT can change treatment plan endometrial cancer patients in 8.6–22.4% of cases, which was afterwards also confirmed by Albano et al. [15] who showed that PET-CT influenced therapeutic approach in 33/157 suspected recurrent endometrial carcinoma patients. Impact of hybrid imaging on therapy decision was also shown in women with suspected recurrent uterine sarcoma [13, 20].

Although CT/MRI negative patients had shorter progression free survival in comparison with CIM positive women treated for uterine cancer in our study, that did not reach statistical significance. However, PET-CT positive patients had significantly shorter PFS than women with normal PET-CT findings (18.3 ± 2.8 months *vs*. 31.4 ± 3.4 months). These results are concordant with findings of Albano et al. [15] which also showed longer PFS but also longer overall survival in patients with suspected recurrent endometrial cancer and negative PET-CT. In addition, in our research we also demonstrated PFS differences in PET-CT positive patients depending on disease spread: in cases

with only loco-regional disease detected PFS was longest (26.3 ± 5.6 months), followed by that in women with distant lesions only (PFS 20.9 ± 4.7 months), and patients with both local and distant active disease present on PET-CT who had shortest PFS of only 9.4 ± 2.4 months. In a study on 61 patients treated for endometrial carcinoma, Chung et al. [21] found that post-treatment PET SUVmax values correlated with disease-free survival, such as that patients with SUVmax < 4.25 had statistically longer disease-free survival. Saga et al. [22] also analyzed possible prognostic role of PET in 21 women treated for endometrial carcinoma, and suggested that negative PET finding could be a predictor of good prognosis.

One of the limitations in our study was relatively small sample size. The other drawback is lack of histopathological confirmation in all of our patients as a gold standard. Furthermore, this study included somewhat heterogeneous sample of tumor histopathological types, which can have different prognosis in general, but with endometrioid type endometrial cancer being most common by far. However, this can better reflect role of FDG PET-CT in everyday clinical practice.

CONCLUSION

Our results suggest important role of FDG PET-CT in clinical practice in follow-up and suspicion for recurrence of uterine cancers, with high sensitivity and overall accuracy. Additional advantage of PET-CT over CT and MRI could be its capability to discover larger number of active tumor sites, and also better specificity. Moreover, PET-CT results in these patients can also have prognostic impact, with PET-CT negative women showing significantly longer progression free survival.

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Дијагностичка улога и прогностички значај позитронске емисионе томографије / компјутеризоване томографије код болесница лечених од малигних тумора утеруса

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САЖЕТАК

Увод/Циљ Циљ ове студије је био да се испита дијагностичка и прогностичка улога позитронске емисионе томографије са компјутеризованом томографијом (ПЕТ-КТ) код болесница претходно лечених од малигних тумора утеруса, уз поређење са конвенционалним методама снимања (КМС).

Метод Анализирано је 37 испитаница које су упућене на преглед ПЕТ-КТ у склопу праћења или сумње на рецидив после лечења малигнитета утеруса хирургијом, хеморадиотерапијом или комбинацијом наведеног. Све болеснице подвргнуте су компјутеризованој томографији или магнетној резонанци пре прегледа ПЕТ-КТ и биле су праћене најмање једну годину.

Резултати Укупна сензитивност, специфичност и дијагностичка тачност у откривању рецидива рака утеруса износиле су 96,3%, 70% и 89,2% за ПЕТ-КТ и 92,6%, 40% и 78,4% за КМС. Корелација ПЕТ-КТ и КМС била је присутна у 29/37 случајева (78%). Код 13 од 25 стварно позитивних испитаница на КМС, ПЕТ-КТ је открила додатне лезије. Позитиван налаз на ПЕТ-КТ био је повезан са краћим преживљавањем без прогресије болести (p = 0,023, лог-ранг тест).

Закључак ПЕТ-КТ представља важно дијагностичко средство код болесница лечених од рака тела материце, показујући високу сензитивност и дијагностичку тачност. У поређењу са КМС, ПЕТ-КТ може открити већи број активних туморских лезија и показује бољу специфичност. Болеснице са позитивним налазима на ПЕТ-КТ имају лошију прогнозу и краће време преживљавања без прогресије болести.

Кључне речи: ендометријални карцином; флуородеоксиглукоза; преживљавање без прогресије болести; сарком утеруса; сензитивност; специфичност

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Intraocular pressure and central corneal thickness in a healthy student population

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SUMMARY

Introduction/Objective Intraocular pressure is an important parameter of eye health, especially when glaucoma is suspected. So far, few studies have been published that aimed to determine the average value of intraocular pressure and central corneal thickness in a healthy population aged 20–30 years. The aim of this study was to determine the distribution of the values of intraocular pressure and central corneal thickness in healthy student population.

Methods In a cross-sectional study, intraocular pressure and central corneal thickness were measured on a sample of a healthy population, aged 22–37 years. Intraocular pressure was measured using the Goldmann applanation tonometry method, while central corneal thickness was measured using ultrasound pachymetry. The analysis of numerical values was done using the methods of descriptive statistics.

Results By measuring intraocular pressure and central corneal thickness in 641 subjects (1282 eyes), the average value of intraocular pressure was determined to be 14.79 ± 2.31 mmHg, and central corneal thickness was 553.92 ± 25.56 μm. By comparing two groups of subjects, one male group and the other one female, we determined that there was no statistically significant difference in the average value of intraocular pressure (t-test, $p > 0.05$), and the average value of central corneal thickness (t-test, $p > 0.05$) between the sexes.

Conclusion The determined average value of intraocular pressure and central corneal thickness is similar to those determined in other cross-sectional studies of this type. No statistically significant difference was found in the intraocular pressure values and the central thickness of the cornea by sex.

Keywords: intraocular pressure; central corneal thickness; students

INTRODUCTION

Intraocular pressure (IOP) is one of the most important parameters of eye health. Its values represent the result of the dynamic balance of aqueous humor production and outflow. Elevated IOP is the most significant risk factor for glaucoma, and factor for the conversion of ocular hypertension to primary open-angle glaucoma [1, 2]. IOP is routinely measured for diagnosis and monitoring of glaucoma suspects and patients [3]. All this indicates the great importance of determining the correct IOP values. Goldmann applanation tonometry (GAT) is the gold standard technique for measuring IOP. However, the accuracy of the results obtained by this procedure can be affected by several factors, the most significant of which is central corneal thickness (CCT) [4, 5]. In general, a thinner cornea leads to a lower IOP reading, while a thicker cornea leads to a higher IOP reading than their actual values [6, 7].

Statistically, an IOP value of 21 mm Hg is widely accepted as the borderline between normal and elevated. When calibrating the Goldmann tonometer, Goldmann assumed a CCT of 0.5 mm and emphasized that variations in corneal thickness could, in theory, affect the measurement [8]. Information on differences in CCT values obtained through *in vivo* measurements subsequently became available [9].

CCT can be measured by different methods, but ultrasound pachymetry is considered the most reliable [10]. Finally, the association between decreased CCT values and readings of apparently decreased IOP values has prompted research into the role of CCT measurements in the early diagnosis of glaucoma [11, 12]. Most of the studies on CCT were performed on the population suffering from glaucoma or other ophthalmic diseases.

There are not many studies that have dealt with normal IOP values in a healthy young population. The aim of the present study was to investigate IOP and CCT values in the healthy population aged 20–40 years.

METHODS

This cross-sectional population-based observational study comprised 641 students of the Faculty of Medicine, University of Belgrade, of both sexes, aged 22–37 years. This study was conducted according to the principles of the Helsinki declaration and the consent of the Ethics Committee of the Faculty of Medicine, Belgrade, Serbia, was acquired. All subjects

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were informed about the test methods before the measurement, and written informed consent was obtained. All subjects underwent a complete ophthalmic examination consisting of a medical history, best corrected visual acuity, slit-lamp biomicroscopy (Haag-Streit AG, Bern, Switzerland), GAT (Haag-Streit AG), fundoscopy, CCT. Exclusion criteria were as follows: any form of glaucoma or systemic disease that might influence IOP values, previous intraocular surgery or trauma, pregnancy, allergy to tetracaine.

Goldmann tonometer, slit lamp mounted (Haag-Streit AG) was used for GAT. Tetracaine 1% and fluorescein sodium 2% strips were used for the GAT measurements. All GAT measurements were done during morning hours (9–11h) of the day, in the sitting position. The mean IOP and CCT value was obtained from three consecutive measurements. PalmScan AP 2000 Ophthalmic Ultrasound 2007 (Micro Medical Devices Inc., Calabasas, CA, USA) was used for CCT measurements after instillation of 1% tetracaine, and the mean of three readings was calculated for each tested eye.

The analysis of numerical values was done using classic methods of descriptive statistics, χ^2 test (for data analysis within groups) and t-test (for analysis between groups), arithmetic mean, median of mean values, and measures of variability with standard deviation, coefficient of variation and standard error, as well as the minimum and maximum value. A value of $p < 0.05$ was considered statistically significant.

RESULTS

The examination was performed on a sample of a healthy student population of 641 subjects (1282 eyes). The average age of the respondents was 24.41 ± 0.99 years.

The determined average values of IOP and CCT are listed in Table 1.

Table 1. Average intraocular pressure and central corneal thickness values in tested students

	IOP	CCT
$\bar{x}$	14.79 ± 2.31 mmHg	553.92 ± 25.56 μm
range	10–24 mmHg	470–697 μm

IOP – intraocular pressure; CCT – central corneal thickness

Table 2. Average intraocular pressure and central corneal thickness by sex

	female	male
Number of subjects/%	414 (828 eyes)/62.81	227 (454 eyes)/37.19
IOP (mmHg)	14.69 ± 0.41	14.932 ± 0.48
CCT (μm)	553.39 ± 4.13	554.99 ± 7.44

IOP – intraocular pressure; CCT – central corneal thickness

Table 3. Average intraocular pressure and central corneal thickness by right/left eye

	Right eye	Left eye	p
IOP (mmHg)	15.13 ± 0.48	15.9 ± 0.45	0.9
CCT (μm)	563.64 ± 5.82	563.23 ± 5.23	0.99

IOP – intraocular pressure; CCT – central corneal thickness

By comparing two groups of subjects, one of which was male (227 subjects, 454 eyes) and the other female (414 subjects, 828 eyes), it was determined that there was no statistically significant difference in the average value of IOP (Student's t-test, $p > 0.05$), and the average value of CCT (Students's t-test, $p > 0.05$) between the sexes (Table 2).

Analysis of the average values of IOP and CCT of the right and left eyes revealed no statistically significant differences ($p > 0.05$) (Table 3).

DISCUSSION

The role of IOP and its connection with glaucoma has been the focus of scientific research practically since the first definition of glaucoma as an eye disease. While this definition of glaucoma currently rests more on structural and functional damage [13], IOP measurement is still used as a mandatory, simple, accessible and economical method in approaching high-risk patients. Many studies have documented an association of increased incidence of glaucoma with increasing IOP values [14, 15], and especially with values above 20–23 mmHg [16, 17]. However, a study on a Latino population found this association with IOP values above 30 mmHg [18]. There are numerous data in the literature for the average statistical normal value of IOP, but few studies have addressed this question in different age groups, especially in the age group of 20–30 years [19]. In the study by Dane et al. [20], which was done on 125 subjects, finding of higher IOP in women was explained by estrogen effects. Some of published studies aimed to study IOP daily fluctuations in young people or the influence of sleeping position on IOP values, but all of them are characterized by a small number of subjects (10 or 20) [21, 22].

Normal IOP ranges 10–22 mmHg, with an average of 16 mmHg. Values for normal IOP have been obtained by examining large population groups. One of the largest studies was conducted on the population in Serbia in 1970, when Cvetković et al. [23] examined 3550 people of both sexes over 40 years of age in the municipality of Opovo. Measurements were made with a Schiøtz impression tonometer, and mean IOP values of 16.85 ± 3 mmHg were obtained. There was no statistically significant difference in the IOP level according to sex (although the IOP in women was slightly higher, 17 mmHg, compared to men, where it was 16.7 mmHg). As part of the aforementioned project, part of the examination was performed using the applanation tonometry method, but on a smaller sample (512 subjects of both sexes), with very similar results – the average IOP value was 16.47 ± 3 mmHg. The mean IOP value measured in this study (15.11 ± 2.35 mmHg) corresponds to those recorded in earlier studies of this type involving healthy Caucasian subjects of approximately the same age [24]. One of the larger studies made in the territory of the Republic of Serbia was conducted in the period 2007–2012 in the territory of the City of Novi Sad, but on the population of people who were being treated for glaucoma in the ophthalmology services of primary health care centers [25].

IOP is a dynamic parameter that changes depending on heart action (systole/diastole), inspiratory/expiratory pressure, extraocular muscle tone, hormonal status, daily rhythm of vagotonia and sympathicotonia, body position, and is even related to the seasons. Also, IOP is known to change with age. In newborn children and infants, and during the entire first decade of life, lower IOP values than those determined for the adult population are considered to be normal [26]. In children in the first years of life, the average normal IOP is below 15 mmHg, from the age of 6–12 years it is 11 ± 2.5 mmHg [27], and in the decades after the 50s, the average IOP value gradually increases, but without statistically significant differences.

The IOP in the right and left eye of the same person is practically the same, and 3 mmHg is accepted as a normal difference. When measuring, it is usual to measure the IOP first in the right eye, then in the left eye, and it is noted that repeated measurement in the right eye usually gives lower values. Probably one of the reasons is the relaxation of the extraocular muscles during the repeated measurement, or the discrete opening of the chamber angle due to the pressure of the prism on the cornea. In our study, it was found that there is no significant difference between the average IOP value between the right and left eyes, which agrees with the results of earlier studies [28].

As for sex differences, it was found that women have slightly higher IOP on average, but without statistical significance. In our study, the determined average value of IOP in female subjects is 15.23 ± 0.43 mmHg, while in male subjects it is 14.89 ± 0.52 mmHg, with a difference that is not statistically significant ($p > 0.05$).

CCT is routinely measured in clinical settings before corneal refractive procedures, but also because it can potentially significantly influence the reading of real IOP values and consequently the classification and therapy of glaucoma.

The average CCT value measured in this study (563.65 ± 27.74 μm) confirms the values documented in earlier studies performed on a similar sample [10, 29, 30]. In earlier clinical studies, the average value of CCT varied from 520 μm when CCT was determined by optical pachymetry to 540 μm when determined by ultrasound [9, 29, 30]. By comparing the average CCT values between the sexes, we found that there is no statistically significant difference in the CCT value in the healthy population sample, which confirms previous studies, although the average value was slightly higher in female subjects [9].

In this study, the average value of CCT between the right and left eyes was determined, which was statistically not significantly different. Previous studies with optical pachymetry have shown that there is a systematic difference between the right and left eyes [30]. This may be due to measurement error in the optical method when the measurement is not positioned normal to the cornea. Such measurement errors do not occur when using an ultrasonic pachymeter because it reads a value only when the probe is directed normally to the cornea. Indeed, other studies using ultrasound pachymetry also found no statistically significant difference between the right and left eyes [30].

CONCLUSION

In this study, we determined the average values of IOP and CCT in a healthy student population, that is – the age group from 22 to 37 years old. So far, similar studies have not been done in our population. The average values of IOP and CCT in our sample did not differ significantly from the values obtained in similar previously published studies.

Conflict of interest: None declared.

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Висина интраокуларног притиска и централна дебљина рожњаче код здраве студентске популације

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САЖЕТАК

Увод/Циљ Интраокуларни притисак је значајан параметар здравља ока, а посебно када постоји сумња на глауком. Ретке су до сада објављене студије које су имале за циљ утврђивање просечне вредности интраокуларног притиска и централне дебљине рожњаче на здравој популацији старости 20–30 година.

Циљ ове студије је утврђивање дистрибуције вредности интраокуларног притиска и централне дебљине рожњаче код особа здраве студентске популације.

Методе У студији пресека вршено је мерење вредности интраокуларног притиска и централне дебљине рожњаче на узорку здраве популације, старости 22–37 година. Мерење интраокуларног притиска вршено је методом Голдманове апланационе тонометрије, док је мерење централне дебљине рожњаче вршено ултразвучном пахиметријом. Анализа нумеричких вредности рађена је методама описне статистике.

Резултати Мерењем интраокуларног притиска и централне дебљине рожњаче на 641 испитанику (1282 ока) утврђена је просечна вредност интраокуларног притиска од 14,79 ± 2,31 *mmHg* и централне дебљине рожњаче од 553,92 ± 25,56 *μm*. Поређењем две групе испитаника, од којих је једна група била мушког пола а друга женског, утврђено је да нема статистички значајне разлике у просечној вредности интраокуларног притиска (*t*-тест, $p > 0,05$) и просечној вредности централне дебљине рожњаче (*t*-тест, $p > 0,05$) између полова.

Закључак Утврђена просечна вредност интраокуларног притиска и централне дебљине рожњаче је слична онима утврђеним у осталим студијама пресека овог типа. Није утврђена статистички значајна разлика у висини притиска и централне дебљине рожњаче поређењем по полу.

Кључне речи: интраокуларни притисак; централна дебљина рожњаче; студенти

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The effect of hemodialysis on the ocular anterior morphometry and intraocular pressure

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SUMMARY

Introduction/Objective This study evaluates the effects of hemodialysis (HD) on intraocular pressure (IOP) and ocular anterior chamber morphometry in end-stage renal disease (ESRD) patients.

Methods In total, 32 ESRD patients (50 eyes) who were on regular HD program, underwent ocular examination. To all of them, 30 minutes before HD and 30 minutes after the end of the HD session, central corneal thickness (CCT), keratometric values (K1, K2), axial length (AL), anterior chamber depth (ACD), and lens thickness (LT) were measured using the Lenstar 900 Haag-Streit USA device (Haag-Streit Group, Köniz, Switzerland). IOP was measured using Goldman applanation tonometry.

Results IOP before HD was 15.74 ± 3,043 while after HD it was 15.14 ± 3,07 (p = 0.125); K1 and K2 values were 43.11 ± 1.68 vs. 43.13 ± 1.73 (p = 0.688) and 43.11 ± 1.60 vs. 43.11 ± 1.66 (p = 0.158); AL increase from 23.25 ± 0.68 to 23.27 ± 0.68 in postHD (p = 0.158) as well as AL from 23.25 ± 0.68 to 23.27 ± 0.68 (p = 0.264); ACD decrease insignificantly from 3.14 ± 0.40 to 3.10 ± 0.42 (p = 0.063); mean LT before HD was 4.66 ± 0.38 while after HD it was 4.67 ± 0.36 (p = 0.290) and CCT was 563.68 ± 42.02 *vs*. 563.34 ± 42.26 (p = 0.777).

Conclusion HD has no significant influences on ocular anterior segment structures such as on CCT, ACD, LT, AL, K values as well as IOP.

Keywords: hemodialysis; eye; ocular morphometry

INTRODUCTION

The end-stage renal disease (ESRD) represents the last stage of irreversible kidney disease which requires transplantation or dialysis. The main purpose of hemodialysis (HD) is to regulate the composition and volume of body fluids by removing excess water and dissolved substances such as urea, potassium, and phosphorus from the body. Consequently, after HD, there is a decrease in systemic blood pressure, plasma volume, and body weight. An increase in the colloid osmotic pressure of the plasma, caused by increased plasma protein concentration, leads to water entering the plasma through the interstitial space. These fluctuations in systemic blood pressure and metabolic parameters can result in refraction changes, dry eye, "red eye syndrome" [1], calcium accumulation in the conjunctiva, band-shaped keratopathies, intraocular pressure (IOP) fluctuations, lens opacities, increased tear osmolarity, corneal endothelium changes, central corneal thickness (CCT) alterations, and variations in the thickness of the retina and choroid in patients undergoing HD. Additionally, signs of retinopathy may manifest due to hypertension, anemia, uremia, and diabetes mellitus. Patients on HD have also shown disturbances in choroidal perfusion [2].

Various results have also been published regarding the influence of HD on the biometric parameters of the eye, such as keratometry values, CCT, anterior chamber depth (ACD), lens thickness (LT) central macular thickness, and axial eye length.

This study evaluates the effects of HD on IOP, central macular thickness, corneal morphometry, anterior chamber (AC) morphometry, and axial length (AL) in patients with cataracts and ESRD.

METHOD

Fifty eyes of 32 ESRD patients who were on regular HD (three times weekly, approximately four hours per session), and who were dialyzed at the Zvezdara University Clinical Center, Belgrade, Serbia, underwent ocular examination including slit lamp examination and ophthalmoscopy. To all of them, 30 minutes before HD and 30 minutes after the end of the HD session, IOP, CCT, keratometry values (K1, K2), AL, ACD, and LT were measured. For the measurement of the above-mentioned parameters, the Lenstar 900 Haag-Streit USA device (Haag-Streit Group, Köniz, Switzerland) was used. IOP was measured using a Goldmann applanation tonometer (Haag-Streit Group). All patients who underwent gonioscopy with narrow and closed angles were excluded from the study because angle structure could be an

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independent risk factor for IOP elevation. Statistical analysis were performed by SPSS Statistics for Windows, Version 17.0. (SPSS Inc., Chicago, IL, USA), and p values < 0.05 were considered statistically significant.

This study has been approved by the institutional Ethics Committee and patients' written consent was obtained, according to the Declaration of Helsinki (1/01/24).

RESULTS

In total, 50 eyes of 32 ESRD patients undergoing regular HD treatment, including 20 males (62.5%) and 12 females (37.5%) were enrolled in the study. The mean age of the patients was 70.03 years ± 6.2 (range 59–83 years). Among them, 22 (68.8% of all) had systemic hypertension, and six (18.8% of all) had type 2 diabetes.

There are no differences between observed parameters before and immediately after HD as shown in Table 1.

Table 1. Biometric characteristics of anterior eye segment before and after hemodialysis (HD)

Parameter	Unit	Before HD	After HD	p
Intraocular pressure	mmHg	15.74 ± 3.043	15.14 ± 3.07	0.125
K1	diopters	43.11 ± 1.68	43.13 ± 1.73	0.688
K2	diopters	43.11 ± 1.60	43.11 ± 1.66	0.158
Axial length	mm	23.25 ± 0.68	23.27 ± 0.68	0.264
Anterior chamber depth	mm	3.14 ± 0.40	3.10 ± 0.42	0.063
Lens thickness	mm	4.66 ± 0.38	4.67 ± 0.36	0.290
Corneal thickness	μm	563.68 ± 42.02	563.34 ± 42.26	0.777

K1 and K2 – keratometric values

According to the aforementioned, HD does not influence anterior segment parameters as well as macula thickness.

DISCUSSION

The majority of studies have examined the effect of HD on IOP. The results obtained are conflicting, ranging from those indicating an increase in IOP after HD, those that do not record a change in IOP values, to those suggesting a statistically significant decrease in IOP after HD [3].

The IOP remains stable, while at a narrow and especially at a closed angle, the IOP increases significantly [4]. The mechanism of IOP elevation includes an increase of colloid osmotic pressure and aqueous humor production, consequently [5]. The second mechanism includes the osmotic difference between the lens and the aqueous humor-water enters into the lens which became thicker, AC reduces depth and narrow-angle leads to a reduced outflow. The final result is an increase in IOP. Wang et al. [5] reported that IOP was significantly increased after two hours of HD in the extremely narrow-angle group and returned to pre-hemodialyzed values 30 minutes after HD.

Since HD induces dehydration and ultrafiltration, it could cause a decrease in the iris and ciliary body thickness with widening of the AC [6, 7]. Consequently, increasing aqueous humor drainage through a widely open angle can reduce IOP. Thus, many factors can increase and decrease IOP during HD [8]. In our study, IOP remains the same in the immediate post-HD period in comparison with the baseline level. The same results were reported by other authors [9, 10, 11] as well as Lim et al. [10], who also found out that the visual field improved after HD. We did not measure IOP during HD sessions as some other authors did such as Wang et al. [5] reported in their study. Recently, case with neovascular glaucoma due to proliferative diabetic retinopathy with unilateral pain in the left eye was reported. Authors concluded that limited outflow during HD in such cases indicates urgent intervention [12]. In conclusion, HD patients with narrow angles, neovascular or exfoliative glaucoma, could have a significant IOP increase at the end of HD because of the osmolar gradient between the plasma and ocular tissue.

The interest of many authors has arisen in recent years regarding the HD effect on CCT and anterior segment morphometry. Asiedu et al. [1] reported that the mean CCT decreased significantly following hemodialysis while Wang et al. [8] found out CCT significant mean decrease. Kanawa et al. [13] also reported a significant decrease in corneal thickness measured in 50 eyes after HD. In our study, CCT remains the same after HD but seems that CCT should be measured during HD session. Elbay et al. [14] found out that CCT and IOP significantly increased in the second hour of HD but at the end of the HD session, there were no significant changes in comparison with baseline.

Keratometric values remain stable after HD in our study. Similar results have been published previously [14]. Elbay et al. [14] also reported that ACD remain stable during the HD session, similar to the result of our study. However, some authors [15] found significant decrease in AC parameters. They measured AC angle parameters such as the angle opening distance (AOD) and the trabecular-iris space area (TISA) by AS-OCT to 20 HD patients. Almaznai et al. [16] reported a significant decrease in ACD and LT after HD.

Yin et al. [17] reported the same results as we did: HD had no significant effect on K readings, CCT, ACD, LT, or IOP. Some authors found that the mean AL was significantly reduced with an average value of 0.26 ± 0.15 mm while others found out that HD increased AL.

CONCLUSIONS

According to our results, HD has no significant influences on ocular anterior segment structures such as on CCT, ACD, LT, AL, K values as well as IOP. Due to possible fluctuations, according to the reliable data, it is recommended to measure the mentioned parameter during the HD session.

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Утицај хемодијализе на морфометрију предњег сегмента ока и интраокуларни притисак

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САЖЕТАК

Увод/Циљ Ова студија процењује ефекте хемодијализе (ХД) на интраокуларни притисак и морфометрију предњег сегмента ока код болесника у терминалној фази бубрежне инсуфицијенције.

Методе Укупно 32 болесника у терминалној фази бубрежне инсуфицијенције (50 очију) који су били на редовном програму ХД подвргнути су очном прегледу. Свима њима су 30 минута пре ХД и 30 минута после завршетка сесије ХД мерене централна дебљина рожњаче, кератометријске вредности (К1, К2), аксијална дужина, дубина предње коморе и дебљина сочива помоћу уређаја *Lenstar 900 Haag-Streit USA* (*Haag-Streit Group*, Кениц, Швајцарска). Интраокуларни притисак мерен је помоћу Голдманове апланационе тонометрије.

Резултати Интраокуларни притисак пре ХД био је 15,74 ± 3,043, док је после ХД био 15,14 ± 3,07 ($p = 0,125$); вредности К1 и К2 биле су 43,11 ± 1,68 према 43,13 ± 1,73 ($p = 0,688$) и 43,11 ± 1,60 према 43,11 ± 1,66 ($p = 0,158$); аксијална дужина се повећала са 23,25 ± 0,68 на 23,27 ± 0,68 у постХД ($p = 0,158$), као и аксијална дужина са 23,25 ± 0,68 на 23,27 ± 0,68 ($p = 0,264$); безначајно се смањила дубина предње коморе – са 3,14 ± 0,40 на 3,10 ± 0,42 ($p = 0,063$); средња дебљина сочива пре ХД била је 4,66 ± 0,38, док је после ХД била 4,67 ± 0,36 ($p = 0,290$), а централна дебљина рожњаче је била 563,68 ± 42,02 наспрам 563,34 ± 42,26 ($p = 0,777$).

Закључак ХД нема значајних утицаја на структуре предњег очног сегмента као што су вредности централне дебљине рожњаче, дубина предње коморе, дебљина сочива, аксијална дужина, кератометријске вредности, као и интраокуларни притисак.

Кључне речи: хемодијализа; око; окуларна морфометрија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Severe neurological complications in a child with multisystem inflammatory syndrome in children after asymptomatic COVID-19

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SUMMARY
Introduction Coronavirus disease-2019 (COVID-19) usually leads to a mild infectious disease course in children, but serious neurological complications have been described in association with both acute infection and the multisystem inflammatory syndrome in children (MIS-C). Cerebrovascular disorders (CVD) in children are rare complication of MIS-C, and various potential mechanisms of CVD in MIS-C have been hypothesized.
Case outline In an eight-year old girl, diagnosis of MIS-C was made according to clinical features of prolonged fever, circulatory shock, heart and renal insufficiency, skin abnormalities, conjunctival hyperemia, and stomach pain associated with laboratory findings (increased CRP, D-dimers, pro BNP, troponins, IL-6), supported by positive contact with SARS-CoV2 one month before the disease onset and increased IgG and IgM anti-SARS-CoV2 antibodies. From the second day of hospitalization, left-side hemiplegia was observed, and using brain CT and MR, CVD was diagnosed. Together with cardiovascular support, corticosteroids and intravenous immunoglobulin were administered. On the fourth day of hospitalization, diagnosis of cerebral salt wasting syndrome (CSWS) was made according to severe dehydration, polyuria, hyponatremia, increased natriuria, and increased urine: serum osmolality ratio. CSWS had very severe course lasting more than one month. The girl was discharged with stable vital signs, normal diuresis and hemiparesis.
Conclusion This is the first case in the literature presenting association of severe CSWS and CVD in a child with MIS-C after COVID-19.
Keywords: COVID-19; MIS-C; cerebrovascular disease; cerebral salt wasting syndrome

INTRODUCTION

Coronavirus disease-2019 (COVID-19) usually leads to a mild infectious disease course in children, but serious neurological complications have been described in association with both acute infection and multisystem inflammatory syndrome in children (MIS-C) [1]. The criteria for MIS-C are fever, evidence of inflammation, at least two organs involved, no other active infection that could explain condition, associated with plausible epidemiologic link to SARS-CoV-2 through a positive laboratory test (PCR, antigen or antibody) or confirmed exposure [2]. Cerebrovascular disorders (CVD) in children are rare complication of MIS-C, while various potential mechanisms of CVD in MIS-C have been hypothesized.

This is the first case in the literature with the association of CVD and long-lasting life-threatening cerebral salt wasting syndrome (CSWS) in a child with MIS-C after COVID-19.

CASE REPORT

In an eight-year-old girl, MIS-C after asymptomatic COVID-19 was complicated by CVD and life-threatening CSWS lasting for more than four weeks.

The onset of the disease was eight days before admission, with everyday fever (up to 39.8°C) and vomiting. Three days after fever onset, the girl felt severe stomach pain. Two days before admission the girl became very exhausted and had submandibular and auricular exanthema followed by target-skin changes on limbs. Ceftriaxone was administered intravenously. In the medical history, we found out that the father had suffered from COVID-19 with positive PCR test for SARS CoV-2 one month earlier, while our patient was asymptomatic and PCR-negative at the same period. The girl was healthy before her current disease, with normal psychomotor development, regularly vaccinated according to the schedule.

On admission to the Institute, the girl was in poor condition, exhausted, with Glasgow coma score of 14, she was answering questions and complaining on severe stomach pain, heart rate was 132 beats/minute, blood pressure was 73/35 mmHg, she had poor capillary filling, gallop heart rhythm and systole murmur of 2/6. The breathing rate was 28 breaths/minute, SpO was 97%, with normal auscultator finding. Erythema multiform on the neck, palms,

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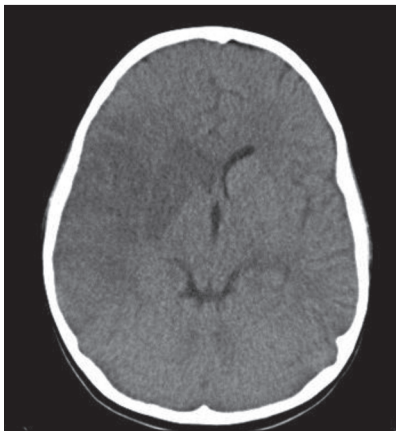

Figure 1. Computerized tomography showed a huge zone of non-homogenous hypodensity of the brain parenchyma involving cortex and white matter in the right frontal-parietal-temporal regions, nucleus caudate, putamen, and globus pallidus; involved zones are edematous with compression to the right ventricle

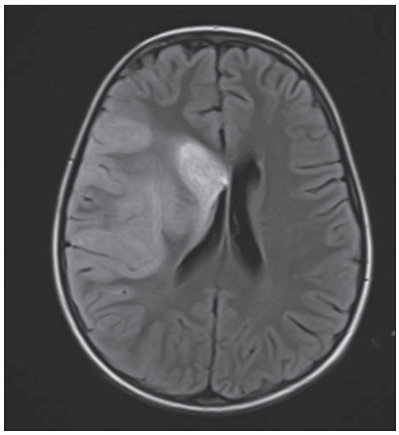

Figure 2. Seven days after the left-side hemiplegia onset, brain MR showed a huge zone of inflammation with cytotoxic edema involving grey and white matters of the right hemisphere involving lateral aspect of the inferior, and the entire middle frontal gyrus, right temporal gyrus, insula, capsule external and internal, part of corticospinal tract, and the right basal ganglia

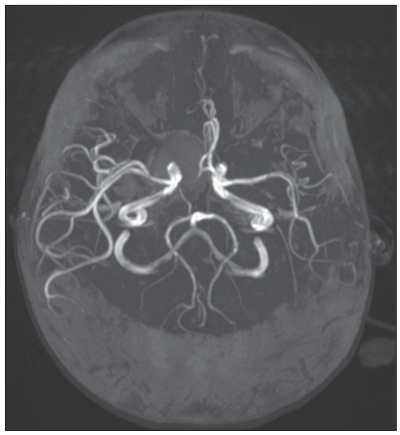

Figure 3. Brain MR angiography done seven days after left-side hemiplegia onset showed decreased signal flow through the A1 segment of the right anterior cerebral artery

dorsal side of the feet and gluteal regions was present. Lips and tongue were "strawberry-like" and conjunctive was hyperemic. No visceromegaly was present. Edema of dorsal side of feet and distal part of the legs was observed. The girl was dehydrated, with oliguria during the first day of hospitalization. No lateralization in neurological finding was observed on admission. Abnormal laboratory findings included the following: C-reactive protein 44.2 mg/l; platelet count 133; Gly 8.3 mmol/l; urea 32 mmol/l; Cr 97 mmol/l; tCO_2 13 mmol/l; potassium 2.8 mmol/l; sodium 122 mmol/l; chlorine 87 mmol/l; uric acid 609 mcmol/l; gamma-glutamyl transferase 9 IJ/l; NT-proBNP 1244 pg/ml (increased); troponin I 0.689 (normal < 0.3); troponin T 0.127 (normal < 0.1); proBNP 22,742 (normal < 125); IL-6: 11.6 pg/ml (normal < 7); D-dimer 1970 (normal < 230); serology for SARS-CoV2: IgM 44 (positive), IgG 84 (positive), PCR negative.

Heart ultrasound on admission showed damaged function of the left ventricle with ejection fraction of 45%. During heart ultrasound follow-up, the recovery of the heart function was observed within the next seven days. The renal function was normalized on the second day after admission.

Diagnosis of MIS-C was made according to clinical features of prolonged fever, circulatory shock, heart and renal insufficiency, skin abnormalities, conjunctival hyperemia, and stomach pain associated with laboratory findings (increased CRP, D-dimers, proBNP, troponins, IL-6), supported by positive contact with SARS-CoV2 one month before the disease onset and increased IgG and IgM anti-SARS-CoV2 antibodies.

The initial treatment of MIS-C included the following: parenteral hydration and electrolyte disturbances correction, inotropic stimulation (dopamine), decongestive therapy (spironolactone and furosemide), antibiotics (ceftriaxone), high doses of methyl-prednisolone and Fraxiparine (Aspen Notre-Dame-de-Bondeville, Notre-Dame-de-Bondeville, France).

The day after admission, the girl became somnolent with left side hemiparesis. Brain native computerized tomography (CT) scan was done urgently (Figure 1). Anti-aquaporin-4 Ab, anti-MOG-Ab, anti-NMDA-Ab were negative.

On the seventh day after hemiplegia appearance, MR (Figure 2) and MR angiography (Figure 3) of the brain were done. Intravenous immunoglobulins were introduced seven days after the onset of hemiparesis in the dosage of 0.4 g/kg/day, for five days. The administration of low-molecular heparin was stopped on the 11th day of hospitalization and acetylsalicylic acid was introduced. In the further course, the girl was treated with prednisone 2 mg/kg per day. Five days' cure of methyl-prednisolone in the dosage of 500 mg/m^2/day was repeated after three weeks from the first cure. Four weeks after the first treatment of intravenous immunoglobulin (IVIG), the one-day infusion of 1g IVIG was given. The improvement of severe left side hemiparesis was very slow during the first four weeks of the disease, while cognitive functions were normal all the time.

From the fourth day of hospitalization, severe dehydration was observed due to polyuria associated with hyponatremia, increased natriuria > 100 mmol/l, increased ratio of urine: serum osmolality (osmolality: urine 604 mOsm/kg, serum 294 mOsm/kg). Since renal and endocrinology causes for polyuria were excluded, diagnosis of CSWS was made. With the increased intake of sodium, fluids and low dosage of mineralocorticoids, the balance was hardly achieved. The managing of CSWS was frustrating, with the duration of more than four weeks, with diuresis up to 10 mg/kg/h, and fluid intake of up to six liters per day. Any decrease of fluid intake led to severe dehydration. The girl was discharged with stable vital signs, normal diuresis and hemiparesis.

The subject's parents' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by the competent institutional ethics committee (No. 8/131), and conforms to the legal standards.

DISCUSSION

Cerebrovascular disorder in children is a rare complication of either COVID-19 or MIS-C after COVID-19 [3]. The results of studies from the early pandemic showed the risk of stroke in children and adolescents from 0.29% to 0.62%. The prevalence of SARS-CoV-2 infection among children with arterial ischemic stroke tested by PCR or serology was 6.1% and 6.9%, respectively [3]. Our patient suffered CVD after asymptomatic SARS-CoV-2 infection. Similarly, it was described by Beslow et al. [3] that 13 of 23 cases with stroke had asymptomatic SARS-CoV-2 infections, and among the patients with symptoms, there was a broad range of periods between viral symptom onset and stroke.

The inflammatory-mediated thrombosis has been identified as a mechanism for the SARS-CoV-2-associated stroke. The children with elevated inflammatory markers or MIS-C may be at particularly high risk of stroke [3]. The use of Fraxiparine (Aspen Notre-Dame-de-Bondeville) as soon as the diagnosis of MIS-C was established did not prevent CVD in our patient. O'Loughlin et al. [4] reviewed published cases of pediatric patients with severe neurological issues and a coexisting positive SARS-CoV-2 test. MIS-C was diagnosed in 65 out of 159 cases with severe neurological manifestations, while CVD was diagnosed in 38 cases. In some of the cases with stroke associated with COVID-19, underlying disorders had existed, while our patient was healthy, with normal neurodevelopment before current disease.

The underlying pathophysiology of neurological complications of MIS-C is the cytokine storm, characterized by high levels of tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, IL-12, and interferon gamma (INFγ) [5]. The hyperinflammatory state contributes to a pro-coagulable state: initial vasculitis causes the disruption of vascular integrity, the exposure of thrombogenic basement membrane, and, finally, the activation of the clotting cascade [1].

The mechanism of CVD in our case is unclear. Inflammatory-mediated mechanism is supposed, since the elevated inflammatory markers, the presence of MIS-C, and very early appearance (on the same day as hemiparesis occurred) of neuroimaging finding of a huge ischemic lesion and cerebral edema, suggested considering that vessels' occlusion is not the only mechanism. Clinical signs of the cytokine storm including cardio-circulatory shock together with increased inflammatory biomarkers (CRP, IL-6, D-dimers) which preceded the neurological abnormalities, strongly suggested the role of inflammation in CVD in our case.

The preferred treatment strategy has to be more aggressive at the diagnosis of MIS-C, to block the cytokine cascade [6]. Maggio et al. [6] described favorable prognosis in 22 children with MIS-C treated by IVIG and steroids as the first-line treatment, suggesting that this approach could explain the favorable prognosis. Despite the same treatment in our case, neurological complications were in fact taking place. Recovery rates, including occurrence and resolution of coronary artery aneurysms, were similar for primary treatment with IVIG when compared to glucocorticoids or IVIG plus glucocorticoids [7].

Clinical and laboratory findings after admission of our patient presented prerenal type of acute renal impairment with signs of acute tubular damage due to dehydration and renal involvement of MIS-C. Two days later, global renal function was normalized including tubular function. In further course, extreme polyuria and dehydration dominated with normal renal function, low uric acid in serum and relatively decreased urine osmolality suggested CSWS. N-terminal pro-brain natriuretic peptide (NT-proBNP) plays vital roles in the regulation of the volume status. There is no data if an increased level of brain natriuretic peptide in children with MIS-C might be a contributing factor in CSWS associated with MIS-C and CVD, so further investigations are necessary to explain this possibility. Despite early recognition and treatment of CSWS in our case, the duration of CSWS was very long and additionally complicated the recovery of the patient.

MIS-C has a wide range of clinical symptoms including neurological symptoms and prognosis [8, 9]. In the study by de Faries et al. [10], death occurred in 21.5% of children with COVID-19 and MISC, reporting that the mortality was associated with higher levels of the vasoactive-inotropic score, the presence of acute respiratory distress syndrome, higher levels of erythrocyte sedimentation rate, and thrombocytopenia.

There is no literature data about CSWS associated with CVD in MIS-C. Our case with severe CSWS and CVD shows that COVID-19 might be associated with life-threatening neurological complications in children, even if the acute illness is asymptomatic.

Conflict of interest: None declared.

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Тешке неуролошке компликације мултисистемског инфламаторног синдрома код деце после асимптоматског ковида 19

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САЖЕТАК

Увод Болест изазвана вирусом корона (ковид 19) најчешће има благи клинички ток код деце, али у овом раду су описане тешке неуролошке компликације које су удружене како са акутном инфекцијом, тако и са мултисистемским инфламаторним синдромом код деце (*multisystem inflammatory syndrome in children – MIS-C*) после ковида 19. Цереброваскуларна болест код деце је ретка компликација *MIS-C* и постоје различите претпоставке о могућим механизмима који до ње доводе.

Приказ болесника Код осмогодишње девојчице постављена је дијагноза *MIS-C* на основу: клиничке слике која је обухватала пролонгирану фебрилност, циркулаторни шок, срчану и бубрежну инсуфицијенцију, промене на кожи, конјунктивалну хиперемију и болове у стомаку; резултата лабораторијских анализа (повишени *CRP*, *D*-димери, *proBNP*, тропонини, *IL*-6); података о контакту са вирусом месец дана пре почетка болести и повишених вредности *IgG* и *IgM* антитела на *SARS-CoV*2. Од другог дана хоспитализације запажа се левострана хемипареза, а применом компјутеризоване томографије и магнетне резонанце ендоканијума доказана је цереброваскуларна болест. Поред кардиоваскуларне потпоре, примењени су кортикостероиди и интравенски имуноглобулини. Четвртог дана хоспитализације постављена је дијагноза синдрома церебралног губитка соли на основу тешке дехидрације, полиурије, хипонатријемије, повишене натриурије и повишеног односа осмолалности урина и серума, који је имао тежак клинички ток и трајао је преко месец дана. Девојчица је пуштена стабилних виталних знакова, нормалне диурезе и хемипаретична.

Закључак Удруженост тешког синдрома губитка соли и цереброваскуларне болести код детета са *MIS-C* после ковида 19 није до сада описана, тако да је ово први приказ у литератури.

Кључне речи: ковид 19; *MIS-C*; цереброваскуларна болест; синдром губитка соли

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Photocolorimetry for full crown central incisor shade matching

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SUMMARY

Introduction The objective of this case series report is color matching of the central incisors all-ceramic crowns and determine the color difference between those crowns and contralateral or neighboring intact natural incisor using the ΔE_{ab} value from CIELab formula.

Case Report The subject of this color assessment was all-ceramic crowns for central incisors for three young female patients. The intact natural incisors were used as the target shade for the all-ceramic crown. After tooth preparation and intraoral scan, everything was done at once, regarding the design of restoration and model. For these cases, we used Ivoclar ZirCAD PRIME multi A1 (Ivoclar, Schaan, Liechtenstein) block. For proper shade mapping polarized picture with grey card for digital calibration is necessary as well as one standard picture for mapping the color effects. For tooth color mapping we used the eLAB software (eLAB Prime, Freiburg im Breisgau, Germany). Highest ΔE_{ab} value for all three cases was 2.7 or less, which indicates that the color is clinically acceptable, considering acceptability threshold value of less than 2.7 (three-year follow-up confirmed acceptable color appearance.

Conclusion Following recommended protocol based on the eLAB software software (eLAB Prime), clinically acceptable color of the all-ceramic crown were obtained.

Keywords: dentistry; color; color matching; all-ceramic crown

INTRODUCTION

The delivery of natural looking restoration is one of the most challenging tasks in oral rehabilitation. The shape, texture and color are factors that contributes to a natural appearance. The color matching of the anterior artificial crown to adjacent natural teeth is especially critique for the patient's satisfaction. In the daily dental practice, visual shade matching with a dental shade guide is still one of the most common methods for color determination. Visual shade matching is subjective, tooth is polychromatic and dental materials present limited shade tab [1]. Instrumental methods using electronic devices such as dental spectrophotometers [e.g VITA Easyshade® (VITA Zahnfabrik H. Rauter GmbH & Co., Bad Säckingen, Baden, Germany)] and digital photography have shown higher precision for shade matching and can be used to convey information to the dental technician [2].

The visible color is a mix of three primary colors: red, green, and blue (determine Hue). The addition of some color pigment in the mixture gives a darker effect (determine Value), and addition of another pigment will produce more color intensity (determine Chroma) [1–4].

For color matching of ceramic crowns, the CIELab system (CIE – International Commission on Illumination) is the most commonly used. The colors in this system are represented in a spherical color space through three coordinate values, Figure 1. The vertical dimension "L" indicates lightness (on the upper pole is the pure white, and on the lower pole is the pure black). Chromatic color characteristics are followed along two horizontal axes: "a" expresses the red-green axis, and "b" the blue-yellow axis [1, 5, 6].

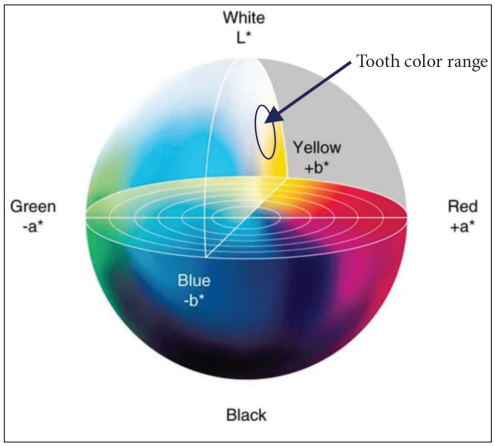

Figure 1. Tooth color range in CIELab color system

The CIELab system is particularly applicable in dental laboratories for determining and reducing color differences, while producing restorations. The degree of diversity, ΔE_{ab} (E – Euclidean distance) is color space with differences in lightness, chroma and hue, and it is determined in this system by the formula:

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$$\Delta E_{ab} = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

The difference between compared colors is represented by the relative value ΔE_{ab}, which is considered as a standard for measuring color differences. It is considered that the threshold of human sensitivity to distinguish shades is at the value of $\Delta E_{ab} = 1$. Color differences lower than this ΔE_{ab} are not perceptible for 50% of the observers, while $\Delta E_{ab} \leq 2.7$ is considered clinically acceptable [1, 7–11].

This case series reports the color matching of all-ceramic central incisors using a photocolorimetry protocol for CIELAB color differences calculation. The procedure for tooth color matching is shown schematically in Figure 2.

CASE REPORTS

Case N° 1: The subject of this color assessment was a maxillary central incisor prepared for a full-ceramic crown of a 37-year-old female patient. The contralateral intact natural incisor was used as the target shade for the all-ceramic crown, Figure 3.

After tooth preparation and intraoral scan everything was done at once, regarding the design of the restoration and model, Figure 4. For this case Ivoclar ZirCAD PRIME multi A1 block (Ivoclar, Schaan, Liechtenstein) was used. For proper shade mapping polarized picture with grey card for digital calibration is necessary as well as one standard picture for mapping the color effects, Figure 5.

For tooth color mapping we used the eLAB software (eLAB Prime, Freiburg im Breisgau, Germany). Values of this grey card are: L:79 lightness, a:00 red, b:00 yellow. These values were used for matching in the next steps. In that manner it was easy to superimpose picture of a tooth shot on the model with the polar filter picture in mouth and digital try-in. The finalization with the layered ceramic has been made by a special recipe combining knowledge and measurements (Figures 6 and 7).

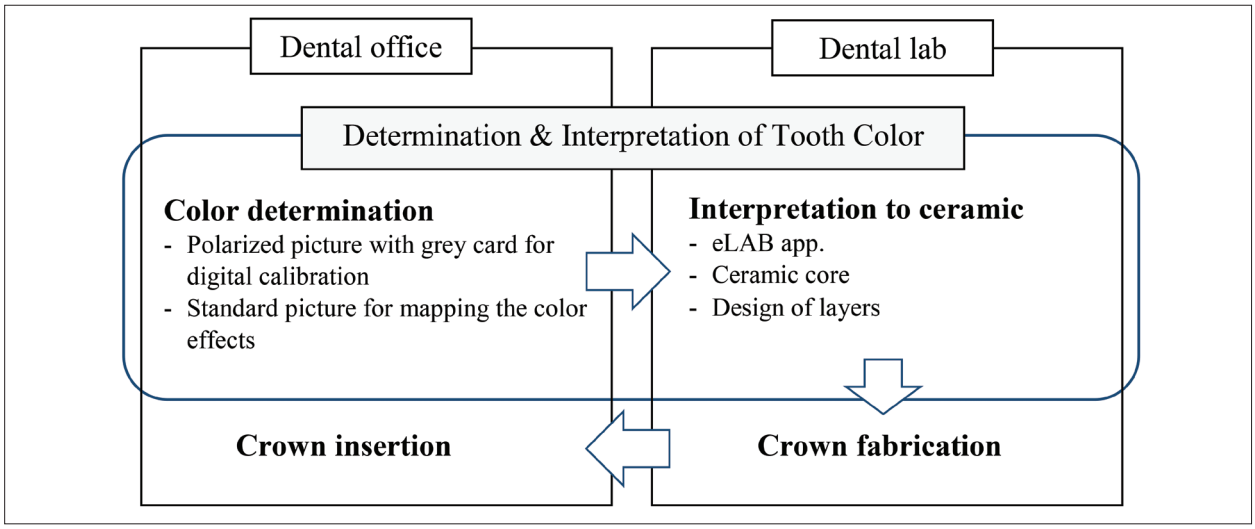

Figure 2. The scheme of communication between dental office and dental technician for tooth color determination, interpretation, and crown fabrication

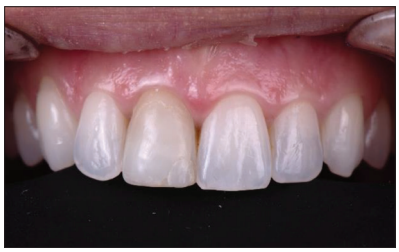

Figure 3. Pre-operative view of the right maxillary central incisor

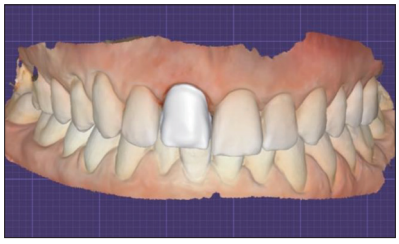

Figure 4. Digital modeling of a all-ceramic crown

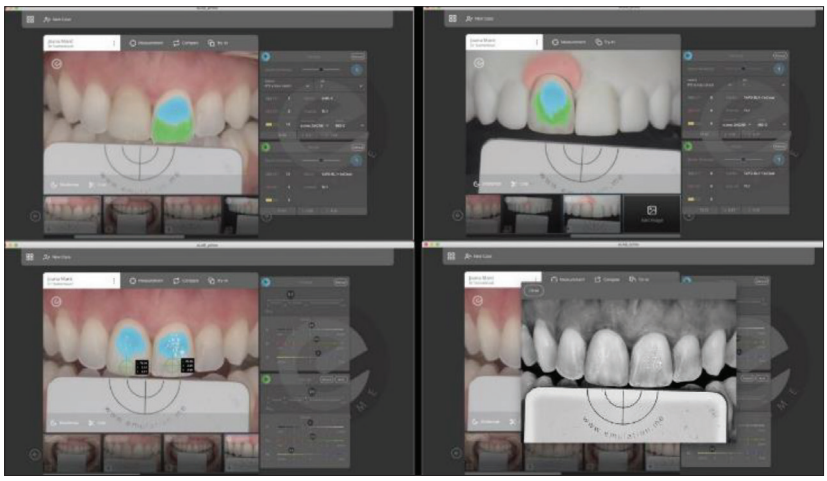

Figure 5. Shade mapping and check

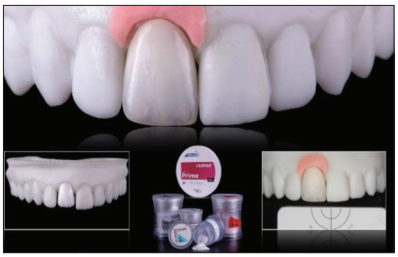

Figure 6. For this case Ivoclar ZirCAD PRIME multi A1 block (Ivoclar, Schaan, Liechtenstein) has been used

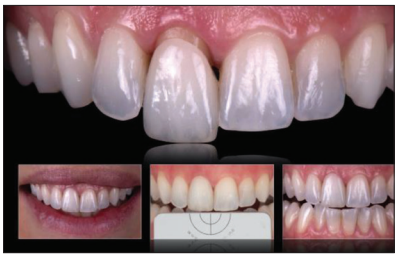

Figure 7. Highest ΔE_{ab} value was 2.7, which indicates that the color is clinically acceptable

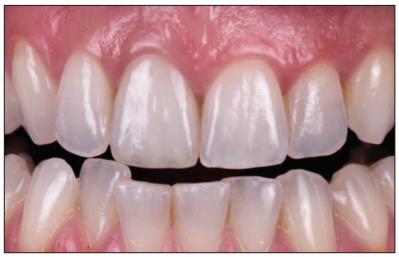

Figure 8. Three-year follow-up confirmed acceptable color appearance

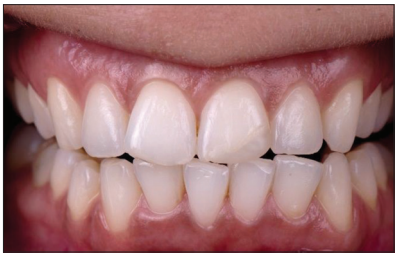

Figure 9. Pre-operative view of both maxillary central incisors

Case Nº 3: Color determination of the lower central incisor and matching it with the remaining natural teeth is also very demanding and creative. However, due to slightly less visibility while speaking and smiling and the vertical overbite of the teeth, determining teeth color is somewhat less demanding compared to maxillary incisors.

In this case color assessment was an all-ceramic crown for mandibular central incisor with 30-year-old female patient. The contralateral intact natural incisor has been used as the target shade for the all-ceramic crown (Figure 12). The procedure for tooth color matching and interpretation was the same as in the previous cases. In this case also, we used the CIELab formula and calculated ΔE_{ab} value. ΔE_{ab} value was less than 2.7 which indicates that the color is clinically acceptable. In all cases patients were extremely satisfied with the tooth color (Figure 13).

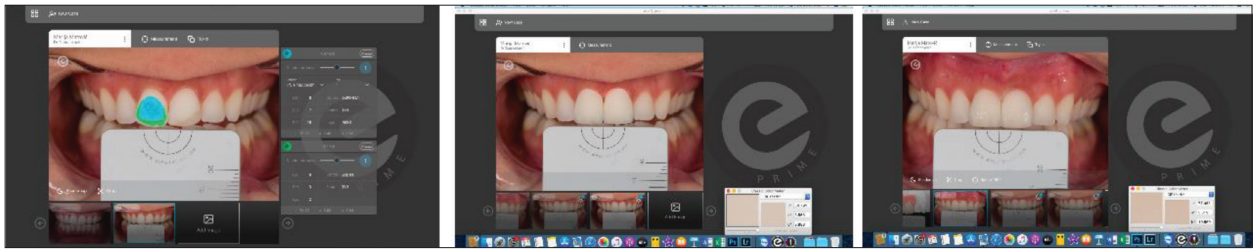

Figure 10. Shade mapping of all-ceramic crowns for both maxillary central incisors

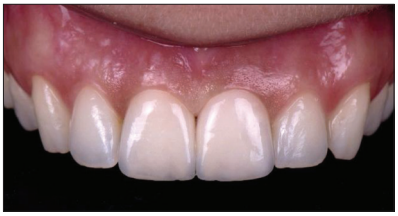

Figure 11. Post-operative view of both maxillary central incisors

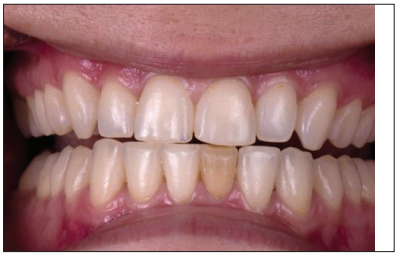

Figure 12. Pre-operative view of the left mandibular central incisor

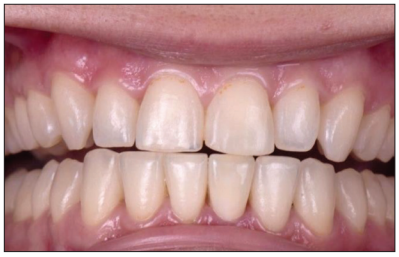

Figure 13. Post-operative view of the left mandibular central incisor all-ceramic crown

Highest ΔE_{ab} value was 2.7, which indicates that the color is clinically acceptable, considering acceptability threshold value of less than 2.7 (three-year follow-up confirmed acceptable color appearance) (Figure 8).

Case Nº 2: As we have shown, the highest challenge was to determine the color and match it with the remaining natural teeth of one upper central incisor. It is demanding, but with a lesser extent, to determine the color of the two upper central incisors and match it with the remaining teeth.

In this case, color assessment were all-ceramic crowns for both maxillary central incisor with a 34-year-old female patient. The intact natural second incisors were used as the target shade, as seen in Figure 9. The procedure for tooth color matching and interpretation was the same as in the previous case (Figures 10 and 11).

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

DISCUSSION

Digital photocolorimetry has shown to improve the communication between the dental professional and technician [3, 12, 13] by delivering a set of protocol-based information besides data obtained with the conventional visual method. The reliability of this protocol depends on the type of camera, its settings, ambient light, flashlight, and size of the captured image [3].

The calculation of ΔE_{ab} color difference through image editing software (eLAB Prime) or data from spectrophotometers (VITA Easyshade®) is of a great help for both, dental technicians, and dental professionals. Although recent studies have established better correlation of color differences calculated with CIEDE 2000 formula, [1, 14–17] dental technicians are used to interpret L, a, b and consequently ΔE_{ab} color differences values. The value for $\Delta E_{ab} = 2.7$ was taken arbitrarily.

The CIELab formula that we used coincides in 75% of cases with the examiner's visual perception, while a new color-difference equation CIEDE2000 matches in 90% of the cases with the examiner's visual perception [15].

In daily dental practice, the use of color difference formula for determining teeth color gives results that both the patient and the entire dental team are satisfied with. A multitude of variables involved (salivary reflections, translucency of dental ceramics, illuminant metamerism between natural teeth and ceramic restorations) are necessary for more serious research.

Color matching is a crucial step in the process of fabricating an aesthetically satisfying restoration. In all our presented cases highest ΔE_{ab} value was ≤ 2.7, which indicates that the color is clinically acceptable, considering acceptability threshold value of less than 2.7. Three-year follow-up confirmed acceptable color appearance.

Conflict of interest: None declared.

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Фотоколориметријско одређивање боје централних секутића

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САЖЕТАК

Увод Циљ овог рада је избор боје керамичких круна централних секутића и утврђивање разлике у боји између керамичких круница и контралатералног или суседног интактног природног секутића коришћењем вредности ΔE_{ab} из формуле *CIELab*.

Приказ болесника Код три пацијенткиње (32–43 год.) вршио се избор боје керамичких круница за централне секутиће. Као циљна нијанса боје за керамичке крунице коришћени су интактни природни секутићи. После припреме зуба и интраоралног скенирања у лабораторији је израђен виртуелни модел и дизајниране су крунице. Коришћен је *Ivoclar ZirCAD PRIME multi A1* блок (*Ivoclar*, Шан, Лихтенштајн). За правилно мапирање нијанси коришћена је поларизована слика са сивом картицом за дигиталну калибрацију, као и једна стандардна слика за мапирање ефеката боја. За мапирање боја зуба коришћен је софтвер *eLAB* (*eLAB Prime*, Фрајбург, Немачка). Највиша ΔE_{ab} вредност за сва три случаја била је 2,7, што указује на то да је боја клинички прихватљива, имајући у виду да је вредност прага прихватљивости мања од 2,7 (трогодишње праћење је потврдило прихватљив изглед боје).

Закључак Применом протокола заснованом на софтверу *eLAB* добијене су клинички прихватљиве боје керамичких круница код све три пацијенткиње.

Кључне речи: стоматологија; боја; избор боје; керамичке крунице

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Kounis syndrome as a cause of acute coronary syndrome

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SUMMARY

Introduction Kounis syndrome (KS) represents an acute coronary syndrome (ACS) induced by a hypersensitivity reaction. First described by Kounis and Zavras in 1991, KS today represents an infrequently diagnosed clinical syndrome. Three different KS variants have been defined: type I vasospastic allergic angina, type II allergic myocardial infarction, and type III stent thrombosis.

Outlines of cases This paper presents three cases of type II KS causing anaphylactic ACS. In the first case, a 66-year-old female presented with dyspnea, dizziness, and electrocardiography findings suggesting ACS after she was stung by a bee. In the second case, we present a 64-year-old female admitted to the Emergency Department with chest pain after an anaphylactic reaction due to an iodine contrast injection used for a thoracic computed tomography scan. In the third case, an 80-year-old female presented with chest pain, palpitation, and skin rash shortly after administration of the intravenous anesthetic propofol during elective malignant colon tumor surgical intervention. All patients were treated at the Cardiology Clinic, University Clinical Center of Serbia.

Conclusion The primary mechanism of KS corresponds to the release of inflammatory mediators during a hypersensitivity reaction triggered by different sources. Although well known, constant reminders of this cause of ACS are needed.

Keywords: acute coronary syndrome; hypersensitivity reaction; anaphylaxis; Kounis syndrome

INTRODUCTION

Kounis syndrome (KS) is defined as simultaneously arising acute coronary syndrome (ACS), myocardial infarction, or stent thrombosis in allergic hypersensitivity, anaphylactic, or anaphylactoid reaction settings. The first descriptions of allergic reactions associated with cardiovascular symptoms and signs appeared more than seven decades ago [1]. However, in 1991, Kounis and Zavras [2] first described "allergic myocardial infarction" caused by mast cell activation following allergic reactions. The lifetime prevalence of allergy and anaphylaxis is increasing, with an estimated lifetime risk of 0.02–2%, with more KS reports in the literature. Most data are sparse and based on isolated clinical cases and series, while exact pathophysiological mechanisms remain unclear [3–7].

Many inflammatory cells are activated during the allergic reaction, releasing their subsequent preformed mediators in circulation, leading to an inflammatory vicious cycle [8]. The entire arterial system seems vulnerable, and besides the coronary arteries, there have been reports of mesenteric and cerebral arteries being affected [6–8].

Since its first description, three different variants of KS have been defined: type I vasospastic allergic angina (without coronary disease), type II allergic myocardial infarction (with prior coronary disease), and type III stent thrombosis (after percutaneous intervention) [3]. It was reported in every age group, race, and diverse geographic location. The treatment strategy for this syndrome may be particularly challenging due to simultaneous cardiac dysfunction and allergic reactions.

We present three cases of anaphylactic ACS treated at the University Clinical Center of Serbia 2019–2021.

REPORTS OF CASES

Case No. 1

A 66-year-old female presented to the Emergency Department (ED) after being stung by a bee while sitting in her yard. Shortly after the sting, she developed dyspnea, palpitation, and dizziness. Her medical history included hypertension, type II diabetes mellitus, hyperlipidemia, and sideropenic anemia.

Emergency medical services found her in hemodynamic compromise. She was administered intravenous epinephrine, methylprednisolone, and chloropyramine, which led to clinical improvement, and sent to the Emergency Department (ED) for further evaluation. Serum tryptase level three hours after the event was 19.8 ng/mL (normal value < 11 ng/ml).

The initial electrocardiogram (ECG) demonstrated ST-elevations in the aVR, and V1

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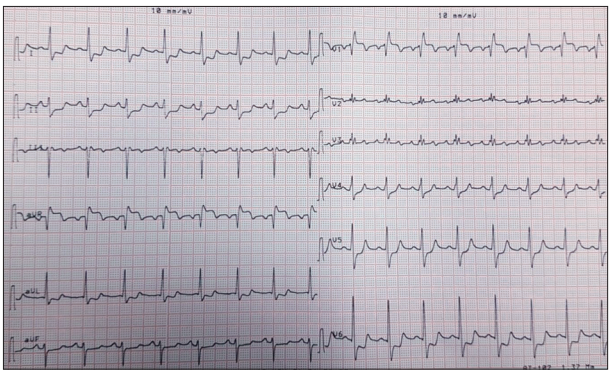

Figure 1. Electrocardiogram at the Emergency Department

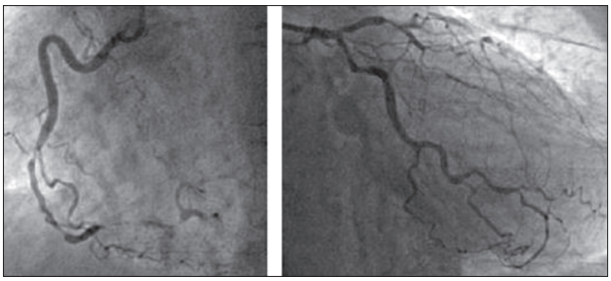

Figure 2. Coronary angiography: triple-vessel disease

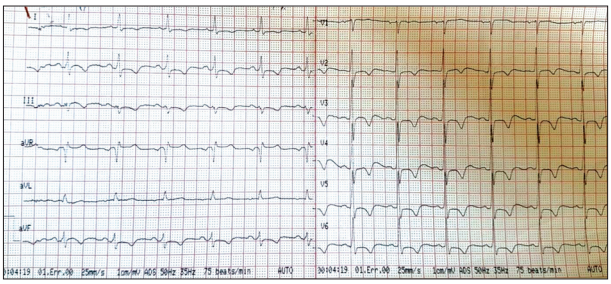

Figure 3. The patient's initial electrocardiogram showed negative T wave in the anterior and inferior wall with ST depression in aVR

leads with ST-depression in D1, D2, aVL, aVF, and V4–V6 leads with right bundle branch block pattern (Figure 1). The patient was admitted to the Coronary Care Unit (CCU), where she was hemodynamically stable with a slightly lower level of arterial pressure (100/60 mmHg). Her high-sensitivity troponin T (hsTnT) was elevated at 289 ng/L (normal value < 14 ng/L). The patient was treated with corticosteroids, antihistamines, and protocol for ACS with dual antiplatelet therapy, acetylsalicylic acid 100 mg and clopidogrel 75 mg, with a beta-blocking agent (bisoprolol 2.5 mg). During the first days of treatment, she did not experience chest pain or heart failure. Transthoracic echocardiography showed mild aortic stenosis with hypokinesis of the inferobasal septum and inferior wall, altered left ventricular systolic function [ejection fraction (EF) 45%], and mild ischemic mitral insufficiency.

Coronary angiography showed triple-vessel disease with left main stenosis of 50–70%, proximal left anterior descending artery (LAD) stenosis of 50–70% and medial LAD stenosis of 50–70%, ostial circumflex artery stenosis of 50–70%, medial right coronary artery (RCA) stenosis of 50–70% with distal occlusion (Figure 2). After initial cardiology treatment and recovery, she was transferred to the Clinic for Cardiac Surgery, where she underwent myocardial revascularization.

Case No. 2

A 64-year-old female presented to the ED with chest pain after an anaphylactic reaction due to an iodine contrast injection used for a thoracic multislice computed tomography. She was regularly followed up after breast cancer, treated surgically and with chemotherapy nine years previously. Her personal history included hypothyroidism. Her medication included capecitabine and acetylsalicylic acid. She was a smoker and had a family history of cardiovascular disease.

Immediately after the intravenous application of the iopromide contrast injection, she experienced dizziness, skin rash, and chest pain. The operating radiologist and emergency medical services administered adrenaline, methylprednisolone, and chloropyramine without improving symptoms. She was transferred to ED with ongoing dizziness, generalized urticaria, chest pain, and hypotension 90/60 mmHg.

An ECG showed sinus rhythm and signs of myocardial injury with a negative T wave in an anterior and inferior wall with ST-segment elevation of 1 mm in aVF with typical angina chest pain (Figure 3). Due to clinical deterioration with hypotension and electrical findings of ACS, she was admitted to the CCU.

Laboratory tests showed the following: serum hsTnT 381 ng/L (normal value < 14 ng/L); creatine kinase 137 U/L; serum glucose 29 mmol/L and no abnormalities in electrolytes, renal function, and routine blood tests. At the time of hospitalization, analysis of blood tryptase was not available. Methylprednisolone, chloropyramine, and dual antiplatelet treatment (acetylsalicylic acid 100 mg, clopidogrel 75 mg with enoxaparin 2 × 0.6 ml subcutaneously) were given. During the next hospital day, our patient's symptoms gradually improved with the maintenance of ECG changes.

Transthoracic echocardiography showed hypokinesis of the medial and apical septum and anterior wall with reduced left ventricular systolic function (EF 40%) and minor ischemic mitral insufficiency. Coronary angiography was postponed due to a hypersensitivity reaction to iodine contrast, withdrawal of symptoms, and stabilization of vital signs. During the further hospital stay, the patient did not have chest pain nor signs and symptoms of heart failure, with normalization of the troponin level. She was discharged stable after seven days.

After four weeks, at the Allergy and Immunology Clinic, drug provocation tests with alternative iodine contrast (ioversol) were performed and were negative. The patient was allowed to use the tested iodine contrast with a corresponding premedication. Following an allergological examination, elective coronary angiography was scheduled. Due to the COVID-19 pandemic, an invasive examination was postponed due to the patient's stable status. A year after the event, selective coronary angiography was scheduled. An angiogram showed a two-vessel disease with

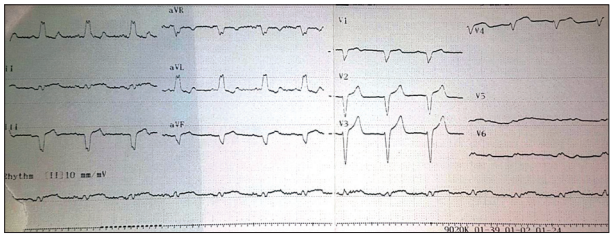

Figure 4. Electrocardiogram after administration of the anesthetic showing left bundle branch block pattern

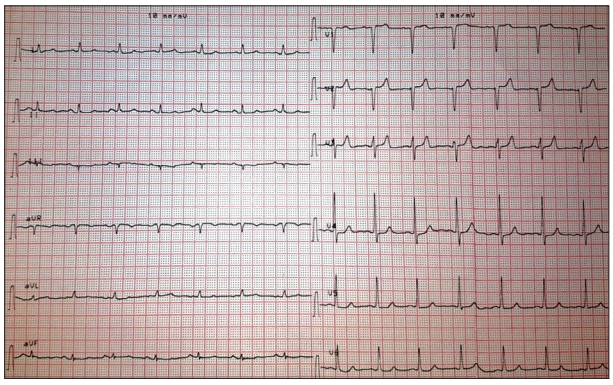

Figure 5. Electrocardiogram on the second hospital day, the same as the preoperative findings

stenosis of the proximal LAD of 80–90% and medial RCA of 70–90%. The percutaneous coronary intervention of the proximal LAD and medial RCA with implantation of two drug-eluting stents was performed.

Case No. 3

An 80-year-old female with known hypertension, type II diabetes mellitus, hyperlipidemia, and chronic obstructive pulmonary disease presented to ED with chest pain, palpitation, and skin rash, which developed shortly after administration of intravenous anesthetic propofol during the elective surgical intervention of malignant colon tumor. Shortly after administration of the drug, the patient was hypotensive (90/60 mmHg). The anesthesiologist administered her methylprednisolone and chloropyramine. The ECG demonstrated a new left bundle branch pattern (Figure 4). The intervention was canceled, and the patient was transferred to ED and further to the CCU with normal arterial pressure without ACS symptoms. Her hsTnT was elevated at 96 ng/l, and the patient received dual antiplatelet therapy with low-molecular-weight heparin, an antihistaminic, and corticosteroids. At the time of hospitalization, the analysis of blood tryptase was not available. On the second day of hospitalization, her ECG returned to the prior normal recorded level (Figure 5).

Transthoracic echocardiography showed a standard dimension of the left ventricle without regional wall motion abnormalities and with borderline systolic function (EF 50%) and minor mitral insufficiency. The patient had no previous history of allergy reactions. Furthermore, until this operation, she had no interventions under total anesthesia.

Coronary angiography was performed. One-vessel disease with the proximal LAD stenosis of 50% and medial sub-occlusion of the LAD of 90–99% was found. After multidisciplinary team consultation, she was transferred to the Clinic for Cardiac Surgery and underwent myocardial revascularization with the left internal mammary artery on the LAD. Previously, allergologic testing for sensitivity to other anesthetics was made. After a short recovery period, she underwent a successful operation on a colon tumor.

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. Written consent to publish all shown material was obtained from the patients.

DISCUSSION

KS is induced by the activation of mast cells and platelets, interacting with inflammatory cells, such as macrophages and T-lymphocytes, leading to a discharge of inflammatory mediators, like histamine, platelet-activating factor, arachidonic acid products, and various cytokines and chemokines during the allergic activation course [8].

Our cases support KS as a recognizable phenomenon by showing three forms of type II KS with myocardial infarction after exposure to different allergens. Iodine contrast, bee sting, and intravenous anesthetic led to the development of allergic myocardial infarction. Our patients had different ECG changes, all with elevated hsTnT levels at the initial presentation.

Furthermore, in Case 1, the patient had no chest pain or typical skin rash known to be an allergic reaction following a bee sting. Without knowing of insect bites, KS would not have been considered. In Case 2, multiple exposures to iodine contrast led to the development of KS with consequent obstacles in further cardiovascular diagnosis and treatment. Also, in Case 3, there were only transient ECG changes and a few symptoms.

This unique disease should be thought of when allergic symptoms, electrocardiographic changes, and elevated cardiac enzymes accompany an acute onset chest pain. All patients referred to ED with chest pain and ST-elevation on ECG should be examined for allergic events. Also, every patient with an allergic reaction in the ED should have an ECG recording.

Dealing with both cardiac and allergic symptoms simultaneously makes treating KS challenging. Treatment with corticosteroids and antihistamines alleviates symptoms in patients with type I KS. For hypersensitivity-induced vasospasm, drugs of choice are vasodilators, such as calcium-channel blockers [9]. Along with type II and type III KS, all standard protocols for ACS should be applied [10.11]. In contrast, the use of morphine and other drugs known to have histamine-liberating properties should be avoided due to the potential of aggravating histamine-induced vasospasm, while epinephrine use must be done with caution due to its potentially vasospastic effect on coronary arteries [11].

Mast cells in allergic reactions interact with macrophages and T-lymphocytes. These cells produce and store secretory granules, released when specific antigens react with IgE antibodies attached to them, inducing degranulation (releasing histamine, leukotrienes, proteases, chymases, and many more mediators) [10]. This process occurs only in around 10% of atopic individuals. All these preformed and newly synthesized inflammatory mediators released locally and running into systemic circulation can cause either coronary artery spasm, which could progress to acute myocardial damage, or coronary plaque erosion or thrombosis, which establishes the main clinical manifestations of KS [11, 12, 13].

KS is doubtlessly a common disease. So far, many case reports support its presence, but knowledge about the pathophysiology still needs to be improved. More studies are required to enhance proper diagnostics and treatment strategies. Correct and prompt treatment is necessary to improve patient prognosis and outcome. In addition to cardiological evaluation and treatment, it is essential to conduct an adequate allergological examination to identify potential disease triggers. We emphasize the importance of ECG in allergic reactions and vice versa, consideration of allergic episodes in patients with ACS.

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Conflicts of interest: None declared.

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Кунисов синдром као узрочник акутног коронарног синдрома

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САЖЕТАК

Увод Кунисов синдром представља акутни коронарни синдром изазван реакцијом преосетљивости. Описан први пут 1991. год. од стране Куниса и Завраса, данас представља ретко дијагностикован клинички синдром. Описне су три различите варијанте Кунисовог синдрома: тип I – вазоспастична алергијска ангина, тип II – алергијски инфаркт миокарда и тип III – тромбоза коронарног стента.

Прикази болесникâ У овом раду приказана су три различита случаја Кунисовог синдрома типа II која су довела до анафилактичког акутног коронарног синдрома. У првом приказу, 66-годишња жена је добила симптоме у виду диспнеје, несвестице и бола у грудима, после уједа пчеле, уз електрокардиографске промене карактеристичне за акутни коронарни синдром. У другом приказу, 64-годишња жена је добила болове у грудима после анафилактичке реакције током употребе јодног контрастног средства у склопу дијагностичке процедуре (компјутеризована томографија грудног коша). У трећем приказу, 80-годишња жена је добила болове у грудима, палпитације и осип по кожи руку и трупа после апликације интравенског анестетика пропофола током елективног хируршког лечења малигне болести дебелог црева. Све болеснице су лечене у Клиници за кардиологију Универзитетског клиничког центра Србије.

Закључак Основа механизма Кунисовог синдрома јесте ослобађање медијатора запаљења током хиперсензитивне реакције изазване различитим узрочницима. Иако је овај синдром прихваћен као узрочник акутног коронарног синдрома, неопходно је константно подсећање да треба мислити и на овај узрок настанка исхемије миокарда.

Кључне речи: акутни коронарни синдром; хиперсензитивна реакција; анафилакса; Кунисов синдром

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Signet-ring colorectal carcinoma

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SUMMARY

Introduction Colorectal cancer is the third most common cancer worldwide. Signet-ring carcinoma is an extremely rare subtype of colorectal cancer, with frequency ranges 0.3–4.6%. The diagnosis of this type of cancer is based on pathohistological analysis.

Case outline A 58-year-old patient was admitted due to abdominal pain and abdominal swelling. The physical findings indicated abdomen above the level of the chest, soft, painfully sensitive in the left hemiabdomen, with positive clinical signs of ascites. Laboratory analyzes indicated positive inflammatory syndrome, elevation of D-dimer and CA-19-9. Ascites analysis showed the presence of malignant cells. Computed tomography revealed hepatomegaly, liver steatosis, as well as multiple secondary deposits in the liver, ascites, and peritoneal implants. Colonoscopy showed ulceration of the right colon, which was covered with fibrin. The pathohistological findings indicated poorly differentiated, invasive adenocarcinoma of the signet ring carcinoma type. The patient was treated with analgesics, diuretics, proton pump inhibitors, beta 2 blockers, angiotensin-converting enzyme inhibitors, low-molecular-weight heparin, antibiotics, and supportive therapy. The patient was discharged after 10 days of hospitalization. He was presented to the multidisciplinary team, which decided on further symptomatic therapy.

Conclusion Signet-ring colon cancer is a rare, aggressive tumor with a poor prognosis. Although it is most often localized in the stomach, it is necessary to think about the colorectal localization of this tumor in the differential diagnosis of patients with colonic complaints, especially if they have "alarm symptoms" and if they are younger.

Keywords: colorectal cancer; signet-ring carcinoma; peritoneal carcinomatosis; ascites; colonoscopy

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide [1]. In terms of mortality, it ranks third, in both sexes [1]. Mortality from CRC is declining, except in Japan, where it is stable [2].

The most common CRC is adenocarcinoma. There are three main types of colorectal adenocarcinoma: conventional adenocarcinoma (AC), mucinous adenocarcinoma, and signet-ring adenocarcinoma (SRAC) [3, 4].

Signet-ring carcinoma is an extremely rare subtype of CRC [3, 5, 6]. Its frequency ranges 0.3–4.6% [3, 7, 8]. Five-year survival ranges 11–46% [9]. This subtype of colon cancer was first described by Laufman and Saphir [10] in 1951.

CASE REPORT

A 58-year-old patient was admitted to the Department for Gastroenterology due to abdominal pain and abdominal swelling. The complaints started a month before admission. The pains were localized in the entire abdomen, occasionally occurring in the left lumbosacral region and spreading to the left leg. The pain occurred in bursts, of the "tearing and pulling" type, with an intensity of 8/10. It got worse when lying down. Stool discharges occurred every two to three days, stools were normally formed, without blood and mucus. Due to the aforementioned complaints, the patient was initially examined by a general practitioner, ultrasound and abdominal and pelvic computed tomography (CT) were performed, and the patient was referred to our clinic for further diagnosis and therapy.

In his medical history, the patient had a cerebrovascular insult two months previously. He has dilated cardiomyopathy, extrasystolic arrhythmia, and hyperlipoproteinemia. He did not take regular therapy, had no surgeries and no allergies. There were no hereditary diseases. The patient was a former smoker, did not consume alcohol.

On admission, the patient was conscious, oriented, afebrile, eupneic, acyanotic, anicteric, hemodynamically stable (TA 120/80 mmHg, HR 90 beats/minute), with normal nutrition. The head and neck were normal, as were auscultatory findings on the heart and lungs. The abdomen above the level of the chest was soft, painfully sensitive in the left hemiabdomen. The liver and the spleen were not palpable. He was positive for clinical signs of ascites. Rectal examination was normal. The findings on the extremities were normal.

Laboratory analyses on admission are shown in Table 1. Malignant cells were detected by the cytological analysis of ascites. Electrocardiogram

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Table 1. Laboratory analyses at admission

Variable	SI unit	Value	Reference range
Leukocytes	10^9/L	22.7	4–10
Erythrocytes	10^{12}/L	5.24	3.8–6
Hemoglobin	g/L	148	115–165
Mean corpuscular volume	fL	83	80–98
Platelets	10^9/L	440	150–400
Blood glucose	mmol/L	6.2	4.1–5.6
Urea	mmol/L	7.4	3–9.2
Creatinine	μmol/L	61	53–115
Sodium	mmol/L	133	136–146
Potassium	mmol/L	5.3	3.5–5.1
Chlorides	mmol/L	90	98–107
Total bilirubin	μmol/L	14.8	3.4–20.5
Aspartate-transaminase	IJ/L	60	11–34
Alanine-transaminase	IJ/L	65	< 45
Gamma-glutamyl transferase	IJ/L	220	< 55
Alkaline phosphatase	U/L	155	40–150
Lactate-dehydrogenase	IJ/L	823	125–220
Total proteins	g/L	73	64–83
Albumins	g/L	47	35–50
Amylase	IJ/L	25	28–100
Lipase	IJ/L	11	< 60
C-reactive protein	mg/L	97.5	< 5
Alpha-fetoprotein	IU/ml	1.6	< 7
Carcinoembryonic antigen	ng/mL	< 0.5	< 10
Carbohydrate antigen 19-9	U/ml	48.87	< 37
Free prostate-specific antigen / total prostate-specific antigen		0.3	> 0.2
B-type natriuretic peptide	pg/ml	1475	< 125
Prothrombin time	s	1.15	11.7–15.5
Activated partial thromboplastin time	s	29.5	24.8–34.4
D-dimer	mg/L	9.88	< 0.7

showed sinus rhythm, right bundle branch block, and q in D2, D3, and aVF. A chest X-ray showed a banded, oblique shadow between the middle and lower left lung fields.

Abdominal ultrasonography showed an enlarged, inhomogeneous liver with multiple heteroechoic lesions with a hypoechoic halo. Ascites was present in the abdomen, as well as diffuse edema of the mesentery. Along the anterior abdominal wall, hypoechoic oval changes with a diameter of 11 mm were visualized.

CT of the abdomen and pelvis was performed before admission to our clinic. The findings indicated hepatomegaly, liver steatosis, as well as multiple secondary deposits in the liver. Ascites and numerous cystic necrotic peritoneal implants were present, predominantly along the anterior and lateral abdominal wall. The colon and rectum were without signs of infiltration, with transverse colon diverticulum and bilateral iliac lymphadenopathy. In addition to the above, an infrarenal aortic aneurysm was detected.

Esophagogastroduodenoscopy showed hyperemia of the antral mucosa with suspicious areas of focal atrophy, hiatus hernia and angiodysplasia of the D2 part of the duodenum, 5 mm in diameter. Pathohistological findings of gastric biopsies indicated reactive gastropathy of the antral mucosa. *H. pylori* status was negative.

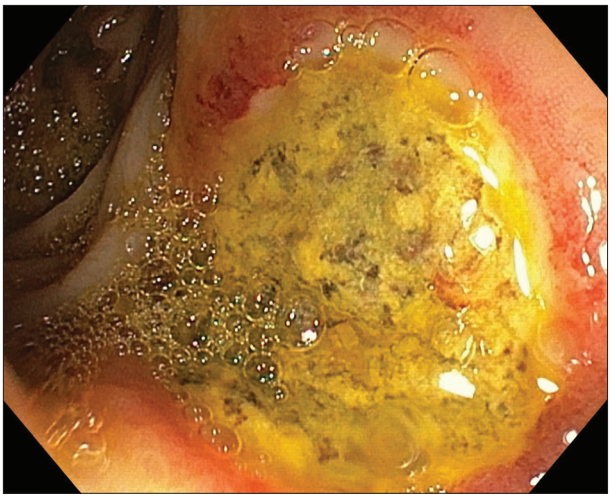

Figure 1. Ulceration of the right colon, covered with fibrin (histopathology: poorly differentiated, invasive adenocarcinoma of the signet-ring carcinoma type)

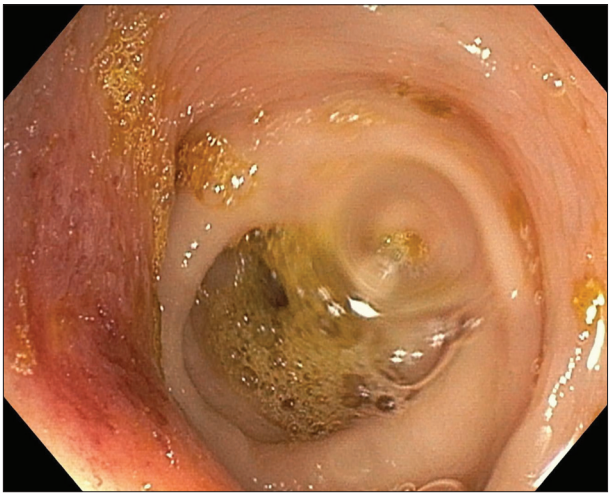

Figure 2. Hyperemic induration of the colonic mucosa (histopathology: interstitial chronic colitis grade I)

A colonoscopy was performed. At 90 cm from the anocutaneous line, an ulceration was detected, covered with fibrin (Figure 1). Biopsies were taken, the pathohistological findings indicated poorly differentiated, invasive adenocarcinoma of the signet-ring carcinoma type. At 45 cm from the anocutaneous line, a hyperemic induration of the mucosa was detected, which is harder during biopsies (Figure 2). Pathohistological findings indicated interstitial chronic colitis (grade I). In addition to the above, diverticula of the transverse colon were detected.

The patient was treated with analgesics, diuretics, proton pump inhibitors, beta 2 blockers, angiotensin-converting enzyme inhibitors, low-molecular-weight heparin, antibiotics, and supportive therapy. The initial analgesics were tramadol, fentanyl, and metamizole, after which tapentadol (50 mg, q6h.), paracetamol IV (1000 mg, q8h) and morphine IV (5 mg, as needed) were administered.

The patient was discharged after 10 days of hospitalization, in a stable general condition. He was presented to the multidisciplinary team, which decided on further symptomatic therapy.

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

DISCUSSION

We present a case of a 58-year-old patient with colonic SRAC. It is worth noting that SRAC occurs more often in the younger population [5, 7, 8, 11, 12, 13]. Cases have also been described in the pediatric population, where CRC is uncommon [14, 15, 16].

In addition to the difference in frequency and time of presentation, there are differences in clinicopathological characteristics between colonic AC and SRAC. Namely, SRAC is more often localized in the right colon [4, 8, 17]. It occurs in the rectum in 20% of patients [7]. In some earlier papers, it was described that the most common localization of this type of cancer is the rectum [18]. In our patient, the cancer was localized in the right colon. Compared to conventional adenocarcinoma, SRAC colon is a more aggressive tumor, usually of larger dimensions, of higher histopathological grade with frequent neurovascular infiltration and involvement of lymph nodes, and greater metastatic potential [8, 11, 19]. In general, the prognosis of this type of tumor is worse than that of colon adenocarcinoma [4, 7, 8, 11, 19]. In addition to being a primary colon cancer, SARC can also be a metastasis of this cancer from other organs (e.g., the stomach) [20].

The clinical presentation of SRAC is similar to conventional colon adenocarcinoma. Very often, patients are asymptomatic. If they are symptomatic, the dominant symptoms are changes in bowel habits (constipation, diarrhea), blood in stool, symptoms/signs of anemia, etc. [7]. Symptomatology is primarily determined by tumor localization. If the tumor is localized in the right colon, it can be asymptomatic until obstruction or perforation occurs. This localization of the tumor can be manifested only by symptoms/signs of anemia [21]. If there is a distal location of the tumor, the dominant symptoms are changes in bowel emptying and blood in stool. Given that the tumor primarily involves the wall of the large intestine, the mucosa may be intact, and the fecal occult blood test may be negative [9]. SRAC colon metastasizes more often in the peritoneum than in the liver [8, 11]. Peritoneal dissemination occurs in approximately 50% of patients [7]. After the lymph nodes, the peritoneum is the most common site of SRAC metastasis [18, 22]. Our patient did not have symptoms typical of colon cancer, which is most likely due to the proximal localization of the tumor. The dominant symptoms were severe abdominal pain and abdominal swelling. The aforementioned complaints are the result of peritoneal dissemination of the disease. Due to the lack of any other complaints, the disease was diagnosed at an advanced stage.

The diagnosis of colonic localization of SRAC is established on the basis of colonoscopy. This type of cancer causes ulcerative lesions in 41.2% of cases [19], which was also the case with our patient. In addition to ulcerative forms, SRAC can also have infiltrative and exophytic forms [19, 23]. Endoscopically, this cancer can look like a depressed, polypoid or, rarely, a flat lesion [17]. Also, the early stage of SRAC can be in the form of a discolored flat-elevated lesion with a central depression [17]. In our patient, another lesion was detected colonoscopically, more distal than the previous one, which macroscopically could correspond to SRAC; however, this was not confirmed by histopathology. The histopathological characteristic of SRAC is the presence of > 50% of tumor cells containing intracytoplasmic mucin [7, 19]. Mucin causes peripheral displacement of the nucleus [8, 19]. This configuration of cells is called a signet ring. The molecular features of SRAC differ from conventional colon adenocarcinoma. Namely, in SRAC there is a higher frequency of microsatellite instability, loss of heterozygosity (e.g., 18q, 3p14.2, etc.), K-ras codon 61 and b-Raf V600E mutations [7, 23, 24].

Most SRAC is diagnosed on CT as a thickening of the longer segment of the colon, while an intraluminal mass is rarely present [19]. This is due to the infiltrative nature of this tumor. Namely, the tumor infiltrates the entire thickness of the wall of the affected segment of the colon, leading to a thickening of the wall. As a result, a rigid and contracted wall occurs, which is a feature of *linitis plastica* [25]. In our patients, CT indicated the presence of peritoneal dissemination of the disease and ascites, while thickening of the colon wall was not detected.

The only curative treatment modality for SRAC is surgery. Surgery can be curative only if it is applied in the early stages of the disease, which is quite rare because this type of cancer is most often diagnosed in advanced stages [6, 22]. An initial treatment with endoscopic mucosal resection was described, however, due to the risk of disease dissemination, the patients subsequently underwent laparoscopic resection [17]. In advanced stages, chemotherapy (5-fluorouracil, capecitabine, oxaliplatin, irinotecan, bevacizumab) can be used with varying success [7, 26, 27, 28]. In the case of peritoneal dissemination, the use of cytoreductive surgery and hyperthermic intraperitoneal chemotherapy has been described [28, 29]. However, there are no clear recommendations.

Signet-ring colon cancer is a rare, aggressive tumor with a poor prognosis. Although it is most often localized in the stomach, it is necessary to think about the colorectal localization of this tumor in the differential diagnosis of patients with colonic complaints, especially if they have "alarm symptoms" and if they are younger.

Conflict of interest: None declared.

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Карцином колона типа печатног прстена

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САЖЕТАК

Увод Карцином дебелог црева је трећи најчешћи карцином у свету. Карцином печатног прстена је изузетно редак подтип колоректалног карцинома, чија учесталост износи 0,3–4,6%. Дијагноза ове врсте карцинома је базирана на патохистолошкој анализи.

Приказ болесника Болесник стар 58 година примљен је на лечење због болова у стомаку и отока стомака. Физикални налаз је указао на абдомен изнад нивоа грудног коша, мек, палпаторно болно осетљив у левом хемиабдомену, са позитивним клиничким знацима асцитеса. Лабораторијски је установљен позитиван запаљенски синдром и повишен *D*-димер и карбохидратни антиген-19-9 (*CA*-19-9). Анализа асцитеса је указала на присуство малигних ћелија. Компјутеризованом томографијом су откривени хепатомегалија, стеатоза јетре, као и бројни секундарни депозити у јетри, асцитес и перитонеални имплантати. Колоноскопијом је откривена улцерација десног колона, која је била прекривена фибрином. Патохистолошки налаз је указао на слабо диферентован, инвазивни аденокарцином типа карцинома печатног прстена. Болесник је лечен аналгетицима, диуретицима, инхибиторима протонске пумпе, бета-2 блокаторима, инхибиторима *ACE*, хепарином ниске молекулске масе, антибиотицима и супортивном терапијом. Отпуштен је после 10 дана хоспитализације. Представљен је онколошком конзилијуму, који је донео одлуку за даљу симптоматску терапију.

Закључак Карцином колона типа печатног прстена је редак, агресиван тумор са лошом прогнозом. Иако је најчешће локализован у желуцу, о колоректалној локализацији овог тумора је потребно размишљати у диференцијалној дијагнози код болесника са колопатским тегобама, нарочито уколико су удружене са „алармним симптомима" и ако се јављају у млађој популацији.

Кључне речи: колоректални карцином; карцином печатног прстена; карциноматоза перитонеума; асцитес; колоноскопија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Surgical treatment of peri-implant femoral fractures – case report and literature review

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SUMMARY

Introduction Peri-implant femoral fractures (PIFF) are defined as fractures of the femur with the presence of previously implanted non-prosthetic osteosynthetic material.
A review of available literature revealed that there are several proposed classifications and sets of guidelines for surgical treatment of PIFF.

Case outline A 49-year-old patient was injured from a fall on the same level, the day before admission to the hospital. The anamnesis at admission showed that six months earlier, he had sustained a pertrochanteric fracture of the left femur, which had been treated surgically with a short cephalomedullary nail. Two years prior to hospital admission, the patient had sustained a tibial plateau fracture of the same leg, which was treated non-surgically with above the knee cast immobilization. After the fracture had healed, paresis of the peroneal nerve was diagnosed, while subsequent follow-up revealed secondary post-traumatic arthrosis of the knee joint. Reduction and fixation of the fracture was performed on a surgical extension table, with the use of fluoroscopy. Previously implanted osteosynthetic material was removed, a short cephalomedullary nail, and fixation of the fracture was carried out with a long cephalomedullary nail. Six months after the operation, the patient can ambulate independently, without assistance. He reports no pain in the left groin and upper leg but reports pain and limitation of movement in the left knee joint.

Conclusion By reviewing the available literature, we found that the patient was cared for in our hospital in keeping with all current recommendations for surgical treatment of this type of fracture.

Keywords: pertrochanteric fracture; cephalomedullary nail; peri-implant fracture

INTRODUCTION

In the overall number of fractures, the incidence of proximal femur fractures is 14%, of which 42% are transtrochanteric fractures. However, the treatment of proximal femur fractures accounts for 72% of the total cost of treating all fractures [1]. The total annual direct medical costs associated with all hip fractures was $50,508 per patient, resulting in a yearly estimate of $5.96 billion to the U.S. healthcare system. Intertrochanteric hip fractures accounted for an annual estimate of $52,512 per patient, corresponding to an overall annual economic burden of $2.63 billion to the U.S. health-care system and representing 44% of all hip fracture costs [2]. Bearing in mind the increase in life expectancy and the incidence of fractures of the trochanteric region, an increase in the number of peri-implant femoral fractures (PIFF) is to be expected. PIFF are defined as fractures of the femur with the presence of previously implanted non-prosthetic osteosynthetic material [3,4]. These fractures most commonly occur in the elderly. In their study, Vilar-Sastre et al. [5] reported a predominance of elderly women with comorbidities and plate fixation. The incidence of PIFF is 1.7% [6], while according to Halonen et al. [7], it is 1.4%. The decision on the method of surgical management of peri-implant fractures is influenced by several factors – primarily the condition of the initial fracture, i.e., whether it has healed, but also by the type of primary osteosynthesis used (plate or nail fixation), as well as by the location of the new fracture. A review of available literature found several proposed classifications and sets of guidelines for surgical treatment of PIFF [4, 8–11]. The aim of this paper is to present the surgical method of treating PIFF in a younger patient, with reference to the classifications and protocols recommended in literature for the surgical management of these types of fractures.

CASE REPORT

A 49-year-old patient was admitted to hospital due to pain in the left thigh, painful and limited movement of the left hip and knee and shortening of the left leg. He was injured from a fall on the same level, which occurred the day before he was admitted to the hospital. Physical examination and radiography of the pelvis and the left upper leg with the knee joint, in two directions, revealed the presence of a short cephalomedullary nail (Figure 1), a PIFF in the projection of

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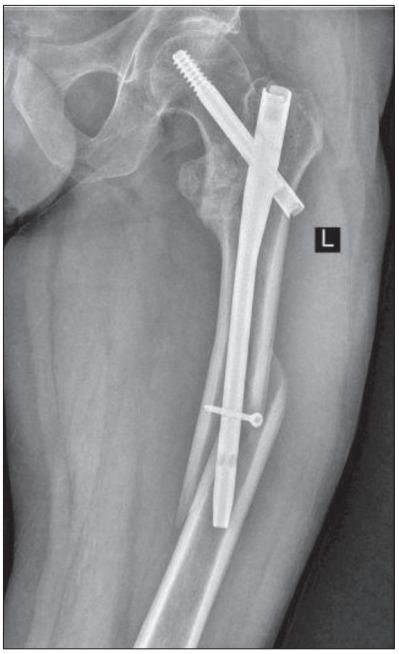

Figure 1. Radiography of the left hip joint and femur on admission; peri-implant femoral fracture at the level of the tip of the nail [source: PACS Bežanijska Kosa UHMC]

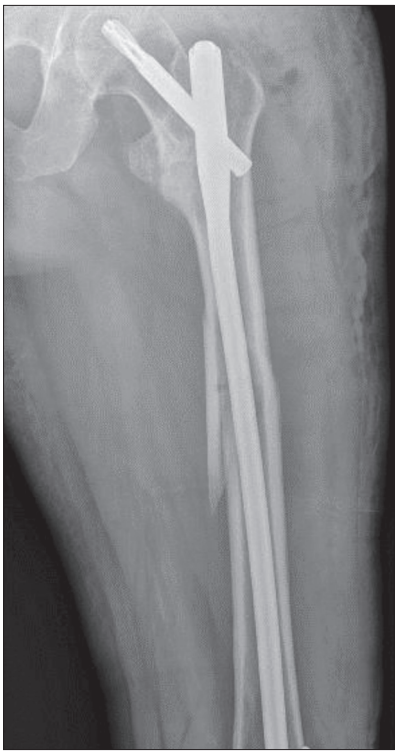

Figure 2. Radiography of the left hip and thigh on the first postoperative day [source: PACS Bežanijska Kosa UHMC]

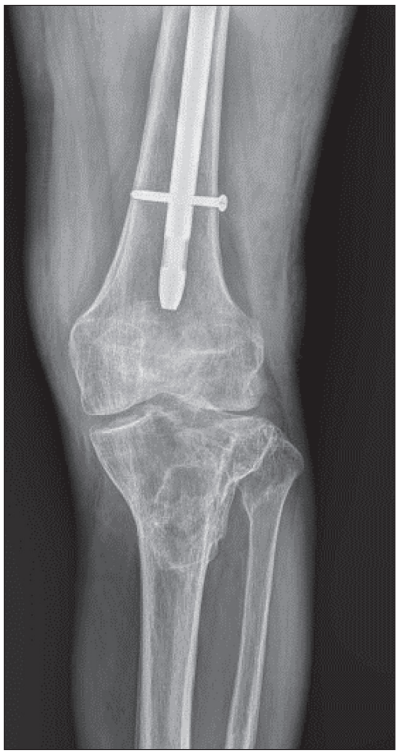

Figure 3. Radiography of the distal end of the femur and the knee joint on the first postoperative day [source: PACS Bežanijska Kosa UHMC]

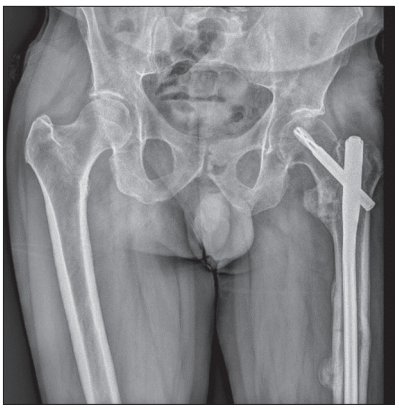

Figure 4. Radiography of the left hip and thigh two months after surgery [source: PACS Bežanijska Kosa UHMC]

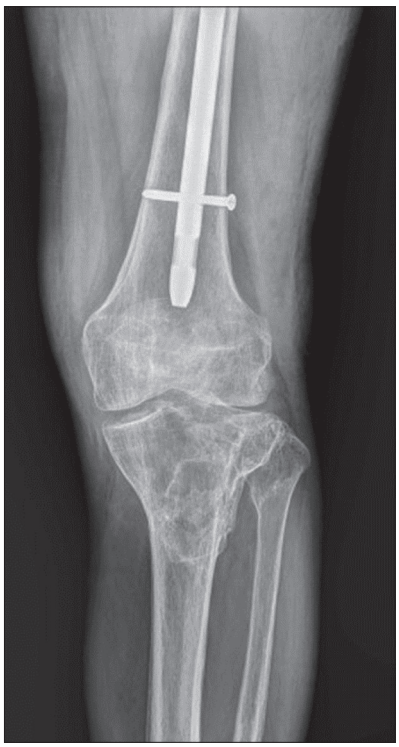

Figure 5. Radiography of the left thigh and knee six months after surgery [source: PACS archive Bežanijska Kosa UHMC]

the tip of the nail, and marked knee joint degenerative changes which we classified as N1A type of fracture according to Chan classification. On admission to the hospital, the patient was fitted with an above-the-knee plaster splint, and analgesic, anticoagulation, and symptomatic therapy was administered. From the anamnestic data taken at admission, we learned that six months before the actual injury, the patient had sustained a pertrochanteric fracture of the left femur, which was treated with a short cephalomedullary nail, at a different hospital. Two years before, during the COVID-19 pandemic, he had sustained a fracture of the tibial plateau of the same leg. He was treated non-operatively at a different hospital, with above-the-knee cast immobilization, after which he developed peroneal nerve paresis. On admission to our hospital, on the X-ray we diagnosed post-traumatic arthrosis of the knee joint. Immediately after admission to the hospital we started with preoperative preparation and planning. An hour before the surgical procedure, two grams of cefazolin were administered. The operation was performed on the orthopedic extension table, with the use of fluoroscopy. We approached the tip of the greater trochanter along the old surgical scar. There we encountered the problem of identifying the proximal end of the nail, due to the fact that during the primary osteosynthesis an end cap was not inserted. After debridement and "release" of the tip of the greater trochanter, we attached the insertion handle, with fluoroscopic guidance. After this, we approached the lag screw through the old surgical scar, removed it, and did the same with the distal static screw. After that, we extracted the nail itself. The removed nail was 240 mm long, 11 mm wide, with a lag screw that was 105 mm long and with a 130° angle. After removing the nail components, swabs of the femoral neck and canal were

taken. With fluoroscopic guidance, we inserted, without femoral canal reaming, a proximal femoral antirotation nail (Proximal Femoral Nail Antirotation – PFNA ® – DePuy Synthes GmbH, Oberdorf, Switzerland), 420 mm long, 12 mm wide, with a 105 mm blade, and an angle of 130°; a distal static screw, 44 mm in length; and an end cap with extension 0 (Figures 2 and 3). Operative wounds were sutured in the standard manner. Physical therapy and rehabilitation of the patient began on the first postoperative day. Walking with crutches was permitted with non-weight bearing on the surgically treated leg. Postoperative recovery was uneventful, the dressings on the wounds were changed regularly, and they healed *per primam*. Swab samples taken intraoperatively were sterile. On the seventh postoperative day the patient was discharged in good general condition. The sutures were removed in the outpatient clinic of our hospital, on the 13th postoperative day. Upon the completion of stationary physical therapy, two months after surgery, the patient was ambulatory with the help of an axillary crutch, used with the opposite, i.e., right arm. Radiographic evidence of healing was visible (Figures 4 and 5) and the patient was, therefore, allowed to walk with full weight bearing on the surgically treated leg, with the support of a cane. At the six-month follow-up, the patient was able to walk independently, without walking aids, but complained of severe pain in the left knee.

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

DISCUSSION

A PIFF in the projection of the tip of the cephalomedullary nail indicates that there was a "stress riser" in that location [4]. Bearing in mind the anamnestic data confirming that directly before the fall the patient had been ambulatory without walking aids, but with pain and limited movement of the knee joint, as well as the X-ray of the injured upper leg and hip at admission, we concluded that the pertrochanteric fracture had healed. According to the proposed classification by Chan et al. [4], we classified this fracture in the N1A group, i.e., in group 32BNP according to Videla et al. [8, 9]. Therefore, as an option for surgical treatment, the possibility of replacing the short cephalomedullary nail with a long intramedullary nail was considered. However, removal of the lag screw would have left a "cavity" in the neck and would potentially represent a weak point at the primary fracture site, so although classified as N1A, we treated the fracture as an N1B type, which is in keeping with the recommendations [12]. Therefore, we decided to replace the existing short nail with a long cephalomedullary nail, with the same angle of 130°, but with a larger diameter (12 mm), without prior femoral canal reaming, because we took care not to damage the endosteal vascularization of the femur. Also, we locked the nail distally, as unlocked nails do not guarantee sufficient stability [13]. One of the potential methods of surgical treatment was the use of a distal femoral plate with locking screws and the use of cables, but due to the extensiveness of the approach and the presence of secondary, post-traumatic arthrosis of the knee joint, we abandoned that option. Considering the clinical and radiographic signs of post-traumatic knee arthrosis, the plan is to replace the degenerative joint with an artificial one. The inserted end cap will allow easier access to the tip of the greater trochanter and the nail itself. This will facilitate the removal of the cephalomedullary nail, which is necessary, in order to perform the implantation of a total endoprosthesis of the knee. PIFFs most often occur in the elderly population. In the case presented here, the most likely cause of PIFF due to low-energy trauma in a person of a younger age is a stress riser on the distal end of the nail combined with post-traumatic arthrosis of the knee joint, accompanied by severe pain and instability. By reviewing the available literature, we found that the patient was cared for in our hospital in keeping with all current recommendations for surgical treatment of these types of fractures. However, the replacement of a short nail with a long one, after PIFF at the tip of a short nail, may be associated with increased patient morbidity [14]. Surgical treatment of PIFF is a challenge because the fracture occurs in the presence of pre-existing non-prosthetic implanted material, often accompanied by osteoporosis, and there is also a high risk of iatrogenic fracture. All this becomes even more significant when we take into account the fact that orthopedic trauma associations still have no uniform position regarding the method of classification and the treatment protocol for these fractures.

Conflict of interest: None declared.

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Хируршко лечење периимплантних прелома бутне кости – приказ болесника и преглед литературе

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САЖЕТАК

Увод Периимплантни преломи фемура дефинисани су као преломи бутне кости уз присуство претходно уграђеног непротетског остеосинтетског материјала. Прегледом литературе установљено је да постоји неколико предлога класификација и водича за оперативно лечење периимплантних прелома фемура.

Приказ болесника Болесник стар 49 година повређен је падом на истом нивоу дан пре пријема у болницу. Анамнестички, на пријему, наводи да је шест месеци пре наведене повреде задобио пертрохантерни прелом леве бутне кости који је лечен хируршки, кратким цефаломедуларним клином. Две године пре пријема болесник је имао прелом горњег окрајка голењаче исте ноге, који је лечен неоперативно, натколеном гипсаном имобилизацијом. По санацији прелома дијагностикована је пареза перонеалног живца, док су накнадни прегледи открили секундарну, посттрауматску артрозу зглоба колена. Репозиција прелома и његова фиксација изведена је на екстензионом столу под контролом флуороскопа. Том приликом је одстрањен претходно имплантирани остеосинтетски материјал – кратки цефаломедуларни клин, а прелом је фиксиран дугим цефаломедуларним клином.

Шест месеци после операције болесник је могао да се креће самостално, без помоћи. Негирао је бол у левој препони и натколеници, али је наводио бол и ограничење покрета у зглобу левог колена.

Закључак Прегледом доступне литературе установили смо да је болесник у нашој установи збринут по свим тренутно актуелним препорукама за хируршко лечење овог типа прелома.

Кључне речи: пертрохантерни прелом; цефаломедуларни клин; периимплантни прелом

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Autopsy findings in a fetus with monosomy 20 mosaicism

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SUMMARY

Introduction Mosaic monosomy 20 is a rare chromosomal aberration, without characteristic clinical features. We present a case of a fetus with monosomy 20 mosaicism revealed after prenatal ultrasound detection of anhydramnios and multiple anomalies.

Case outline The second pregnancy of a 33-year-old woman, was terminated at 23rd gestational week, because of the multiple fetal anomalies and anhydramnios, detected by ultrasound. The autopsy of a female fetus revealed multiple congenital anomalies: ventriculomegaly, bilateral choroid plexus cysts, perivascular gliosis in periventricular region of cerebri, hydropericardium, severe cardiomegaly, severe myocardial hypertrophy, hydrothorax, glandular/canalicular stage of fetal lung development, bilateral renal and ureter agenesis (Potter syndrome), bladder aplasia, agenesis of the uterus, fallopian tubes and proximal vagina and valgus deformity of left foot (*pes valgus*). Fetal growth was adequate for gestational age with no craniofacial dysmorphia or radiographically visible anomalies of the skeleton, without signs of infection. The umbilical cord was too long for gestational age – 48 cm. Analysis of fetal karyotype from fetal blood sampling revealed monosomy of chromosome 20 in 10% of analyzed cells in metaphase.

Conclusion Revealing the genetic basis of fetal anomalies is at outmost importance not only for further evaluation of pregnancy, but also for proper genetic informing of patients.

Keywords: fetus; autopsies; monosomy of chromosome 20

INTRODUCTION

Chromosomal mosaicism is the presence of two or more genetically distinct cell lines. It may occur in various genetic changes, including chromosomal aberrations, single-nucleotide variations or small insertions/deletions. Such changes can either go unnoticed or underlie genetic diseases. Chromosomal mosaicism may refer to the presence of two or more different abnormal cell lines (e.g., aneuploid/aneuploid), or a normal and an abnormal cell line (e.g., euploid/aneuploid) [1].

Mosaicism happens because a mutation occurs after the zygote is created. Frequent mitotic errors after fertilization contribute to prevalent aneuploidy in human embryos, including cell cycle dysregulation, defective chromatid cohesion, and centrosome overduplication [2, 3].

The fitness consequences of mosaicism are less precise than those of meiotic origin – aneuploidy. Just because an embryo is a mosaic does not mean those cell lines will propagate throughout development. The influence of mosaicism during development may depend on the degree of aneuploidy, the tissues involved, and the particular chromosome complement. While mosaicism is associated with adverse pregnancy outcomes, some mosaic embryos are viable, and low-level mosaicism may be a regular feature of human development [3].

Chromosomal mosaicism in pregnancies and live births has been reported for cytogenetic aberrations, including trisomies, monosomies, deletions, duplications and other rare alterations. Mosaicism with the loss of an entire autosome is extremely rare in liveborn babies.

We present a case of a fetus with monosomy 20 mosaicism revealed after prenatal ultrasound detection of anhydramnios and multiple anomalies.

Ethical approval was obtained by the Ethics Committee of the University Clinical Center of Serbia, and the study followed Helsinki Declaration principles (Number 68/14). Written consent was obtained from the patient to publish this case report and any accompanying images.

CASE REPORT

A 33-year-old woman, at 21st gestation week of her second pregnancy, was referred to our clinic because of multiple fetal abnormalities diagnosed at her prior hospital. The couple was both healthy and not consanguineous. They had one healthy child and no family history of genetic diseases or congenital malformations. The mother denied being exposed to teratogenic agents or irradiation during the pregnancy. First-trimester

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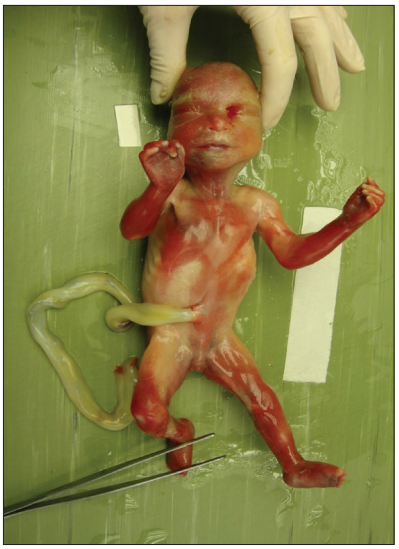

Figure 1. Phenotype of a fetus with monosomy 20 mosaicism

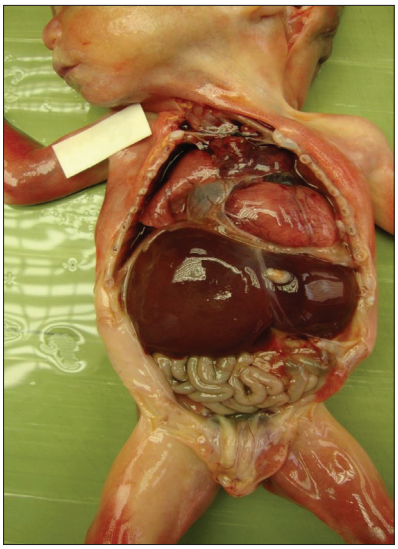

Figure 2. Cardiomegaly and hepatomagaly

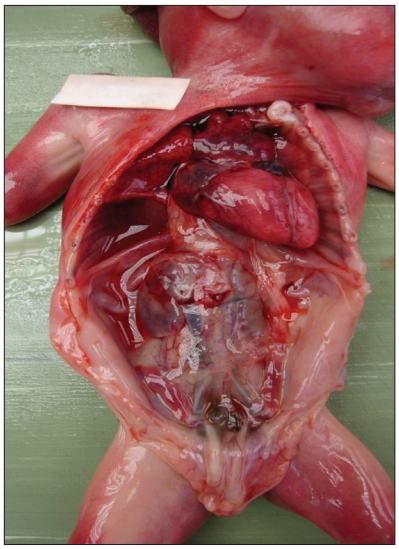

Figure 3. Bilateral renal and ureter agenesis, bladder aplasia, agenesis of the uterus, fallopian tubes, and proximal vagina

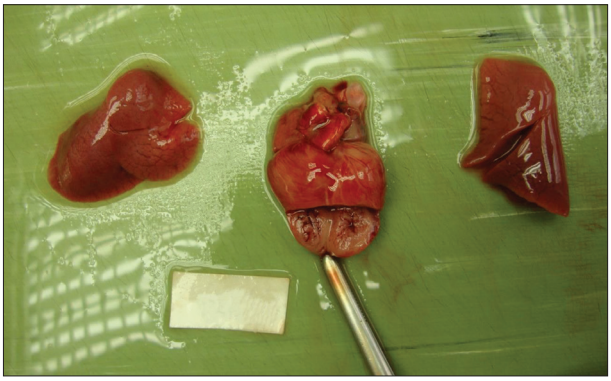

Figure 4. Severe cardiomegaly and myocardial hypertrophy

screening for aneuploidies revealed the low-risk range. No prenatal invasive test was indicated before the patient was observed.

At referral, a fetal comprehensive transabdominal ultrasound exam was performed at 22 weeks of gestation by two experienced maternal-fetal medicine physician sonographers.

Ultrasound examination showed an anhydramnios, ventriculomegaly, bilateral choroid plexus cysts, pleural effusions, fetal heart failure and bilateral renal agenesis.

A sample of fetal blood was analyzed for chromosome abnormalities. The sample was taken by cordocentesis and processed using standard techniques. All specimens were G-banded using trypsin – Giemsa. One hundred metaphase cells were analyzed for chromosomal constitution. In 10 cells (10%), monosomy of chromosome 20 was found, so the karyotype was 45, XX,-20/46, XX (10%:90%). In addition, the parental karyotypes were normal.

On the parent's demand, after genetic counselling and ethics committee approval, the pregnancy was terminated.

Autopsy of a female fetus after inducted abortion (with Prostaglandin E2 and Prostaglandin E3) revealed multiple anomalies: ventriculomegaly, bilateral choroid plexus cysts, perivascular gliosis in periventricular region of cerebri, hydropericardium, severe cardiomegaly, severe myocardial hypertrophy, hydrothorax, glandular/canalicular stage of fetal lung development, bilateral renal and ureter agenesis (Potter syndrome), bladder aplasia, agenesis of the uterus, fallopian tubes and proximal vagina and valgus deformity of left foot (*pes valgus*) (Figures 1–4). Fetal growth was adequate for gestational age with no craniofacial dysmorphia or radiographically visible skeleton anomalies without signs of infection. The umbilical cord was too long for the gestational age – 48 cm.

DISCUSSION

Historically, prenatal diagnosis has focused on detecting chromosomal abnormalities, particularly trisomy 21, using metaphase karyotype.

Pathological biomarkers of the fetus are routinely collected via percutaneous umbilical cord blood sampling. The key applications of this procedure are diagnosis and identification of fetal infections, karyotype analysis, diagnosis of hematologic conditions, fetal growth retardation, and metabolic analysis. This procedure has become more popular recently since it provides direct data on fetal blood status [4].

Clinical testing to determine the underlying etiological factors involved in fetal death currently involves the complex integration of family and obstetric history, radiographic imaging and macroscopic and histological examination of the body and placenta, along with laboratory investigations such as biochemistry, microbiology and genetic testing [5]. Failure to detect low-level mosaicism is a concern since microarrays for detecting genomic imbalances have supplanted karyotyping as the first genomic investigation for patients with developmental delay or multiple congenital anomalies.

A fetal autopsy is the backbone for fetal phenotyping in the molecular era and contributes to the limited data on fetal phenotypes of various genetic disorders. Reverse phenotyping requires detailing fetal characteristics, including dysmorphism, that may not be apparent on ultrasound. Thus, fetal autopsy plays an essential role in better understanding phenotypic and genotypic relationships and complements the field of molecular autopsy in diagnosing genetic diseases [6].

To our knowledge, only five cases of liveborns with monosomy 20 mosaicism have been previously reported. In most cases, only peripheral blood was sampled. The phenotype of patients with monosomy 20 mosaicism ranged from clinically normal to delayed motor and intellectual development, with mild dysmorphic signs and asymmetry. There were no common abnormalities except for an intergluteal cleft asymmetry. There was no correlation between the percentage of aneuploid cells in cultured lymphocytes and the severity of the phenotype in the five patients with monosomy 20 mosaicism, with the highest percentage (25%) found in a normal woman [7, 8].

Stefanou et al. [9] found a significant number of monosomy 20 cells (39 out of 50) in the urine sediment of a boy with bilateral vesicoureteric reflux. They suggested that monosomy 20 causes renal tract abnormalities and trisomy 20. Our case supports this thesis since the fetus we examined had bilateral agenesis of kidneys and ureters, with secondary aplasia of the bladder [9].

Mosaicism arises from mitotic errors occurring after fertilization, during post-zygotic development, usually after the first three cleavage divisions. The best-characterized types of mitotic errors resulting in mosaicism are sister chromatid malsegregations: anaphase lagging, mainly resulting in one normal and one monosomic daughter cell, and non-disjunction, leading to reciprocal trisomic and monosomic daughter cells [10]. The observation that monosomies are commonly found without reciprocal trisomies in mosaic embryos indicates that anaphase lagging might be more frequent than non-disjunction during mitotic errors [11, 12, 13]. The specific method by which mosaicism arises can result in distinctly different outcomes because the impact on fetal development depends on the percentage of mosaicism, specific chromosomes involved, monosomy versus trisomy and inclusion of complete or segmental chromosome mosaicism [11, 12, 13]. We assume that mosaicism anaphase lagging occurred in the case of monosomy 20. If the mosaicism resulted from a cell division error after fertilization, recurrence risk for the mosaic chromosome is very low**.**

The devastating impact of pregnancy loss, terminations and perinatal death on families and the wider community is often compounded by the uncertainty of the cause of death and the subsequent recurrence risk for future pregnancies [5].

We should agree with McCoy [3] that future research should focus on understanding the risks associated with various forms of mosaicism to guide the implementation of genetic screening approaches.

Percutaneous blood sampling allows direct access to the fetal circulation, thus spreading new prenatal diagnosis and therapy areas. Revealing the genetic basis of fetal anomalies is of foremost importance not only for further evaluation of pregnancy but also for proper genetic informing of patients. Identifying a genetic diagnosis in the fetus is valuable to aid in pregnancy management decisions and can be critical for the medical management of the newborn.

Conflict of interest: None declared.

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Аутопсијски налази фетуса са мозаичном монозомијом хромозома 20

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САЖЕТАК

Увод Мозаична монозомија хромозома 20 је ретка хромозомска аберација, која нема карактеристичну клиничку слику, будући да она зависи од процента аберантних ћелија у различитим ткивима.

Приказ болесника Друга трудноћа 33-годишње жене прекинута је у 23. гестацијској недељи због вишеструких аномалија плода и недостатка плодове воде, откривених ултразвучним прегледом. Аутопсијом плода женског пола утврђено је постојање вишеструких урођених аномалија: Потеров синдром – билатерална агенезија бубрега и уретера са секундарном аплазијом мокраћне бешике; агенезија утеруса и вагине; цисте хороидног плексуса; вентрикуломегалија. Плод је био одговарајућег интраутерусног раста за гестацијску старост, без краниофацијалне дисморфије, без радиолошки видљивих аномалија скелета, са знацима инсуфицијенције срца тешког степена, без знакова инфекције. Пупчана врпца била је превелике дужине за гестацијску старост – 48 *cm*. Анализа кариотипа плода из узорка феталне крви открила је монозомију хромозома 20 у 10% анализираних ћелија у метафази.

Закључак Утврђивање генетичке основе конгениталних аномалија важно је како ради даље евалуације трудноће, тако и због исправног генетичког информисања пацијената.

Кључне речи: фетус; аутопсија; монозомија хромозома 20

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

The importance of re-biopsy in the era of molecular therapy for lung cancer

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SUMMARY

Introduction Recent epidemiological data highlight that lung cancer incidence and mortality rates remain alarmingly high globally for both men and women. Over the last 10 years, the evolution in treatment corresponds to identifying specific driver mutations within lung tumors and developing inhibitors targeting these mutations.

Case outline A 73-year-old woman was diagnosed with lung adenocarcinoma staged as T4N2M1b at the Institute for Pulmonary Diseases of Vojvodina in February 2019. The Oncology Board recommended molecular analysis of the tumor and palliative radiation therapy for spinal metastases. Molecular testing identified an exon 19 deletion in the epidermal growth factor receptor (*EGFR*) gene. Following radiation treatment of the spine metastases, the patient began treatment with afatinib in May 2019. After 35 cycles of the aforementioned therapy, in April 2022, a computed tomography scan of the thorax and abdomen showed that the disease had progressed. Despite three liquid biopsies failing to detect the T790M mutation, a subsequent bronchoscopy and tissue re-biopsy confirmed its presence, prompting the initiation of osimertinib treatment. Twelve months into osimertinib therapy, the patient continues to be monitored.

Conclusion *EGFR* is a crucial predictive biomarker for non-small cell lung cancer. The introduction of specific tyrosine kinase inhibitors – first-generation agents like gefitinib and erlotinib, second-generation afatinib, and introduction of third-generation (osimertinib or lolatinib) when initial treatments are met with resistance, has led to significant therapeutic breakthroughs.

Keywords: lung adenocarcinoma; liquid biopsy; T790M; tissue biopsy

INTRODUCTION

Current international guidelines recommend conducting molecular testing at the initial diagnosis of advanced non-small cell lung cancer (NSCLC), and it is considered obligatory for cases of lung adenocarcinoma (ADC) [1]. Over recent decades, there has been a significant increase in the identification of oncogenic drivers, facilitating the prediction of clinical responses to targeted therapies in NSCLC. The molecular characterization of lung ADC, as opposed to mere histological classification, has paved the way for fully personalized treatment strategies [2]. In 2004, the epidermal growth factor receptor (EGFR) was recognized as a predictive biomarker for NSCLC [3]. EGFR, a 170-kDa tyrosine kinase receptor and member of the ERbB family, has been associated with mutation-driven oncogenesis in NSCLC. Approximately 10–15% of NSCLC patients with mutations predominantly in exons 19 and 21 exhibit profound responses to targeted inhibition by first-generation tyrosine kinase inhibitors (TKIs) such as gefitinib and erlotinib, the second-generation drug afatinib, and third-generation drugs like osimertinib and lorlatinib [4]. However, prolonged treatment with these TKIs can lead to the development of resistant neoplastic cells due to mutations in the targeted *EGFR* gene, consequently diminishing the initial high expectations for targeted therapy. Specific mechanisms which lead to acquired resistance to TKIs were uncovered. A notable example is the T790M mutation within the *EGFR* gene in lung ADC, which is found in over 50% of patients after treatment with first- or second-generation EGFR TKIs [5]. Subsequent generations of drugs, like osimertinib, have been designed to precisely target this specific mutation [6]. Re-biopsy of the lung tumor tissue has proven to be useful in identifying particular genetic changes and targeted mutations. This process offers more detailed information compared to liquid biopsy and is essential for reassessing patients to ensure the continuation of effective treatment [7]. Illustrating the potential for long-term success with this approach, we report the case of a female patient with metastatic ADC. After developing resistance to the first line of molecular therapy, she was administered a new generation TKI in her second-line treatment regimen, highlighting the evolving landscape of personalized cancer treatment.

CASE REPORT

A 73-year-old non-smoking female with a history of hypertension, complete right bundle

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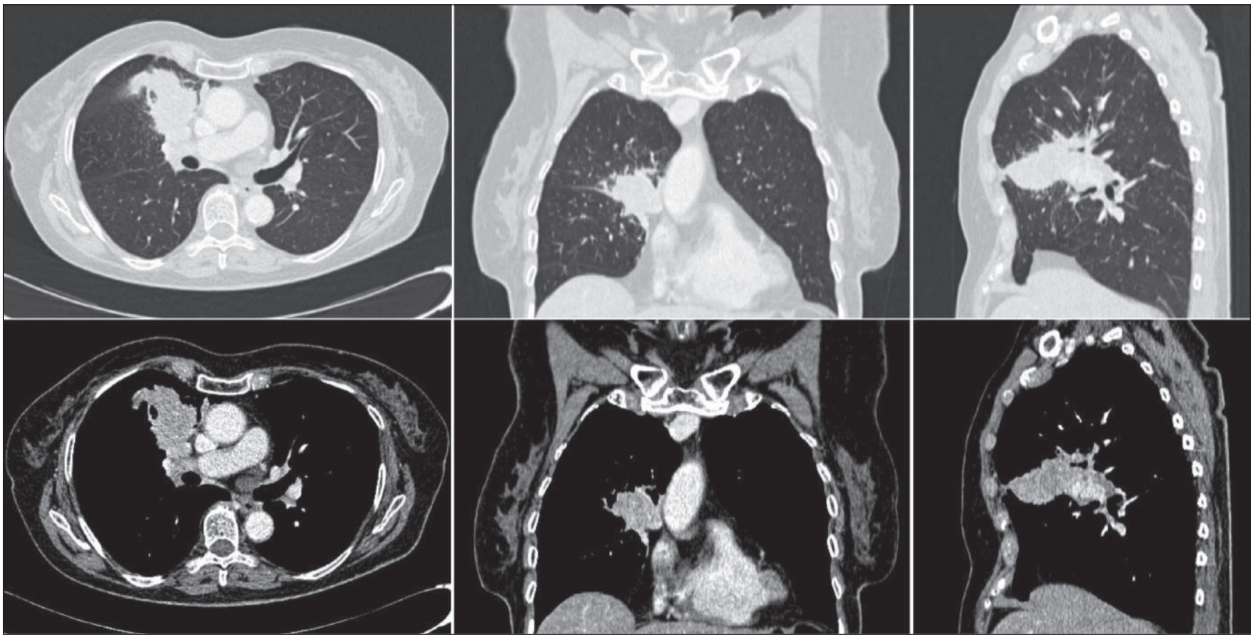

Figure 1. Initial chest CT at the time of diagnosis

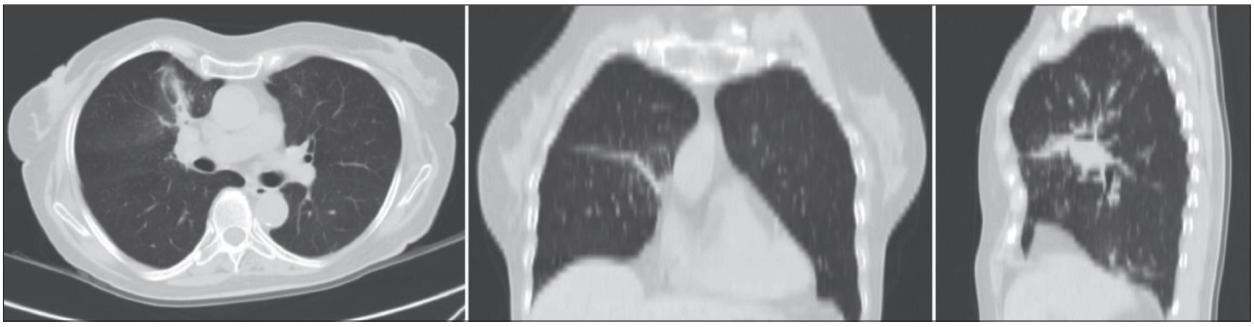

Figure 2. Chest CT after four months of treatment with afatinib

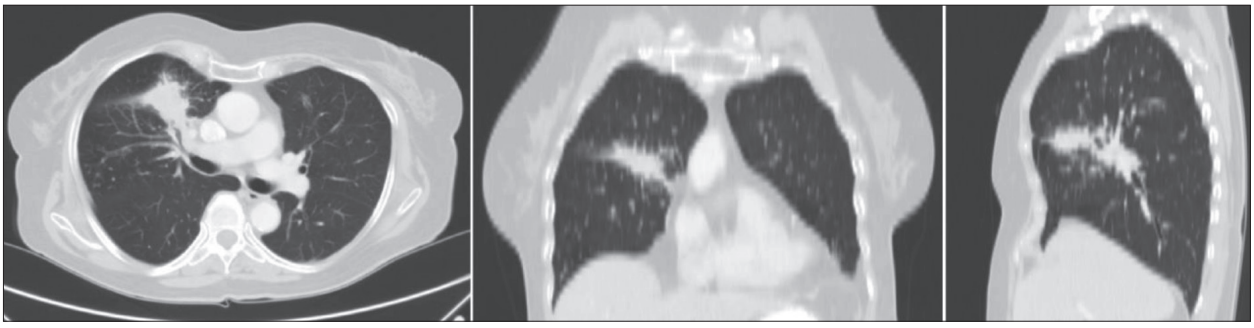

Figure 3. Chest CT at the time of disease progression to the drug afatinib

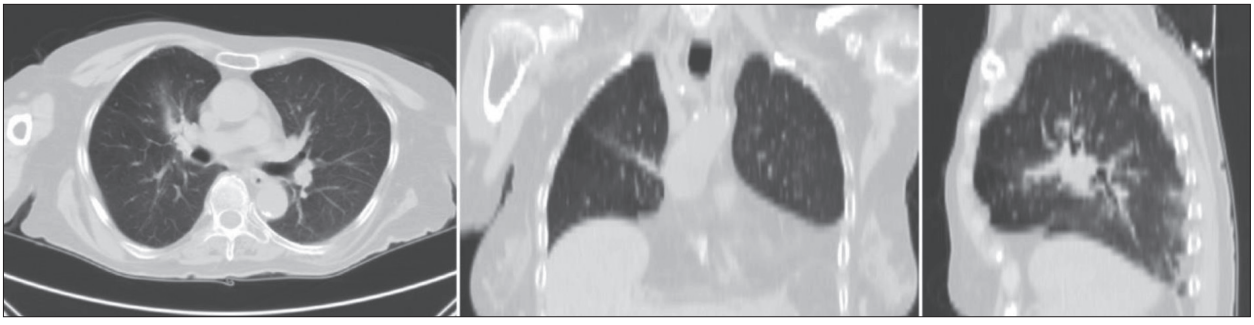

Figure 4. Chest CT after 12 months of treatment with osimertinib

branch block, and type 2 diabetes, developed symptoms in the form of difficulty breathing, and persistent dry cough in January 2019. An initial chest X-ray disclosed a sizable tumor, approximately 7 cm in diameter, located in the right parahilar region. Further investigation using a computed tomography (CT) scan of the chest and abdomen identified an infiltrative mass in the S3 segment of the right upper lung lobe, with dimensions of 78 × 53 mm. The lesion extended into the mediastinal pleura, mediastinal fatty tissue, and interlobar fissure, accompanied by pronounced pneumonitis and lymphangitis. Additionally, bilateral nodular alterations in the lung were evident (Figure 1). There was a suspicion of involvement with potential secondary deposits in the left iliac bone, as well as vertebral levels, VTh11 and VL3–5.

In February 2019, bronchoscopy was performed and the cytological analysis of samples obtained via transbronchial needle aspiration of tumor bronchial compression confirmed the presence of lung ADC. The disease was clinically and radiologically staged as T4N2M1b. In the following month, March 2019, the Oncology Board at the Institute for Pulmonary Diseases of Vojvodina (OB IPBV) recommended molecular testing of the tumor biopsy to identify potential targets for therapy. The patient's tumor sample was tested for *EGFR* mutations using the Cobas EGFR Mutation Test v2 (CE-IVD). This test confirmed a deletion in exon 19 of the *EGFR* gene. In April 2019, the patient underwent conformal radiation therapy, targeting the tumor metastases in the thoracic and lumbar vertebrae. Following radiotherapy, the patient began treatment with afatinib in May 2019. The first follow-up CT scan of the chest and abdomen in July 2019, assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, documented partial regression of both target and non-target lesions (Figure 2), indicating a positive response to the afatinib therapy.

For an extended period of 35 months from the beginning of afatinib therapy, according to RECIST criteria, stable disease was observed on all control findings of the CT of the chest and abdomen. In April 2022, however, a chest and abdomen CT scan showed a progression of the disease per RECIST criteria (Figure 3).

In May and June 2022, a liquid biopsy was carried out on the patient's blood plasma to check for *EGFR* mutation status. These tests reaffirmed the presence of a deletion in exon 19 of the *EGFR* gene, while at the same time, the circulating tumor DNA (ctDNA) plasma result was negative for the T790M mutation. The progression of the disease at this stage was classified as oligoprogression and the decision was made to continue with the afatinib treatment. However, a subsequent CT scan of the chest and abdomen in July 2022 verified further disease progression according to the RECIST criteria. The OB IPBV suspended the administration of the drug afatinib and made a decision for repeated bronchoscopy and re-biopsy.

The bronchoscopy performed in August 2022 confirmed endoscopic evidence of disease progression. Subsequent pathohistological analysis confirmed the diagnosis of lung ADC. Molecular testing identified the previously detected deletion in exon 19 and an additional T790M mutation in exon 20. With the emergence of the T790M mutation, the OB IPBV advised initiating treatment with osimertinib, a third-generation EGFR TKI, as a second-line therapy. The patient has been adhering to the prescribed osimertinib regimen in October 2022. The latest check-up control CT scan of the thorax and abdomen was performed in September 2023, showing partial regression according to RECIST (Figure 4).

Summarized, the beginning of the application of molecular therapy in our patient in whom metastatic disease existed since the date of established the diagnose was in May 2019 as the first-line therapy with the drug afatinib for 35 months, and the second line of therapy with the drug osimertinib for 12 months, up to the date of publication of the paper. She feels well, regularly performs all daily activities, and has no side effects from the currently applied therapy.

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. Written consent to publish all shown material was obtained from the patient.

DISCUSSION

According to the most recent epidemiological data, the global incidence of lung cancer remains alarmingly high. It is the most common cancer among men and the second most common one among women. In 2020, there were approximately 2.2 million new cases of lung cancer diagnosed worldwide [8]. NSCLC is the predominant form, and the past decade has seen significant advances in the treatment of lung cancer type due to the identification of driver mutations, facilitating a personalized approach to therapy [9]. Tyrosine kinase inhibitors targeting the EGFR (EGFR TKIs) have been notably effective in patients with mutations in the EGFR gene. Among these "positive patients," especially those with lung ADC, response rates to EGFR TKIs can be as high as 80%, with progression-free survival (PFS) typically ranging 10–14 months [5]. The most prevalent activating mutations in the EGFR gene are deletions in exon 19 and the L858R point mutation in exon 21, which combined make up over 80% of all known activating *EGFR* mutations [10]. The presence of deletions in exon 19 or the L858R point mutation in exon 21 of the *EGFR* gene correlates with an enhanced response to targeted therapy using EGFR TKIs [11]. For our patient, who was diagnosed with metastatic ADC of the lung, the OB IPBV recommended molecular profiling using the real-time PCR Cobas EGFR Mutation Test v2 (CE-IVD). This test is capable of detecting 42 distinct mutations across exons 18–21 of the *EGFR* gene, including the T790M resistance mutation, but it has a reduced sensitivity for detecting 50% of *EGFR* exon 20 insertion mutations when compared to the more comprehensive next-generation sequencing method, which our Institution has not yet adopted [12]. Upon receiving the molecular test results indicating an *EGFR* exon 19 deletion, our patient

commenced targeted treatment with a second-generation TKI afatinib. Second-generation TKIs, including afatinib and dacomitinib, have been evaluated in phase III randomized trials. Results from Lux-Lung 3, Lux-Lung 6, and Lux-Lung 7 trials consistently demonstrated a significantly longer median PFS for patients treated with afatinib [13]. For the patient discussed, the PFS reached impressive 35 months. Throughout this period, based on the RECIST version 1.1, our patient's follow-up imaging consistently showed either partial regression or stable disease.

Acquired resistance to EGFR TKIs eventually develops in most patients undergoing this treatment modality. Typically, resistance that is EGFR-dependent arises in patients who have been treated with first or second-generation TKIs. In contrast, resistance associated with third-generation TKIs occurs less frequently [14]. The T790M mutation in exon 20 of the *EGFR* gene is particularly notable, emerging in about 50–60% of patients treated with first- or second-generation TKIs. Interestingly, this mutation appears more commonly in patients who initially present with a deletion in exon 19 of the *EGFR* gene as opposed to those with the L858R mutation [15]. The detection of the T790M mutation can be conducted through two principal methods: a liquid biopsy of the patient's blood plasma or a re-biopsy of the tumor tissue. By examining blood plasma or other bodily fluids such as pleural effusion, circulating tumor DNA (ctDNA) carrying the mutation can be identified. However, the sensitivity of liquid biopsies might be lower in some metastatic lung ADC since ctDNA might not be present in large quantities in peripheral blood, which can lead to false-negative results. For the patient in question, three liquid biopsies failed to detect the T790M mutation, yet a re-biopsy of the tumor obtained during a new bronchoscopic procedure did reveal the mutation in exon 20 and the T790M mutation. In the AURA2 study, the sensitivity and specificity of liquid biopsy for detecting the T790M mutation were 61% and 79%, respectively, compered to tissue biopsy, which boasts a higher sensitivity of 90% and a specificity of 91% [16]. The AURA2 study explored the efficacy and safety of osimertinib, a third-generation EGFR TKI, for patients who had developed resistance to first- and second-line EGFR TKI treatments and had the T790M mutation in exon 20 of the *EGFR* gene. AURA3 phase III clinical trial demonstrated that for patients harboring the T790M mutation and treated with osimertinib, the median PFS ranged 10.1–14.2 months [17]. The detection of T790M mutations varies based on the method employed: 30–40% are identified through pathological or cytological examination of tumor tissue, while plasma samples account for about 20% of detections [18]. This disparity highlights the ongoing need to re-biopsy using refined diagnostic tools to ensure accurate mutation status assessment and to guide therapeutic decision-making.

Conflict of interest: None declared.

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Значај ребиопсије у ери молекуларне терапије карцинома плућа

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САЖЕТАК

Увод Према последњим епидемиолошким подацима, инциденца карцинома плућа у свету је и даље висока, као и стопе морталитета за оба пола. Пре више од деценије, открићем такозваних покретачких мутација у ткиву тумора омогућено је терапијско деловање њиховим инхибиторима.

Приказ болесника Код болеснице старости 73 године је у фебруару 2019. године бронхоскопијом у Институту за плућне болести Војводине дијагностикован аденокарцином плућа, клиничко-радиолошког стадијума болести *cT4N2M1b*. Онколошки конзилијум Института за плућне болести Војводине донео је одлуку за молекуларно тестирање ткива тумора и примену палијативног зрачења метастаза у кичми. Тестирањем рецептора епидермалног фактора раста добијен је резултат делеције у егзону 19. После примењене зрачне терапије на метастазе у кичменим пршљеновима, у мају 2019. године започета је терапија применом лека афатиниб. На компјутеризованој томографији торакса и абдомена у априлу 2022. описана је прогресија болести. Упркос томе што три течне биопсије нису успеле да открију мутацију тумора T790M, накнадна бронхоскопија и ребиопсија потврдиле су њено присуство у ткиву тумора и отпочела је терапија леком осимертинибом. До објављивања рада болесница је била 12 месеци на терапији леком осимертинибом.

Закључак Рецептор епидермалног фактора раста је предиктивни биомаркер за немикроцелуларни карцином плућа. Примењени специфични инхибитори тирозинске киназе прве генерације као што су лекови гефитиниб, ерлотиниб, друге генерације – лек афатиниб појавом резистенције тумора на инхибиторе тирозинске киназе, треће генерације – лекови осимертиниб и лорлатиниб, дају одличне терапијске одговоре.

Кључне речи: аденокарцином плућа; течна биопсија; T790M; ткивна биопсија

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Continuous glucose monitoring in pregnancy

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SUMMARY
Pregnancies complicated with either pregestational or gestational diabetes mellitus deserve great attention due to their complexity and potential subsequent complications for both mother and the fetus. Based on already proven role of glycemic variability in the development of these, improving glucose monitoring continues to be an important step towards preventing adverse outcomes. Besides already well-established self-monitoring of glycemia, newer devices in the form of continuous glucose monitoring have found their place due to their proven preciseness and non-invasiveness. This paper has the aim to analyze results and conclusions of obtained, newer studies focused on these methods of glucose monitoring and to also give a closer insight of their usability and limitations.
Keywords: gestational diabetes mellitus; pregestational diabetes mellitus; glycemia tracking

INTRODUCTION

Diabetes mellitus (DM) in pregnancy holds great importance due to numerous reasons, including its rising prevalence and overall high perinatal morbidity and mortality [1]. Whether as pregestational (type 1 or type 2 DM) or gestational DM (GDM), the proportion of pregnancies complicated by this disease continues to rise, mostly due to the rising prevalence of type 2 DM [2]. Additionally, recent data suggests that one in six pregnancies is of higher risk due to being complicated by maternal hyperglycemia [3]. In Belgrade, Serbia, the prevalence of pregestational DM had increased in the past decade among pregnant and non-pregnant women and it is expected to further increase in the following decades [4]. Furthermore, the importance of pregestational DM (especially when poorly controlled) also lies in possible adverse outcomes for both mother (higher risk of preeclampsia, diabetic ketoacidosis, etc.) and the fetus (possible spontaneous abortions, preterm births, major congenital malformations, stillbirth, macrosomia, neonatal hypoglycemia, etc.). GDM, due to its time of onset, is mainly associated with macrosomia and neonatal hypoglycemia [2]. It is also proven that diabetes in pregnancy can have an impact not only on the blood flow in the placenta but also on placental structure and metabolism [5]. Nevertheless, these comorbidities during pregnancy could have a severe impact on children's health later in life, by putting them at a higher risk of developing various chronical diseases (due to the hypothesis of the fetal programming) [6]. In order to most adequately prevent these outcomes, it is suggested that existing screening regimes for GDM remain in the domain of each country due to its possibilities and population characteristics [7]. When already affected, minimizing the risk of these outcomes through adequate glycemic control remains the main goal, so different societies provided their recommendations regarding optimal goal glycemia values in these pregnancies. The National Institute for Health and Care Excellence (NICE) optimal values of glycemia in pregnancies complicated by any type of DM are as follows: fasting glucose < 5.3 mmol/l, one hour after meal < 7.8 mmol/l; two hours after meal < 6.4 mmol/l [8]. American Diabetes Association (ADA) from 2023 suggests the following goal levels: fasting glucose < 5.3 mmol/l, postprandial after one hour < 7.8 mmol/, and after two hours < 6.7 mmol/l. Regarding continuous glucose monitoring (CGM), ADA also suggests spending time in range (TIR) (3.5–7.8 mmol/l) > 70%, time below range (< 3.5 mmol/l < 4%, and < 3.0 mmol/l < 1%) and time above range (TAR) (> 7.8 mmol/l) < 25% [9]. Therefore, to minimalize glycemic variability (GV) (defined as range of glucose variations in one patient over one day or in-between days), which proved to be a contributing factor to adverse outcomes, research focused on finding the most optimal ways of glycemia monitoring [10].

REAL-TIME CONTINUOUS GLUCOSE MONITORING AND INTERMITTENTLY SCANNED ("FLASH") CONTINUOUS GLUCOSE MONITORING

One of the oldest and more conventional approaches to measuring glycemia is self-monitoring of blood glucose (SMBG) with the help of glucometers. Newer, more promising

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techniques that have been developed are through continuous monitoring of glucose – either by real-time continuous glucose monitoring (rtCGM) or by intermittently scanned ("flash") continuous monitoring (isCGM, FGM). Although sometimes perceived as identical, these two hold reasonable differences, and manufacturers even position FGM as a "third" category not truly comparable to either the rtCGM system or the usual glucometers [11]. CGM as such measures blood glucose either in a minimally invasive way (through continuous measurement of interstitial fluid) or completely non-invasively (by applying electromagnetic radiation throughout the skin, that reaches blood vessels). A sensor can be inserted subcutaneously and measure interstitial fluid *in situ* or an external sensor is placed. After a device-specific calibration process (either manually by aligning with SMBG values or already factory-calibrated), each device provides blood glucose readings every 1–10 minutes for up to 72 hours (with the minimally invasive technology) and even up to three months (with the non-invasive technology), overall up to 288 measurements per day. Maximal duration of use of the FGM sensor is 14 days, and seven days for the rtCGM. Some benefits of these devices include the unnecessity of painful finger lancing and quick results' readings. Even if the glucose measurements are not constantly updated in FGM (as they are in rtCGM) and therefore no alarms are triggered when glycemia values reach certain points, current values can still be quickly obtained when needed [11]. Results are monitored on a display, on which these data are transmitted, which shows the current sensor-detected levels, along with the glucose trend arrow and glucose variability in past hours. Obtaining glucose data is possible at any time or it can be automatically obtained and stored [11, 12].

RECENT EXPERIENCE IN CLINICAL PRACTICE

RtCGM has so far primarily been used by patients with type 1, and FGM by patients with type 2 DM who performed only a few measurements daily (training sessions are needed either way), but despite the benefits, not many have been using them long-term due to relatively high costs [11, 12]. Lai et al. [12] even suggested that for women with GDM that have HbA1c < 6%, SMBG (already widely spread) might be more economical that CGM. Further benefits come from their interoperability – these systems can also be used by those on insulin therapy, with particular significance for patients using continuous subcutaneous insulin injections (CSII), as a part of a closed-loop system [13]. Their significance and potential were also recognized by NICE, who included them in their guidelines and now recommends that rtCGM is offered to all pregnant women with type 1 DM, as well as to all pregnant women on insulin therapy that keep experiencing problems with achieving adequate glycemic control, and to those with hypoglycemias. If women are unable to use or tolerate CGM, it is reasonable to offer FGM [8]. Guided by these recommendations, all pregnant women with type 1 DM in the United Kingdom will receive government-funded rtCGM for one year, which will enable them to continue this type of monitoring for some time even after delivery [14]. These guidelines had a prominent role in further studies, such as CONCEPTT, a multicenter randomized controlled trial regarding the use of rtCGM in women with type 1 DM. It showed that the use of rtCGM was associated with lower HbA1c at 34 weeks, suggesting that this type of monitoring might lead to better maternal glycemic control during the late second and early third trimesters (without increasing maternal hypoglycemia). Also, authors observed reduction in large-for-gestational-age (LGA) infants, neonatal hypoglycemia, and neonatal intensive care unit (NICU) admissions [15]. By applying functional data analysis to CONCEPTT data, it was able to better enlighten daily oscillations of maternal glucose. In doing so, rtCGM users showed lower glucose during the day than women using only SMBG, and it was also observed that giving birth to LGA babies was associated with maintaining a higher glucose level throughout pregnancy [16]. A systematic review that combined data from CONCEPTT with comparable data from the GlucoMOMS trial also reported a reduction in preeclampsia [17]. Sobhani et al. [18] reported similarly in this group of women and emphasized the importance of spending TIR as long as possible, mainly in early pregnancy, because it seems that even small improvements in time spent in range can significantly reduce the incidence of preeclampsia and LGA children. Sanusi et al. [19] also highlighted the importance of TIR, and supported ADA recommendations of aiming for 70% TIR, because even 5%-point increase in TIR can reduce neonatal morbidity rates. Furthermore, Scott et al. [20] analyzed over 10.5 million glycemia values from pregnant women with type 1 DM and showed that normal birth weight (BW) is associated with achieving significantly lower mean CGM glucose concentration across 24 hours and that aiming for TIR of ≥ 55–60% (with aiming to achieve 70% thereafter), a mean glucose of ≤ 7 mmol/l and TAR < 35% by 10 gestational weeks may be sufficient for adequate fetal growth. Therefore, authors propose that the focus in everyday clinical management of these pregnancies shifts on optimizing glycemia from early pregnancy [20]. Their superiority over SMBG in type 1 DM pregnancies was also shown throughout a retrospective cohort that reported lower HbA1c values in CGM users not only during pregnancy but also postpartum, as well as less often noted macrosomia and NICU admissions than in SMBG users [21]. Regarding their use in GDM, Bitar et al. [22] concluded that in GDM and type 2 DM spending longer TIR ≤ 70% is associated with various adverse maternal and neonatal outcomes. A meta-analysis implied that GDM women using CGM have lower average glucose levels, maternal gestational weight gain (GWG) and neonatal BW compared to SMBG women [23]. In support of these findings, another meta-analysis brought out further benefits of these devices (CGM group having lower HbA1c levels, maternal GWG, and caesarean section rates than the SMBG users) [24]. These devices might also be beneficial in monitoring GDM women on insulin therapy, because better dynamic and lower HbA1c values in CGM than SMBG group were noted (without

increasing severe hypoglycemia) [25]. Their benefits have been proven even in women with type 1 DM on CSII, by two studies done in Poland, which showed that the addition of CGM to this type of insulin application has its benefits – among other findings, it results in improved glycemic control, lower HbA1c levels during pregnancy, and lower rates of LGA [26, 27]. Regarding the FGM use, the FLAMINGO trial, a non-blinded randomized controlled trial, assessed its efficiency in GDM. During its first month, no significant correlation was found between mean fasting glucose nor postprandial glucose and BW. However, FGM application seemed to improve glycemic control in the third and fourth week of this study, and had no impact on GWG, HbA1c, caesarean section prevalence, qualification to insulin therapy, or its dosage. It decreased macrosomia incidence but no significant impact on BW percentile or neonatal hypoglycemia incidence was observed [28]. Also, Pikee et al. [29] observed that FGM is truly better in detecting GV, as well as frequency and duration of asymptomatic or nocturnal hypoglycemias, and has improved patient satisfaction compared to SMBG. Even in pregestational DM, isCGM can reduce hyperglycemia exposure and was also found to better detect nocturnal hypoglycemia than SMBG [30].

CONCLUSION

Great efforts have been made in researching methods of glycemia monitoring in DM and GDM-complicated pregnancies, with the aim of reaching adequate glycemic control and minimizing GV. Future holds hope that CGM and FGM as newer and more accurate methods by monitoring in real time and by being safe and non-invasive might in some cases replace SMBG. Nevertheless, further enlightening of the potential, safety, and possible wider implementation of these devices still remains an elusive goal, which deserves great attention and holds inexhaustible research potential.

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

Conflict of interest: None declared.

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Континуирано праћење гликемије у трудноћи

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САЖЕТАК

Трудноће компликоване прегестацијским или гестацијским дијабетесом мелитусом заслужују велику пажњу због своје комплексности и могућих следујућих компликација за мајку и фетус. Заснивајући се на већ доказаној улози гликемијске варијабилности у развоју ових компликација, побољшавање праћења гликемије наставља да буде важан корак у спречавању лоших исхода. Сем већ добро установљеног самосталног праћења гликемије, нови апарати базирани на континуираном мерењу гликемије пронашли су своје место због доказане прецизности и неинвазивности. Циљ овог рада је анализа резултата и закључака прикупљених новијих студија и обезбеђивање ближег увида у могућности употребе и могућа ограничења ових метода.

Кључне речи: гестацијски дијабетес мелитус; прегестацијски дијабетес мелитус; праћење гликемије

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REVIEW OF LITERATURE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Identification and prevention of refeeding syndrome in pediatric intensive care

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SUMMARY
"Refeeding syndrome" is described in the literature as a range of metabolic and electrolyte disorders that result from starting nutritional rehabilitation in malnourished patients. Without a universally accepted definition, data on "refeeding syndrome" incidence are heterogeneous. In most cases, a clinician will subjectively identify "refeeding syndrome," many authors have developed their purposes and criteria for it in their studies. Using the PubMed database and the appropriate filters ("refeeding syndrome"-related terms: refeeding syndrome, pediatrics, child, nutrition support, nutrition assessment, malnutrition), a search of the published literature was conducted. The American Society for Parenteral and Enteral Nutrition's 2020 recommendations are the only guidelines for identifying children with or at risk for "refeeding syndrome". High-quality scientific evidence regarding the clinical syndrome is absent, so we need further research in all "refeeding syndrome"-related areas, from validation to better identification of risk factors, definitions of "refeeding syndrome," and standardization of treatment protocols. For now, clinicians must remain vigilant to protect patients from the potentially devastating consequences of the "refeeding syndrome."
Keywords: refeeding syndrome; child; nutrition support; nutrition assessment; malnutrition

INTRODUCTION

"Refeeding syndrome" (RFS) is defined in the literature as a group of metabolic and electrolyte disorders that occur in response to nutritional rehabilitation in malnourished patients [1, 2]. It was first mentioned in the journals of starving Asian prisoners during World War II, Keys et al. [3] reported on a prospective randomized control trial examining the physiologic consequences of protracted malnourishment. Even though RFS is most often written about in the adult population, it can also occur in childhood [4]. The physiology and pathophysiology of RFS are well known, while clinical signs, symptoms, and treatment are less known. When risk factors are not identified promptly, negative consequences such as hydro electrolyte imbalances, metabolic disorders, respiratory failure, cardiac arrhythmias, encephalopathy, coma, and death can occur [5, 6]. The consequences of a rapid refeeding scheme in the presence of malnutrition include disturbances in potassium, magnesium, thiamin, and phosphate levels; vitamin deficiencies; glucose and fluid intolerance; and cardiac, pulmonary, hematologic, and neuromuscular dysfunction [7]. This condition is frequently undiagnosed, particularly in the pediatric population, so becoming familiar with the pathophysiology, clinical manifestations, and treatment models will help clinicians avoid unnecessary life-threatening conditions. The criteria developed for predicting RFS have been published in previous years but scored poorly for sensitivity or specificity [8]. The American Society for Parenteral and Enteral Nutrition (ASPEN) Consensus in 2020 advocated for new rules for defining RFS and screening procedures that entail stratification of criteria in response to the challenges brought up by the individual definition of RFS. The authors suggest that the diagnostic criteria for RFS should be as follows: a decrease of serum phosphorus, potassium, and/or magnesium levels by 10–20% (mild), 20–30% (moderate), or > 30% and/or organ dysfunction caused by a decrease in any of these and/or thiamin deficiency (severe) within five days of reintroducing calories [9]. Novel criteria sets such as those proposed by the ASPEN may be predictive for RFS in pediatric patients [10]. The crucial point in preventing the occurrence of RFS is to be aware of its existence.

EPIDEMIOLOGY OF REFEEDING SYNDROME IN PEDIATRICS

The epidemiological data on RFS are heterogeneous due to a lack of universally accepted defining criteria; a clinician usually identifies RFS subjectively, and many authors have created their own definitions and standards in their studies, often using hypophosphatemia as the

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single diagnostic criteria [11]. Consequently, the data on RFS incidence in the pediatric population is challenging. The overall prevalence of RFS in various hospital populations has been cited with a wide range of estimations, from 0.43% to 34% [12, 13].

The cohort study made by Dunn et al. [14] reported that within 72 hours of the beginning, the incidence of "electrolyte shifts" in the whole population was 27% (eight out of 15) in the population at risk of those patients who developed hypophosphatemia, three developed lethargy, and cardiac dysfunction. Two neonatal studies found that rates of hypophosphatemia were significantly higher in patients of early gestational age [15, 16]. In two other studies, hypophosphatemia and hypokalemia were discovered in neonates receiving parenteral nutrition (PN) [17, 18, 19].

PATHOPHYSIOLOGY

Understanding the pathophysiology of malnutrition is crucial for comprehending what occurs during refeeding [20]. Starvation can be defined as a catabolic state where the body shifts from carbohydrate utilization to fat and protein metabolism. With this shift, the pancreas' production of insulin declines in the absence of available carbohydrates [21]. The following change in the metabolic pathway is that ketone bodies and free fatty acids will replace glucose as the primary energy fuel. Further catabolism leads to a continuing and progressive wasting of cellular and muscle mass, resulting in hypotrophy, atrophy of vital organs, and, consequently, dysfunction. As a result, lower renal concentration capacity, hydro electrolytic disbalances, a decline in metabolic rate and hemoglobin level, and reductions in respiratory and cardiac function may all result in serious complications.

When we start refeeding, increased glucose levels increase insulin secretion, stimulating glycogen, fat, and protein synthesis. This increment in insulin release and its anabolic activity are the keys to the pathophysiology [22]. This anabolic process requires electrolytes, primarily phosphorus, magnesium, and potassium, and cofactors, such as thiamine, to be taken into cells. The consequence of this alteration in metabolism can be a life-compromising extracellular depletion of these electrolytes. Phosphate is essential for all intracellular processes, cell membranes' structural integrity, adenosine triphosphate production, DNA, RNA, and 2,3-diphosphoglycerate. Hypokalemia (below 3.5 mEq/L) and hypomagnesemia (below 1.8 mg/dL) are also commonly related to electrolyte imbalances with RFS [23]. A mild reduction of potassium and magnesium serum levels may induce nausea, vomiting, constipation, diarrhea, muscle twitching, or weakness. In contrast, a more severe reduction of the serum levels of these electrolytes can cause dysrhythmias, cardiac dysfunction, skeletal muscle weakness, seizures, and metabolic acidosis.

Children may suffer more from short periods of starvation because their bodies need more energy to grow, while adults may be able to handle more prolonged periods of starvation better [24].

PREDICTIVE CRITERIA

An example of criteria especially designed for predicting RFS and nutritional support in adults – Britain's National Institute for Health and Care Excellence (NICE) guideline was published in 2006 [8]. This guideline was established based on previously reported reviews and the authors' expertise and agreed upon based on an unofficial consensus. The short nutritional assessment questionnaire is an example of screening criteria designed for malnutrition that are validated for diagnosing malnutrition and have also been validated for predictive value in RFS [25]; the usefulness of these two previously mentioned tools is questionable because their contribution to predicting less severe hypophosphatemia, hypokalemia, or hypomagnesemia is undefined, and their performance in predicting severe hypophosphatemia is poor [26].

In 2017, the ASPEN, the Parenteral Nutrition Safety Committee, and the Clinical Practice Committee established consensus recommendations for discovering patients with or at risk for RFS (Figure 1) and recommendations for the avoidance and treatment of RFS in at-risk pediatric patients (Table 1) [6]. As of today, these are the only recommendations for the pediatric population. Still, the predictive validity of these unique and novel recommendations has yet to be studied [9].

CLINICAL FEATURES

Severe hypophosphatemia causes impaired neuromuscular function with paresthesia, seizures, cramps, weakness, impaired muscular contractility, and rhabdomyolysis. The consequence for the respiratory system is hypoventilation, which may be followed by respiratory failure [27]. It can also present as a central nervous system dysfunction in the form of confusion or coma. Phosphate deficiency also leads to hematologic disorders such as thrombocytopenia, damaged clotting, and leukocyte dysfunction, and the red blood cells show a deteriorated capacity to release oxygen [28].

Both hypomagnesemia and hypokalemia lead to neuromuscular dysfunction, which presents as weakness, paralysis, paresthesia, confusion, rhabdomyolysis, respiratory depression, cardiac arrhythmias, and cardiac arrest. Additionally, due to starvation, stress, inflammation, and increased insulin release, sodium retention increases, and the consequence is extracellular fluid expansion followed by edema. As mentioned above, the disorders, when associated with thiamine deficiency, lead to tachyarrhythmias, enlargement of the heart, severe edema, and finally, congestive cardiac failure with lung edema. Thiamine deficiency also causes Wernicke–Korsakoff syndrome in the central nervous system and neuropathy in the peripheral nervous system [29].

Most clinical signs and symptoms during an RFS are nonspecific (Figure 2) [30]. The primary and most common symptoms are tachycardia, tachypnea, and peripheral edema. However, such signs may also be due to other

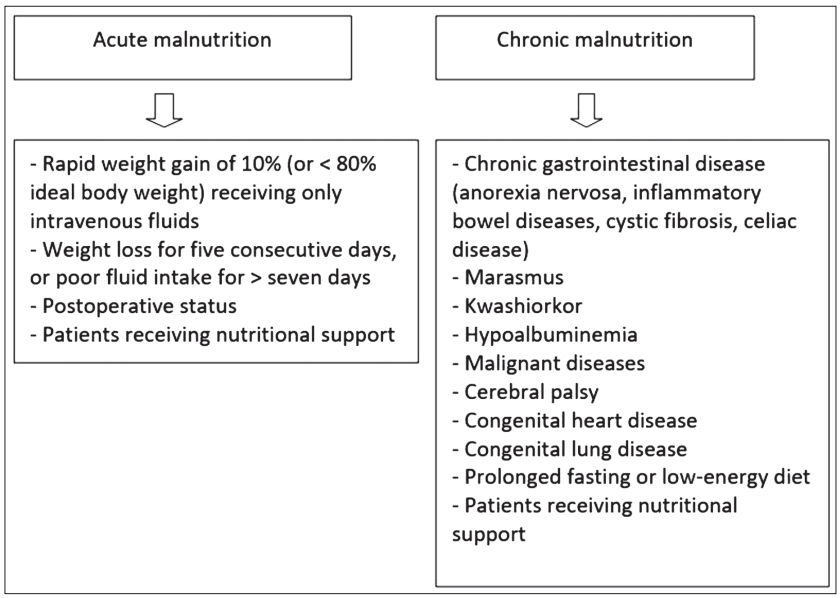

Figure 1. Risk factors for refeeding syndrome [1]

conditions in hospitalized patients with different diseases, especially those in intensive care departments.

AVOIDANCE AND TREATMENT

Prevention and early recognition of at-risk patients, careful monitoring before and during refeeding, and proper individualized nutrition rehabilitation are the keys to successful management and outcome [31]. Various studies have evaluated preventive approaches for RFS, mainly guided by hypocaloric nutrition, electrolyte substitution, and thiamin infusions. Management of confirmed RFS should be conducted in two ways: by improving the underlying electrolyte imbalances, and by lowering or slowing the advancement of calories, according to the final aim. RFS can be seen with any pattern of nutritional support: oral diet, enteral nutrition, PN, or intravenous dextrose solutions [32]. The ASPEN consensus recommendations for avoiding and treating RFS presented in Table 2 are universal. They should be adapted to individuals and particular populations, such as those with decreased renal function. These recommendations are based on consensus and, in the future, will need to be investigated in randomized clinical trials in general and specific populations with different comorbidities to define their actual benefits and utility [33].

Most clinical trials investigating risk factors for RFS are conducted according to criteria developed by the NICE guidelines. However, Goyale et al. [34] and Zeki et al. [35] found these factors had low sensitivity and specificity in predicting RFS. According to the NICE guidelines, feeding should be started gradually (maximum 0.042 MJ / kg / 24 hours) and individually adapted for patients at high risk of developing RFS [8]. Also, the NICE guidelines advocate that in very undernourished patients (body mass index ≤ 14 or insignificant food intake for more than two weeks), refeeding should start at a maximum of 0.021 MJ / kg / 24 hours, with cautious monitoring on an electrocardiogram. The NICE recommendations also state that correcting hydroelectrolyte imbalances should be done in conjunction with refeeding; doing it before starting with feeding is not imperative. All guidelines agree that vitamin supplementation should be started promptly before and for

Table 1. ASPEN Consensus Criteria for Identifying Pediatric Patients at Risk for Refeeding Syndrome [9]

Parameters	Mild Risk: 3 Risk Categories Needed	Moderate Risk: 2 Risk Criteria Needed	Significant Risk: 1 Risk Criteria Needed
Weight-for-length *z*-score (1–24 months) or BMI-for-age *z*-score (2–20 years)	-1 to -1.9 *z*-score that is a change from baseline	-2 to -2.9 *z*-score that is a change from baseline	-3 *z*-score or greater that is a change from baseline
Weight loss	< 75% of norm for expected weight gain	< 50% of norm for expected weight gain	< 25% of norm for expected weight gain
Energy intake	3–5 consecutive days of protein or energy intake < 75% of estimated need	5–7 consecutive days of protein or energy intake < 75% of estimated need	> 7 consecutive days of protein or energy intake < 75% of estimated need
Abnormal prefeeding serum potassium, phosphorus, or magnesium concentrations[b]	Mildly abnormal or decreased to 25% below lower limit of normal	Moderately/significant abnormal or down to 25–50% below lower limit of normal	Moderately/significantly abnormal or down to 25–50% below lower limit of normal
Higher-risk comorbidities (see Table 4)	Mild disease	Moderate disease	Severe disease
Loss of subcutaneous fat	Evidence of mild loss ORMid-upper arm circumference *z*-score of -1 to -1.9 *z*-score	Evidence of moderate loss ORMid-upper arm circumference *z*-score of -2 to -2.9	Evidence of severe loss ORMid-upper arm circumference *z*-score of -3 or greater
Loss of muscle mass	Evidence of mild or moderate loss ORMid-upper arm circumference *z*-score of -2 to -2.9		Evidence of severe loss ORMid-upper arm circumference *z*-score of -3 or greater

ASPEN – American Society for Parenteral and Enteral Nutrition; BMI – body mass index;
[a]not intended for use in patients at ≤ 28 days of life or ≤ 44 weeks corrected gestational age;
[b]please note that electrolytes may be normal despite total-body deficiency, which is believed to increase risk of refeeding syndrome

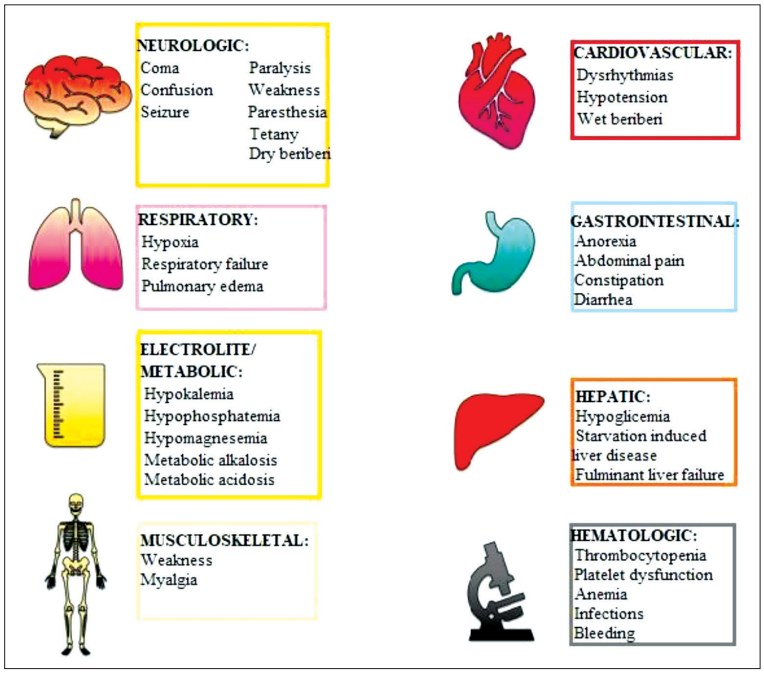

Figure 2. Signs and symptoms of refeeding syndrome [30]

the first 10 days of nutritional rehabilitation. The required levels of supplements cited by NICE are only grade-level D recommendations.

Additionally, the circulatory volume should also be reestablished. Regarding further patient monitoring, in the first week, electrolyte levels should be controlled once a day and at least three times in the following week. Urine electrolytes could also be checked for closer monitoring and assessment of hydro electrolyte status, wasting, and replacement.

CONCLUSION

Even though RFS is reported most often in adults, it can also occur in children. This condition is frequently undiagnosed, particularly in the pediatric population, so becoming familiar with the pathophysiology, clinical manifestations, and treatment

Table 2. ASPEN consensus recommendations for avoidance and treatment of RFS in at-risk pediatric patients [9]

Aspect of Care	Recommendations
Initiation of nutrition	- Initiate nutrition at a maximum of 40–50% goal, but usually starting the glucose infusion rate around 4–6 mg/kg/min and advancing by 1–2 mg/kg/min daily as blood glucose levels allow until you reach a max of 14–18 mg/kg/min. This includes enteral as well as parenteral glucose. - Calories from IV dextrose solutions and medications being infused in dextrose should be considered in the limits above and/or initiated with caution in patients at moderate to severe risk for RFS. If the patient is already receiving IV dextrose for several days and/or medications in dextrose and has been asymptomatic with stable electrolytes, calories from nutrition may be reintroduced at a higher amount than recommended above.
Fluid restriction	No recommendation
Sodium restriction	No recommendation
Protein restriction	No recommendation
Electrolytes	- Check serum potassium, magnesium, and phosphorus before initiation of nutrition. - Monitor every 12 hours for the first three days in high-risk patients. May be more frequent based on clinical picture. - Replete low electrolytes based on established standards of care. - No recommendation can be made for whether prophylactic dosing of electrolytes should be given if prefeeding levels are normal. - If electrolytes become difficult to correct or drop precipitously during the initiation of nutrition, decrease calories/grams of dextrose by 50% and advance the dextrose/calories by approximately 33% of goal every 1–2 days based on clinical presentation. Recommendations may be changed based on practitioner judgment and clinical presentation, and cessation of nutrition support may be considered when electrolyte levels are severely and/or life-threateningly low or dropping precipitously.
Thiamin and multivitamins	- Thiamin 2 mg/kg to a max of 100–200 mg/d before feeding commences or before initiating IV fluids containing dextrose in high-risk patients. - Continue thiamin supplementation for 5–7 days or longer in patients with severe starvation, chronic alcoholism, or other high risk for deficiency and/or signs of thiamin deficiency. - Routine thiamin levels are unlikely to be of value. - Multivitamin injectable is added to parenteral nutrition daily, unless contraindicated, as long as parenteral nutrition is continued. For patients receiving oral/enteral nourishment, add complete oral/enteral multivitamin once daily for 10 days or greater based on clinical status and mode of therapy. - Once patient is within adult weight ranges, refer to adult multivitamin recommendations
Monitoring and long-term care	- Recommend vital signs every four hours for the first 24 hours after initiation in those at risk. - Cardiorespiratory monitoring is recommended for unstable patients or those with severe deficiencies, based on established standards of care. - Daily weights with monitored intake and output. - Estimation of energy requirements as needed for oral feeding patients. - Evaluate short- and long-term goals for nutrition care daily during the first several days until the patient is deemed stabilized (e.g., no requirement for electrolyte supplementation for two days) and then based on institutional standards of care.

ASPEN – American Society for Parenteral and Enteral Nutrition; IV – intravenous; RFS – refeeding syndrome

models will help clinicians avoid unnecessary life-threatening conditions. New sets of criteria, like those suggested by ASPEN, may be able to predict RFS in children. High-quality scientific evidence regarding the clinical syndrome is absent, so we need further research in all areas related to RFS, from validation to better identification of risk factors, definitions of RFS, and standardization of treatment protocols. Even though ASPEN's recommendations have been provided, their most significant shortcomings are that they are based on consensus and must be examined in randomized controlled trials in general and specific populations with different comorbidities to define their utility in pediatric and adult populations. For now, clinicians must remain vigilant to protect patients from the potentially devastating consequences of RFS.

The authors declare that the article was written according to ethical standards of the Serbian Archives of Medicine as well as ethical standards of medical facilities for each author involved.

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САЖЕТАК

Синдром дохране је описан у литератури као спектар метаболичких и електролитних поремећаја који настају као последица започињања исхране код потхрањеног пацијента. Не постоји универзално прихваћена дефиниција, а подаци о инциденци су хетерогени. У највећем броју случајева клиничари ће субјективном проценом идентификовати синдром дохране, а многи аутори су у студијама развили сопствене критеријуме за постављање дијагнозе. Користећи базу података *PubMed* и одговарајуће филтере (појмови повезани са синдромом дохране: синдром дохране, педијатрија, деца, нутритивна подршка, нутритивна процена, неухрањеност), претражили смо публиковану литературу. Препоруке Америчког друштва за парентералну и ентералну исхрану из 2020. године су једине препоруке које омогућавају идентификацију деце са синдромом дохране или деце која су у ризику. Недостају висококвалитетни научни докази о овом клиничком синдрому, што указује на потребу за даљим истраживањима у области синдрома дохране, од валидације до боље идентификације фактора ризика, дефиниције синдрома дохране и стандардизације протокола за лечење ових пацијената. За сада клиничари морају да остану на опрезу како би заштитили пацијенте од могућих разарајућих последица синдрома дохране.

Кључне речи: синдром дохране; деца; нутритивна подршка; нутритивна процена; неухрањеност

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.

АДРЕСА:
Српско лекарско друштво
Уредништво часописа „Српски архив за целокупно лекарство“
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11000 Београд
Србија

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The papers are always submitted with Summary in both English and Serbian, included in the manuscript file. The text of the manuscript should be typed in *MS Word* using the *Times New Roman* typeface, and font size 12 pt. The text should be prepared with margins set to 25 mm and onto A4 paper size, with double line spacing, aligned left and the initial lines of all paragraphs indented 10 mm, without hyphenation. Tabs and successive blank spaces are not to be used for text alignment; instead, ruler alignment control tool and *Toolbars* are suggested. In order to start a new page within the document, *Page Break* option should be used instead of consecutive enters. Only one space follows after any punctuation mark. If special signs (symbols) are used in the text, use the *Symbol* font. References cited in the text are numbered with Arabic numerals within parenthesis (for example: [1, 2]), in order of appearance in the text. Pages are numbered consecutively in the right bottom corner, beginning from the title page.

When writing text in English, linguistic standard American English should be observed. Write short and clear sentences. Generic names should be exclusively used for the names of drugs. Devices (apparatuses, instruments) are termed by trade names, while their name and place of production should be indicated in the brackets. If a letter-number combination is used, the number should be precisely designated in superscript or subscript (i.e., 99Tc, IL-6, O2, B12, CD8). If something is commonly written in italics, such as genes (e.g. BRCA1), it should be written in this manner in the paper as well.

If a paper is a part of a master's or doctoral thesis, or a research project, that should be designated in a separate note at the end of the text. Also, if the article was previously presented at any scientific meeting, the name, venue and time of the meeting should be stated, as well as the manner in which the paper had been published (e.g. changed title or abstract).

CLINICAL TRIALS. Clinical trial is defined as any research related to one or more health related interventions in order to evaluate the effects on health outcomes. The trial registration number should be included as the last line of the Summary.

ETHICAL APPROVAL. Manuscripts with human medical research should contain a statement that the subjects' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by competent ethics committee, and conforms to the legal standards. Experimental studies with human material and animal studies should contain statement of the institutional ethics committee and meet legal standards.

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The authors should enclose the description of contribution to the article of every co-author individually (within the Submission Letter). Funding, collection of data or general supervision of the research group alone cannot justify authorship. All other individuals having contributed to the preparation of the article should be mentioned in the *Acknowledgment* section, with description of their contribution to the paper, with their written consent.

PLAGIARISM. Since January 1, 2019 all manuscripts have been submitted via SCIndeks Assistant to Cross Check (software iThenticate) for plagiarism and auto-plagiarism control. The manuscripts with approved plagiarism/auto-plagiarism will be rejected and authors will not be welcome to publish in Serbian Achieves of Medicine.

TITLE PAGE. The first page of the manuscript (cover sheet) should include the following: title of the paper without any abbreviations; suggested running title; each author's full names and family names (no titles), indexed by numbers; official name, place and country of the institution in which authors work (in order corresponding to the indexed numbers of the authors); at the bottom of the page: name and family name, address, phone and fax number, and e-mail address of a corresponding author.

SUMMARY. Along with the original article, preliminary and short communication, review article, case report, article on history of medicine, current topic article, article for language of medicine and article for practitioners, the summary not exceeding 100–250 words should be typed on the second page of the manuscript. In original articles, the summary should have the following structure: Introduction/Objective, Methods, Results, Conclusion. Each segment should be typed in a separate paragraph using boldface. The most significant results (numerical values), statistical analysis and level of significance are to be included. The conclusion must not be generalized, it needs to point directly to the results of the study. In case reports, the summary should consist of the following: Introduction (final sentence is to state the objective), Case Outline (Outline of Cases), Conclusion. Each segment should be typed in a separate paragraph using boldface. In other types of papers, the summary has no special outline.

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TRANSLATION INTO SERBIAN. The third page of the manuscript should include: title of the paper in the Serbian language; each author's full name and family name (no titles), indexed by numbers; official name, place and country of the institution in which authors work. On the fourth page of the manuscript the summary (100–250 words) and keywords (3–6) should be typed, but this refers only to papers in which a summary and keywords are compulsory. The terms taken from foreign literature should be translated into comprehensible Serbian. All foreign words or syntagms that have a corresponding term in Serbian should be replaced by that term.

If an article is entirely in Serbian (e.g. article on history of medicine, article for "Language of medicine," etc.), captions and legends of all enclosures (tables, graphs, photographs, schemes) – if any – should be translated into English as well.

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All enclosures (tables, graphs, photographs, etc.) should be placed at the end of the manuscript, while in the body of the text a particular enclosure should only be mentioned and its preferred place indicated. The final arrangement (position) of the enclosures will depend on page layout.

ABBREVIATIONS. To be used only if appropriate, for very long names of chemical compounds, or as well-known abbreviations (standard abbreviations such as DNA, AIDS, HIV, ATP, etc.). Full meaning of each abbreviation should be indicated when it is first mentioned in the text unless it is a standard unit of measure. No abbreviations are allowed in the title. Abbreviations in the summary should be avoided, but if they have to be used, each of them should be explained when first mentioned in the text of the paper.

DECIMAL NUMBERS. In papers written in English, including text of the manuscript and all enclosures, a decimal point should be used in decimal numbers (e.g. 12.5 ± 3.8), while in Serbian papers a decimal comma should be used (e.g. 12,5 ± 3,8). Wherever applicable, a number should be rounded up to one decimal place.

UNITS OF MEASURE. Length, height, weight and volume should be expressed in metric units (meter – m, kilogram – kg, gram – g, liter – l) or subunits. Temperature should be in Celsius degrees (°C), quantity of substance in moles (mol), and blood pressure in millimeters of mercury column (mm Hg). All results of hematological, clinical and biochemical measurements should be expressed in the metric system according to the International System of Units (SI units).

LENGTH OF PAPER. The entire text of the manuscript – title page, summary, the whole text, list of references, all

enclosures including captions and legends (tables, photographs, graphs, schemes, sketches), title page and summary in Serbian – must not exceed 5,000 words for original articles, review articles and articles on history of medicine, and 3,000 words for case reports, preliminary and short communications, current topics, articles for practitioners, educational articles and articles for "Language of medicine", congress and scientific meeting reports; for any other section maximum is 1,500 words.

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