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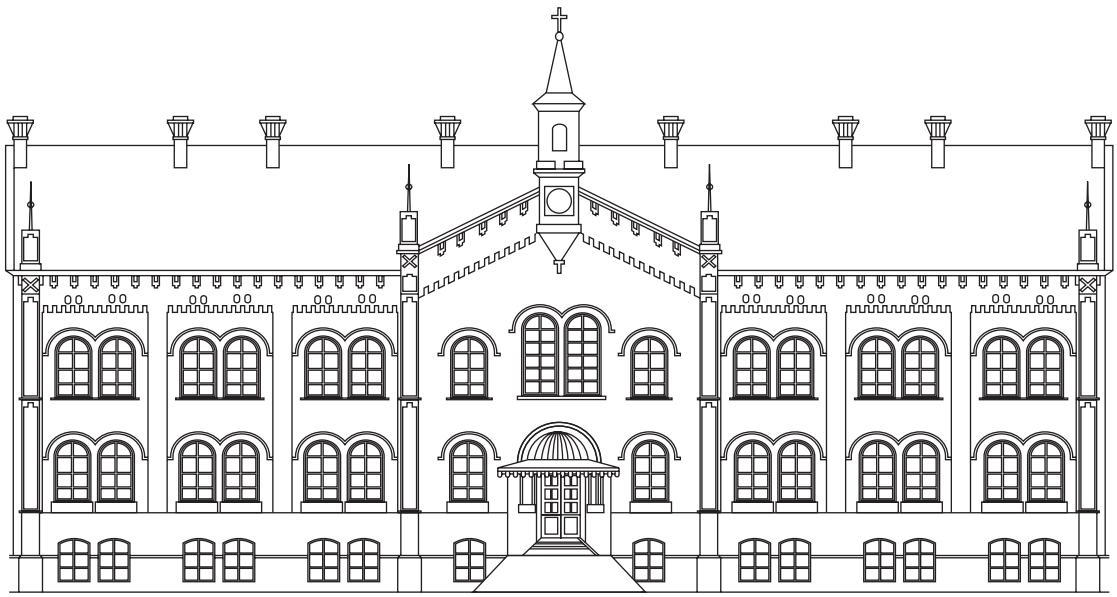

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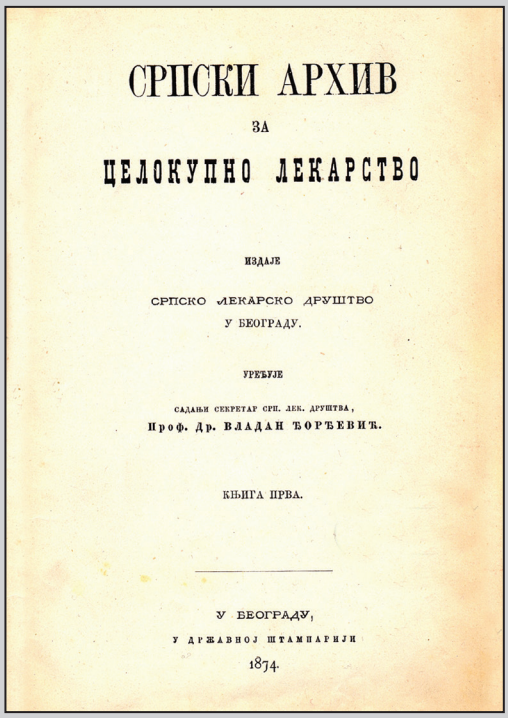

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

ИЗДАЈЕ

СРПСКО ЛЕКАРСКО ДРУШТВО

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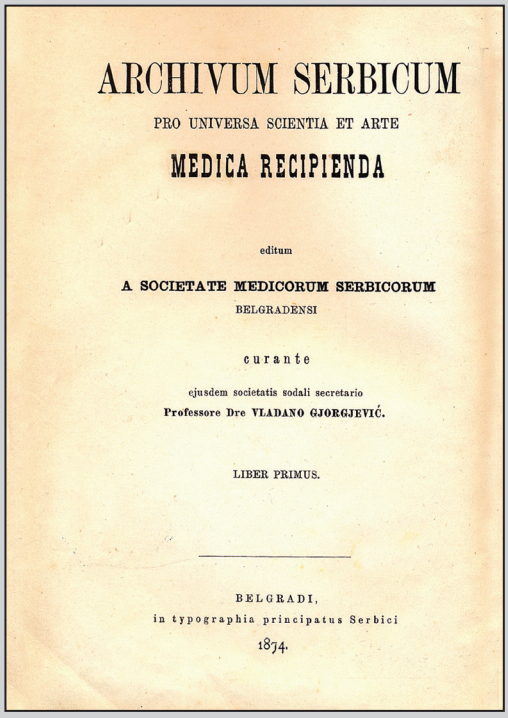
У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

Прва страна првог броја часописа на српском језику



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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Clinical efficacy of casein phosphopeptide – amorphous calcium phosphate and casein phosphopeptide – amorphous calcium fluoride phosphate and their influence on the quality of life in patients with Sjögren's syndrome

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SUMMARY

Introduction/Objective The purpose of this study was to compare clinical efficacy of casein phosphopeptide – amorphous calcium phosphate (CPP-ACP) and casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP) with 0.05% NaF, and to assess their influence on the quality of life among individuals with Sjögren's syndrome.

Methods Thirty patients were randomized into three groups treated with different remineralizing agents: CPP-ACP, CPP-ACFP, and 0.05% NaF. Oral health was evaluated at the beginning of the study, after 28 days (short-term effects), and after six months. The diagnosis of dental caries was performed using the decayed, missing, and filled teeth (DMFT) / decayed and filled surfaces (DFS) criteria. Enamel demineralization was visually examined using the white spot lesion index (Gorelick). The gingival health was evaluated with the gingival index (Löe–Silness). Assessment of oral hygiene was done using the simplified oral hygiene index (Greene–Vermilion). The Xerostomia Inventory was used to quantify dry-mouth symptoms. The oral health-related quality of life was analyzed using the short form of the Oral Health Impact Profile (OHIP-14).

Results During the evaluation period, caries increment was not significant. Considerable regression of white spot lesions was noted in all three experimental groups ($p < 0.001$). No significant improvement in gingival health and oral hygiene was observed. Physical pain was decreased in all three experimental groups, and subjective feeling of dry mouth was reduced in CPP-ACP and CPP-ACFP groups.

Conclusion CPP-ACP and CPP-ACFP may reduce the caries activity and relieve the dry-mouth symptoms in patients with Sjögren's syndrome.

Keywords: caries; casein phosphopeptide – amorphous calcium phosphate; casein phosphopeptide – amorphous calcium fluoride phosphate; dry mouth; Sjögren's syndrome

INTRODUCTION

Sjögren's syndrome (SS) is a chronic systemic autoimmune disorder, characterized by dysfunction of both salivary and lachrymal glands. Exocrine glands are infiltrated by mononuclear cells (lymphocytic infiltration), causing either compromised or even total failure in secretion of saliva and tears [1, 2]. The variety of symptoms makes SS a complex disease. Pronounced and severe oral dysfunction, disability, and discomfort, defined as oral distress, seem to be the most important factors in a patient's perception of oral health status [3].

The majority of published reports on the oral component of SS focused on dry-mouth symptoms. However, not much attention has been given to the incidence of oral pathology in patients with SS. Despite the fact that the prevalence of persons with SS is high (1–23 cases per 10,000 inhabitants), while the prevention and early treatment of oral diseases is of the utmost importance in these patients, no caries prevention protocol has been established so far [4]. Usually, topical application of high-concentration fluoride agents is recommended for caries control in patients with salivary hypofunction [5]. However, decreased concentration of calcium and phosphate ions in saliva and plaque of xerostomic patients may be a limiting factor for remineralization driven by topical fluorides [6]. Papas et al. [7] suggested that aggressive fluoride protocols in patients receiving radiation therapy for head and neck cancer could be modified with adjusting the level of phosphate and calcium ions in remineralizing agents.

One of the most studied calcium phosphate-based nanotechnologies is casein

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phosphopeptide – amorphous calcium phosphate (CPP-ACP). This formulation localizes effectively at the surface of tooth enamel and enables a long enough retention of phosphate and calcium ions at the site where remineralization is wanted [6].

The purpose of the present study was to compare clinical efficacy of CPP-ACP and casein phosphopeptide – amorphous calcium fluoride phosphate (CPP-ACFP) containing pastes with 0.05% NaF oral rinse, and to evaluate the influence of these agents on dry-mouth symptoms and oral health-related quality of life in patients with SS.

METHODS

This longitudinal observational study was approved by the Ethics Committee of the School of Dental Medicine, University of Belgrade (document 36/30). The study was conducted in accordance with the guidelines of the Declaration of Helsinki.

The patients who were referred to the Clinical Centre of Serbia, Institute of Rheumatology and the Clinic of Allergology and Immunology were participants in this study. Dental examination was performed on thirty patients at the School of Dental Medicine of the University of Belgrade. One dentist performed the first dental exam making random treatment assignments. A random number table was used to randomize patients into three groups treated with different agents (n = 10): 1) 10% CPP-ACP (Tooth Mousse, GC Int Corp, Tokyo, Japan), 2) 10% CPP-ACP and 0.9 mg/g fluoride in CPP-ACFP (MI Paste Plus, GC Int), and 3) 0.05% NaF (Curasept ADS 205, Curaden International AG, Kriens, Switzerland). The sample size was calculated with 80% power at the 5% significance level, assuming the 5% dropout rate. The total sample size for the three groups was calculated to be 30 participants. A detailed description of the study material and methods has been published previously [8].

An assessment of oral health status of each participant was conducted at the beginning of the study, after 28 days (short-term effects) and after a six-month use of remineralizing agents. The complete oral exam was performed by two trained dentists, blinded for treatment allocation, using a dental mirror and explorer. The WHO criteria were used for the diagnosis of dental caries [decayed, missing, and filled teeth (DMFT) / decayed and filled surfaces (DFS)] [9]. Enamel demineralization was visually examined using the white spot lesion index (WSL – Gorelick) [10]. The gingival health was evaluated with the gingival index (GI – Löe–Silness) [11]. Assessment of oral hygiene was done using the simplified oral hygiene index (OHI – Greene–Vermilion) [12].

During the investigation, the patients were interviewed about their dietary and oral hygiene habits. The dietary habits questionnaire comprised nine questions about the number of main meals, number and type of cariogenic snacks, use of chewing gum, frequency of beverage intake, and the type of sweetener. The oral hygiene habits analysis included six questions related to regularity of tooth brushing, oral hygiene devices and products, and fluoride uses.

To compliment clinical examination, a self-rating of oral health and its influence on life in general was evaluated. Patients rated their oral health as: excellent, very good, good, fair, or poor. Self-assessed impact of oral health on life in general was recorded as follows: not at all, very little, some, a lot, and very much. The Xerostomia Inventory (XI) was used for measuring xerostomia symptoms [13]. Answers were coded as follows: 1 = never, 2 = hardly ever, 3 = occasionally, 4 = fairly often, and 5 = very often. Oral health-related quality of life was analyzed using a short form of the Oral Health Impact Profile (OHIP-14) [14]. The answers were marked as follows: 0 – never, 1 – hardly ever, 2 – occasionally, 3 – fairly often, and 4 – very often. Results for XI and OHIP-14 were obtained by summing up scores for each question. The prevalence of impacts was estimated by identifying individuals who answered with 'fairly often' and 'very often' [15].

Descriptive statistical analyses were primarily implemented. Kruskal–Wallis test and Wilcoxon test were used to analyze differences within the same treatment group during the experimental period. For the comparisons of frequency distributions, Fisher exact test and χ^2 test were used. The significance level of $p < 0.05$ was used. Statistics software package SPSS was utilized for data processing.

RESULTS

The study included 27 female and three male volunteers aged 15–65 years (mean 39.7 ± 16.6 years). Twenty-two (73%) participants came from urban communities, six (20%) persons were from peri-urban areas, and two (7%) were from rural areas. Education of participants was not equal: three (10%) patients completed primary school, 16 (53%) completed secondary school, five (17%) had a university degree, while six (20%) belonged to the category "pupil/student."

All patients experienced SS symptoms from at least six months to 25 years (average 5.7 ± 5.6 years). Hypertension was present in four patients, diabetes in three, and both conditions in one patient. Medications that have a side effect of reducing salivary flow were taken by 10 (33%) patients: antihypertensives (three patients), anxiolytics (three patients), both antihypertensives and anxiolytics (one patient), and nonsteroidal anti-inflammatory drugs (three patients). The stimulated salivary flow was 0.49 ± 0.20 ml/min. (0.06–0.70 ml/min.).

All participants in the study maintained oral hygiene using a toothbrush and toothpaste. The majority of participants brushed their teeth two (43%) or 2–3 (29%) times a day. Twenty-one (70%) patients did not know precisely what kind of toothbrush they had, and 24 (80%) did not use dental floss. Fluoridated toothpaste has been used by 24 (80%) of participants. However, 26 (86%) have never been recommended additional use of mouth rinse with fluoride. Analysis of dietary habits revealed that patients often had poor eating habits. Apart from drinking water,

Table 1. Caries incidence, white spot lesion index, gingival index (GI), and oral hygiene index-simplified (OHI) values

Parameter	CPP-ACP			CPP-ACFP			0.05% NaF		
	baseline	28 d	6 m	baseline	28 d	6 m	baseline	28 d	6 m
DMFT	15 ± 6.4	15 ± 6.4	15 ± 6.4	16.8 ± 6	16.8 ± 6	16.8 ± 6	16.7 ± 11.3	16.7 ± 11.3	17 ± 11.2
CC (DFS)	20.7 ± 13.5	20.7 ± 13.5	21 ± 13.8	21.4 ± 8.6	21.4 ± 8.6	21.6 ± 8.8	14.5 ± 11.3	14.8 ± 11.7	15.2 ± 12.2
RC (DFS)	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.2 ± 0.4	0.2 ± 0.4	0.2 ± 0.4	3.8 ± 2.8	3.8 ± 2.8	3.8 ± 2.8
WSL	2.9 ± 0.9	2.4 ± 1.2	2.1 ± 1.3*	2.1 ± 0.4	1.4 ± 0.5	1.1 ± 0.4**	2.2 ± 0.4	1.9 ± 0.3	1.6 ± 0.5*
GI	0.6 ± 0.4	0.4 ± 0.2	0.5 ± 0.4	0.4 ± 0.6	0.1 ± 0.1	0.3 ± 0.5	1.4 ± 0.1	0.7 ± 0.3	0.8 ± 0.5*
OHI	0.8 ± 0.6	0.4 ± 0.3	0.4 ± 0.3	1 ± 0.7	0.6 ± 0.5	0.4 ± 0.3	1.1 ± 0.4	0.6 ± 0.4	0.3 ± 0.3*

WSL – white spot lesion index; GI – gingival index; OHI – oral hygiene index-simplified; CC – coronal caries; RC – root caries; DMFT – decayed, missing, and filled teeth; DFS – decayed and filled surfaces;
*significant difference within the group ($p < 0.05$, Kruskal–Wallis test);
**significant difference within the group ($p < 0.00001$, Kruskal–Wallis test)

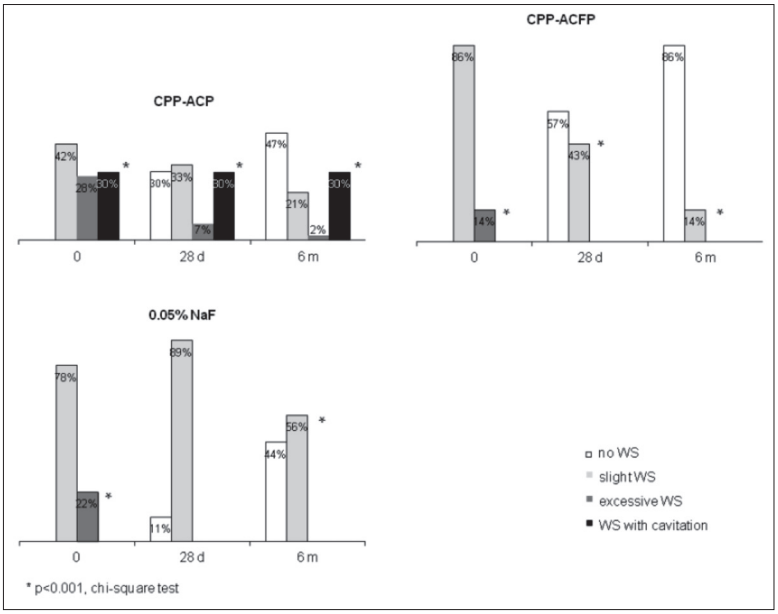

Figure 1. White spot lesion formation;

CPP-ACP – casein phosphopeptide – amorphous calcium phosphate;
CPP-ACFP – casein phosphopeptide – amorphous calcium fluoride phosphate; WS – white spot

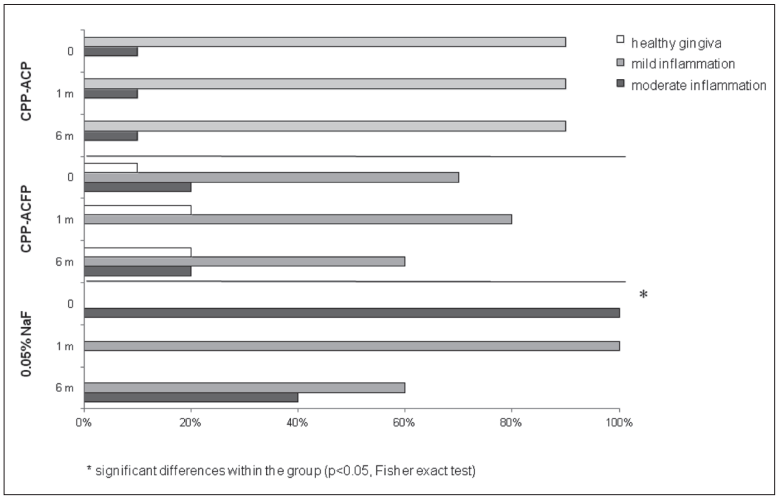

Figure 2. Gingival health

CPP-ACP – casein phosphopeptide – amorphous calcium phosphate;
CPP-ACFP – casein phosphopeptide – amorphous calcium fluoride phosphate

the problem with dry mouth was solved by consuming sweetened beverages (57%) and confectionery products (37%).

Over an experimental period of six months, no significant changes ($p > 0.05$, Kruskal–Wallis test) in caries incidence were documented in the three groups (Table 1). The caries increment was the same in both the CPP-ACP and CPP-ACFP group manifesting as secondary caries on two teeth, and initial caries lesion on one tooth in the NaF group ($p > 0.05$, Fisher exact test). Considerable regression of WSL was noted in all three experimental groups ($p < 0.001$, χ^2 test, Figure 1, Table 1). The influence of investigated agents on gingival health and oral hygiene is shown in Table 1 and Figures 2 and 3.

Three (10%) patients perceived their oral health as excellent, two (7%) described it as very good, 12 (40%) reported good oral health, while 15 (50%) patients described their oral health as being fair. Three (10%) patients believed that condition of their mouth did not affect their lives, one patient (3%) acknowledged very little influence of oral health on his life, and 12 (40%) patients reported some influence. Most of the participants in the study, 14 (47%) of them, reported a big impact of oral health on their lives.

Twenty-three (77%) patients reported that the feeling of dry mouth occured "fairly often" or "very often." Among other XI items, the most prominent were feeling of dry skin (83%), dry eyes (70%), dry lips (70%), and dry nose (60%). Twelve patients (40%) had problems in eating and swallowing.

The mean OHIP-14 score among SS patients was 14.13 ± 11.58 (range 0–34). The prevalence of OHIP-14 individual items is shown in Table 2. During the study, no significant change in OHIP-14 score was

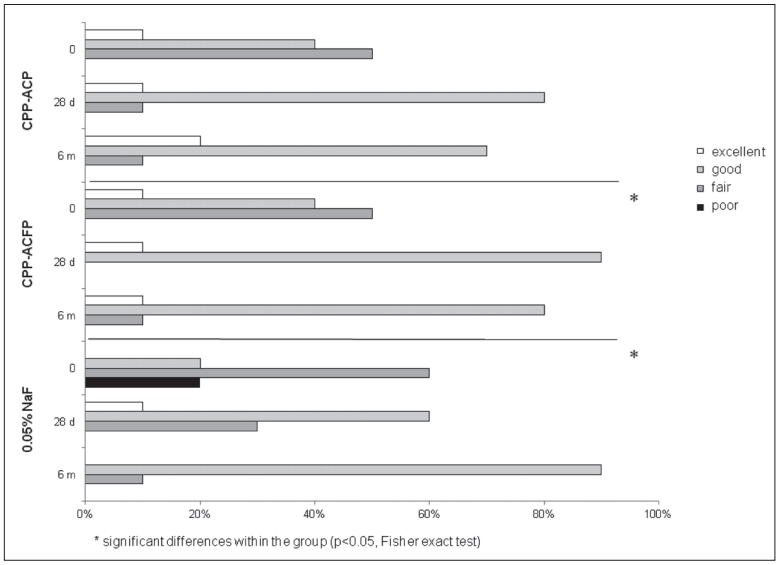

Figure 3. Oral hygiene;

CPP-ACP – casein phosphopeptide – amorphous calcium phosphate;
CPP-ACFP – casein phosphopeptide – amorphous calcium fluoride phosphate

Table 2. Prevalence of OHIP-14 items

OHIP-14 items	Prevalence
Functional limitation	
Have you had trouble pronouncing any words?	8 (27%)
Have you felt your sense of taste has worsened?	4 (13%)
Physical pain	
Have you had painful aching in your mouth?	10 (33%)
Have you found it uncomfortable to eat any foods?	12 (40%)
Psychological discomfort	
Have you been self-conscious?	3 (10%)
Have you felt tense?	2 (7%)
Physical disability	
Has your diet been unsatisfactory?	6 (20%)
Have you had to interrupt meals?	5 (17%)
Psychological disability	
Have you found it difficult to relax?	3 (10%)
Have you been a bit embarrassed?	3 (10%)
Social disability	
Have you been a bit irritable with other people?	3 (10%)
Have you had difficulty doing your usual jobs?	1 (3%)
Handicap	
Have you felt that life in general was less satisfying?	5 (17%)
Have you been totally unable to function?	0

noted in any of three experimental groups ($p > 0.05$, Wilcoxon test).

During the six-month observation period, reduced feeling of dry mouth was reported in CPP-ACP (scores of 3.3 ± 0.8 and 2.9 ± 0.9, respectively), and CPP-ACFP group (scores of 2.1 ± 1 and 1.9 ± 0.8, respectively). In addition, physical pain was reduced in all three experimental groups (2.2 ± 0.4 and 1.8 ± 0.5 for CPP-ACP, 2 ± 1.2 and 1.8 ± 1.1 for CPP-ACFP, 2.4 ± 0.5 and 2.3 ± 0.5 for 0.05% NaF), but without statistically significant differences ($p > 0.05$, Wilcoxon test).

DISCUSSION

Increased susceptibility to oral soft tissue diseases and caries in relation to salivary gland hypofunction has been a long-time problem for patients, as well as for dentists. Even though xerostomic patients tend to be more vigilant about their oral health, higher incidence of coronal caries, root caries, and oral soft tissue pathology comparing to healthy persons has been reported [16]. In the beginning of the present study, high prevalence of dental caries and gingivitis, poor oral hygiene and inadequate diet were found in participants. The higher risk of caries in patients with salivary gland hypofunction is due to poor self-cleaning and reduced salivary defense mechanisms. Dental plaque build-up, associated with increased concentration of pathogens, contribute to further vulnerability of dental tissues. Sometimes, mucositis and dental hypersensitivity can impede even more the maintaining of regular oral hygiene. In an attempt to stimulate salivary flow and overcome chewing and swallowing difficulties, xerostomic patients tend to excessively consume sugar-sweetened beverages and softer, more cariogenic food.

Evidence of clinical efficacy of CPP-ACP for remineralization of early caries lesion has been reported, but clinical superiority of CPP-ACP over fluoride has not been determined yet [6, 17]. In order to investigate the anticariogenic potential of calcium phosphates alone, and in comparison to an already approved remineralizing agent in patients with salivary glands hypofunction, CPP-ACP, CPP-ACFP, and 0.05% NaF have been included in the present study.

Efficacy of CPP-ACP in patients with salivary gland hypofunction has not been extensively studied. Sim et al. [18] reported lower rates of caries progression for both occlusal and smooth surfaces in persons treated with 0.4% stannous fluoride gel supplemented with CPP-ACP-containing crème. However, this study was conducted immediately after head and neck radiotherapy when none of the therapeutic agents was capable of completely preventing decay. Previously we investigated *in situ* caries-preventive potential of calcium phosphate agents in patients with SS [8]. The study revealed reduction in quantity and dimensions of enamel defects. After 28 days of CPP-ACP and CPP-ACFP application, enamel surface showed improved, more uniform and smooth appearance. The present study found no significant caries increment during the observation period. In addition, significant

WSL remineralization was noticed in the calcium phosphate groups in a short time, while the remineralization effect of NaF mouthrinse in the treatment of WSL was noticed after a six-month use.

Efficacy of prophylactic agents evaluated in the present study is probably best shown by the reduction of WSL appearance. WSL has been traditionally evaluated by direct visual examination. However, the method is not quantitative, and can be influenced by a subjective opinion of the evaluator, thus it may not be precise enough. In order to overcome these limitations, several caries quantification methods were proposed. In recent clinical studies, quantitative light-induced fluorescence (QLF) has been considered a gold standard for the detection of WSL and their longitudinal observation. The method enables monitoring and quantifying changes in the mineral content of the tooth enamel and the area of the tooth covered with WSL, but it is time consuming and not cost-effective [19]. Chairside fluorescence-based caries diagnostic methods, i.e. DIAGNOdent, proved to be less sensitive and accurate compared to QLF [20], and no better than visual diagnostics [19]. Therefore, visual evaluation of WSL, although focused only on the severity and not on the size of the lesion, is still the adequate method for everyday clinical practice.

In the present study, improved oral hygiene and subsequently better gingival status was reported in a very short time – after 28 days. Better motivation of patients and conviction that good oral hygiene habits contributed to the improvement of their general health might be the reason for such fast changes. Furthermore, changes in the DMFT/DFS distribution (higher prevalence of filled surfaces) were noted. However, patients' compliance and adherence to the oral hygiene routine tend to decrease over time. Frequent control examinations and re-motivation are of great importance in high caries risk groups. Therefore, adequate preventive, prophylactic and less invasive therapeutic procedures may retain good oral health and prevent difficult complications of salivary glands hypofunction. Another possible way to deliver oral health information to the patients might be the e-health. Today, the availability and the ease of access to the online health information have re-defined the terms of the doctor–patient relationship [21]. Although not without limitations and negative aspects, new technologies can effectively provide information, improve access to healthcare, and help patients share their experiences [22]. However, internet content is usually oriented towards dental diseases rather than prevention and oral health promotion [23]. Reliable and usable information might be of great importance, especially for patients with increased risk for oral diseases such as xerostomic patients. Patients' knowledge on oral hygiene and dietetic regimen could be improved if clinicians referred them to reliable internet educational sources. The cooperation between the specialties is mandatory, as this content should be placed on both dental and web-pages that provide information concerning systemic diseases.

Xerostomia presents an everyday challenge for persons suffering from SS. Dry mouth is usually accompanied with taste changes, burning mouth sensation, difficulties in opening the mouth, swallowing, chewing, and speaking. Often, salivary hypofunction does not only result in uncomfortable feeling, but can seriously threaten oral and general health. The assessment of the quality of life should become an essential part of oral health evaluation. Focusing on social, emotional, and physical aspects of the illness instead of the illness *per se* may contribute to the patient motivation and active participation in the treatment and recovery [24].

In an effort to understand the efficacy of investigated agents to help SS patients overcome disability, both OHIP-14 and XI were used in the present study. Our findings confirmed that xerostomia presented significant and noticeable influence on the patients' quality of life. Almost half of the participants revealed that "fair" description of their oral status had an enormous influence on their perception of well-being and life itself. They usually complained of compromised oral functions such as talking, chewing, and swallowing, while psychological and social disabilities were rarely pointed out. Our results are in accordance with those of Locker [25], who described discomfort with eating, chewing and swallowing in elderly xerostomic patients, while prevalence of psychological and social disabilities was reported to be less than 10%. Thomson et al. [15] reported lower occurrence of functional limitations and higher rates of psychological discomfort and disability in xerostomic adults, which is in disagreement with findings of the present study. Likewise, Ikebe et al. [26] showed significant psychological and social limitations in patients with hyposalivation. The fact that the last two studies used only OHIP-14 as a survey method, may explain the differences. The OHIP-14 contains fewer questions exploring limitations of oral functions, while psychological discomfort and social disabilities are more emphasized. Therefore, more detailed questionnaire aiming to disclose relevant information regarding oral health condition and the need of dental care in persons with salivary gland hypofunction is necessary. In addition, results of the present investigation showed that CPP-ACP and CPP-ACFP agents decrease dry mouth and burning mouth sensations to some extent, which might be helpful in improving the oral discomfort in patients diagnosed with salivary gland dysfunction. Nevertheless, rehabilitation of dry mouth depends on the etiology and the treatment of systemic conditions, rather than just management of the oral symptoms. Therefore, treatment of systemic disease, i.e. adjustment to certain kind of medication, introduction to sialagogues, diet, etc., should be monitored by both the dentist and the general practitioner.

CONCLUSION

Within the limitations of the present study, it has been demonstrated that remineralizing agents containing CPP-ACP and CPP-ACFP show promising results as caries-preventive agents for patients with SS. Even though regression of WSL was noted in three experimental groups, remineralization was faster and more pronounced in groups

treated with CPP-ACP and CPP-ACFP agents. Despite the high caries risk in SS patients, caries increment was low during the six-month observation period. Early improvement of GI and OHI suggested the importance of oral health education, frequent dental exams and motivation in patients with SS. CPP-ACP and CPP-ACFP agents might be helpful in improving the oral discomfort in patients diagnosed with salivary gland dysfunction.

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Клиничка ефикасност казеинског фосфопептида – аморфног калцијум-фосфата и казеинског фосфопептида – аморфног калцијум-флуорофосфата и њихов утицај на квалитет живота оболелих од Шегреновог (*Sjögren*) синдрома

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САЖЕТАК

Увод/Циљ Циљ рада је био да се упореди клиничка ефикасност казеинског фосфопептида – аморфног калцијум-фосфата (*CPP-ACP*) и казеинског фосфопептида – аморфног калцијум-флуорофосфата (*CPP-ACFP*) са 0,05% *NaF* и да се испита њихов утицај на квалитет живота код оболелих од Шегреновог (Sjögren) синдрома.

Методе Тридесет болесника је рандомизовано у три групе које су користиле различита средства за реминерализацију глеђи: *CPP-ACP*, *CPP-ACFP* и 0,05% *NaF*. Орално здравље је анализирано на почетку истраживања, после 28 дана (краткотрајни ефекат испитиваних средстава) и после шест месеци, и то: заступљеност обољења зуба (КЕП/кеп индекс), деминерализација глеђи (Горликов индекс беле мрље), стање гингиве (Лоу–Силнесов гингивални индекс) и ниво оралне хигијене (Грин–Вермилионов индекс). За процену симптома сувих уста и квалитета живота у вези са оралним здрављем коришћени су упитници *Xerostomia Inventory* и *Oral Health Impact Profile*.

Резултати У току истраживања, прираштај каријеса ни у једној од испитиваних група није био значајан. Израженост почетних каријесних лезија значајно је редукована ($p < 0,001$). Испитивана хемиопрофилактичка средства нису значајно утицала на побољшање нивоа оралне хигијене и здравља гингиве. Примена *CPP-ACP* и *CPP-ACFP* допринела је слабијој изражености осећаја сувих уста, као и смањеном осећају печења и жарења.

Закључак *CPP-ACP* и *CPP-ACFP* могу допринети заустављању акутног тока каријеса и смањеној израженост симптома сувих уста код оболелих од Шегреновог синдрома.

Кључне речи: каријес; казеински фосфопептид – аморфни калцијум-фосфат; казеински фосфопептид – аморфни калцијум-флуорофосфат; ксеростомија; Шегренов (Sjögren) синдром

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Pleuropulmonary manifestations of systemic autoimmune diseases – an 84-case series analysis

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SUMMARY

Introduction The systemic autoimmune diseases (SAD) can cause a variety of pulmonary and pleural abnormalities. The aim of this paper is to review clinical and radiological characteristics of a series of patients with a systemic autoimmune disease hospitalized at a tertiary level facility.

Methods In this retrospective study, we reviewed the clinical and imaging findings in patients diagnosed with SAD at the Teaching Hospital of Pulmonology during a nine-year period.

Results An 84-patient group (mean age of 53.8 years) consisted of 64 women and 20 men. Fifty-eight out of 84 patients suffered from collagen vascular disease (CVD) and 26/84 had systemic vasculitis. Fatigue was the dominant symptom (75.8% in CVD, and 69.2% in vasculitis). Cough, hemoptysis, and fever were more frequent in patients with vasculitis. Fibrosis was the most common radiological manifestation of CVD (26/58), followed by pleural effusion (18/58) and consolidation (10/58). Irregular opacities were dominant radiologic finding in vasculitis (10/26), followed by nodules (8/26). Histological confirmation of systemic autoimmune disease was obtained in 28.6% patients, in 58/84 patients the diagnosis was based on a positive serologic test and clinico-radiological manifestations, in two cases on clinical and radiological features according to defined criteria.

Conclusion Pleuropulmonary manifestations of SAD are usually expressed in the sixth decade of life, predominantly in women. Clinical findings and positive serologic tests suggest diagnosis of SAD. Fibrosis is the most common radiologic pattern found in almost one half of the patients with CVD and irregular opacities are the most common findings in vasculitis.

Keywords: autoimmune diseases; vasculitis; pleura; pulmonary; radiology

INTRODUCTION

Systemic autoimmune diseases (SAD) include a heterogeneous group of immunologic disorders whose common characteristic is the presence of an idiopathic systemic autoimmune process. These disorders include collagen vascular diseases (CVD) and the systemic vasculitis. The characteristic thoracic manifestations of the diseases are influenced by the pathophysiologic characteristics of the underlying process. The pleuropulmonary manifestations of systemic diseases are broad and vary according to the specific disease type. Several anatomic locations of the respiratory tract may be involved, including lung parenchyma, airways, vessels, pleura, and respiratory muscles [1, 2]. In some patients, pulmonary involvement belongs to prognostic factors related to mortality. The major causes of morbidity and mortality in CTD are interstitial lung diseases (ILD) and pulmonary arterial hypertension [3, 4]. Although pulmonary complications generally occur in patients with a well-established disease, lung involvement can be the first manifestation of an autoimmune disorder. Patients with CVD are at a higher risk of various malignancies, and the most frequent are breast and lung cancer, the latter most commonly detected at an advanced stage [1, 5]. Therefore, both the general practitioner and the specialist should have broad knowledge of the SAD and their complications because identification of these manifestations may initiate earlier treatment and, possibly, better disease outcome. Diagnosis of the SAD solely on a clinical basis is difficult due to mainly nonspecific presentation. Apart from that, the diagnosis is based on imaging, histopathology, biology, and autoimmune serology [2]. We aimed to analyze a group of patients with SAD in terms of their clinical, immunologic, histologic, and radiological features.

METHODS

Subjects

This retrospective study was performed on 84 patients discharged from the Teaching Hospital of Pulmonology, with diagnoses of pleuropulmonary manifestations of systemic diseases in a nine-year period. The medical files were carefully reviewed for clinical, radiological, immunological, and histological features. Clinical examination included the data of general

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and respiratory physical examination. The radiological examination included plain chest X-ray and high-resolution computed tomography (HRCT) of the thorax. Pulmonary function tests included spirometry: forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), FEV1/FVC ratio, and peak expiratory flow (PEF) [6]. Patients with hemoptysis or severe clinical imaging were not examined spirometrically, but rather pulse oximetry or arterial blood gas analysis were performed. The following investigations were also performed: complete blood count (CBC), routine urine analysis, serum levels of rheumatoid factor (latex agglutination test), antinuclear antibody (ANA) (immunoassay method), c-ANCA (anti-neutrophil cytoplasmic antibodies) and p-ANCA (indirect fluorescence antibody and ELISA method), C-reactive protein assay (latex agglutination test), and biopsies of different organs in 24 patients. The diagnosis was based on the evaluation of clinical and radiological manifestations, serological tests, and histological analyses of the involved organs.

The study was done in accordance with the institutional Committee of Ethics.

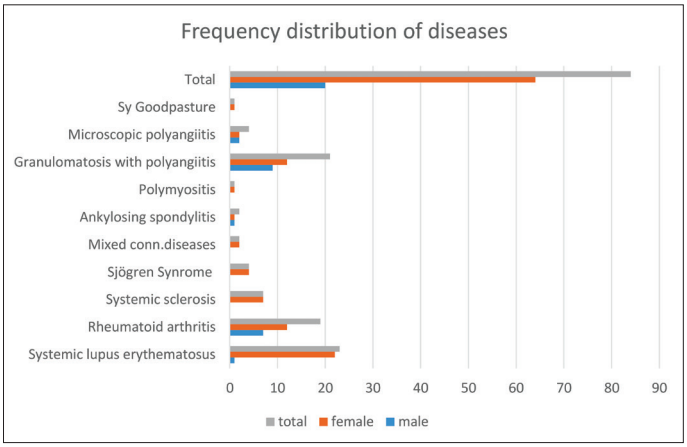

Figure 1. Frequency distribution of diseases

Table 1. Clinical presentation of the patients with systemic autoimmune diseases (n = 84)

Symptoms	CVD	Vasculitis	Total	p
	n (%)	n (%)	n (%)	
Cough	37 (63.8)	24 (92.3)	61 (72.6)	0.0285
Hemoptysis	7 (12.1)	17 (65.4)	24 (28.6)	0.001
Chest pain	23 (39.6)	3 (11.5)	26 (30.9)	0.614
Dyspnea	37 (63.8)	13 (50)	50 (59.5)	0.077
Fever	24 (41.4)	12 (46.2)	36 (42.8)	0.020
Fatigue	44 (75.8)	18 (69.2)	62 (73.8)	0.325
Arthralgia	22 (37.9)	7 (21.9)	29 (34.5)	0.0123
Loss of weight	16 (27.6)	2 (7.7)	18 (21.4)	0.551

CVD – collagen vascular diseases

Statistical analysis

Statistical analysis was performed using the statistical program R-- version 3.1.1 (2014-07-10) "Sock it to Me," Copyright (C) 2014; the R Foundation for Statistical Computing; Platform: x86_64-w64-mingw32/x64 (64-bit); (22.10.2014). Descriptive statistics were used to summarize baseline patients' demographic and clinical characteristics. The results were expressed as mean ± standard deviation for continuous variables and as percentages for categorical variables. Testing of normality of the data with normal distribution was performed using graphics: normal Q-Q plot and histogram, and Kolmogorov–Smirnov and Shapiro–Wilk tests. Continuous variables were compared by the Wilcoxon or the Kruskal–Wallis test. Categorical variables were compared using the χ^2 test and the Fisher's exact test. A p-value < 0.05 was considered statistically significant. In the case of multiple testing on the same data set, Bonferroni correction was used ($\alpha_1 = 0.05/6 = 0.0083$).

RESULTS

The study group of 84 patients with SAD included 76.2% women and 23.8% men. The patients' age ranged from 19 to 83 years (mean being 53.8 ± 13.8 years) with predominance of those between 41 and 70 years. Patients with systemic vasculitis were significantly younger than those with CVD (p < 0.017).

Clinical characteristics

We reviewed 58 patients with CVD and 26 with systemic vasculitis. Frequency distribution of the diseases is shown in Figure 1. Among patients with CVD, female patients prevailed (49/58). There was no significant sex frequency difference in the group of patients with primary systemic vasculitis. The average age at the onset of disease was 43.7 ± 14.05 years in patients with CVD, and 48.3 ± 11.9 years in patients with vasculitis (p = 0.128). Eighty-one (96.4%) patients had two or more symptoms and only three patients with CVD had only one symptom. Overall, the dominant symptom was fatigue. Cough, hemoptysis, and fever were more frequent in patients with vasculitis (Table 1). The duration of symptoms varied from a few weeks to 35 years. Thirty-two patients (38.1%) were non-smokers, 13 (15.4%) were smokers, and 7 (8.3%) ex-smokers. Thirty-four (40.5%) patients were exposed to environmental tobacco smoke. Lung function tests were done in 47/84 patients. Thirty-three of these were patients with CVD. Disorder of pulmonary function was found in 41 (87.2%) patients: in 29 with CVD and in 12 with vasculitis. The most common pulmonary function disorder tested with spirometry was restriction in 18 (38.3%) patients, followed by mixed pulmonary ventilation disorder in 13 (27.7%) and obstruction in 10 (21.3%) patients. Arterial blood gas analysis performed in 37 (44%) patients showed that 27 (81.8%) of the investigated patients experienced combined pO_2 and pCO_2 disorders and six (16.2%) had hypoxemia. The analysis of CBC revealed anemia in six patients with CVD and in 10 with vasculitis. Raised erythrocyte sedimentation rate was found in 46 patients with CVD and in

Table 2. Radiologic presentation of systemic autoimmune diseases

Radiologic finding→ Disease↓	Pleural effusion	Fibrosis	Consolidation	Other	Total
Systemic lupus erythematosus	11	3	7	2 (1 bulla, 1 tracheal stenosis)	23
Rheumatoid arthritis	7	8	3	1 (adhesions)	19
Systemic sclerosis	0	7	0	0	7
Sjögren's syndrome	0	4	0	0	4
Ankylosing spondylitis	0	1	0	1 (bulla)	2
Mixed connective tissue disease	0	2	0	0	2
Polymyositis	0	1	0	0	1
Granulomatosis with polyangiitis	0	0	10	8 nodules 2 thickened bronchovascular bundles 1 ground glass opacity	21
Microscopic polyangiitis	1	1	0	1 thickened bronchovascular bundles 1ground glass opacities	4
Sy Goodpasture	0	0	0	1 alveolar opacity	1
Total	19	27	20	18	84

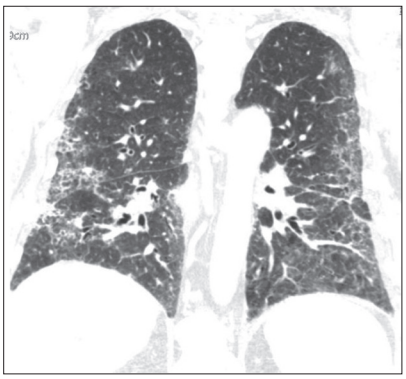

Figure 2. Coronal chest computed tomography view in lung window setting in a patient with systemic sclerosis shows thickened interstitium with ground glass opacities in peripheral parts of both lungs

Table 3. Frequency of interstitial lung diseases in systemic autoimmune diseases

Diseases	NSIP	UIP	OP	Indeterminate	LIP	Total
Systemic sclerosis	6	1	0	0	0	7
Rheumatoid arthritis	2	6	1	0	0	9
Sjögren's syndrome	3	0	0	0	1	4
Systemic lupus erythematosus	0	0	1	1	0	2
Mixed connective tissue disease	0	1	1	0	0	2
Polymyositis	1	0	0	0	0	1
Microscopic polyangiitis	0	1	0	0	0	1
Ankylosing spondylitis	0	0	0	1	0	1
Total	12	9	3	2	1	27

NSIP – nonspecific interstitial pneumonia; UIP – usual interstitial pneumonia; OP – organizing pneumonia; LIP – lymphoid interstitial pneumonia

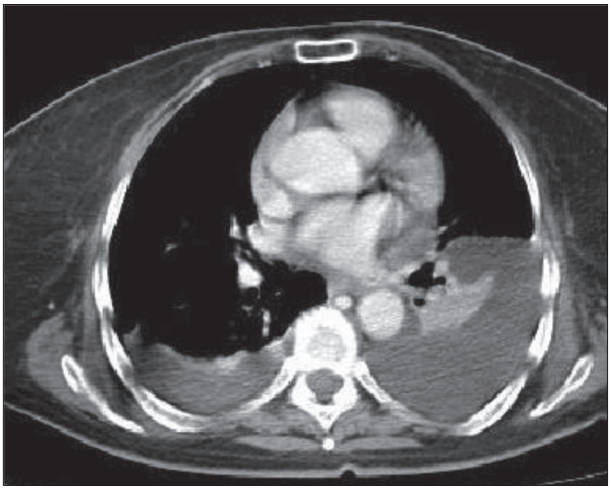

Figure 3. Axial computed tomography scan in soft tissue window shows bilateral pleural effusion, more prominent on the left side in a female patient with systemic lupus erythematosus

20 patients with vasculitis. Elevated levels of serum urea and creatinine were detected in 22 patients. All patients with CVD had positive serologic tests and all but two patients with vasculitis had positive ANCA values. We found concomitant manifestations in 38 patients with CVD: cardiovascular in 14, hematological in nine, kidney failure in six, three patients had pulmonary thromboembolism, and the other three had hypothyreosis. Three of them suffered from carcinoma (endometrium, urinary bladder, and stomach, respectively). Sixteen patients with vasculitis had a generalized form of the disease, including renal failure, and in 10 patients with limited form GPA, upper respiratory tract was also involved.

Radiological characteristics

Lung fibrosis was the most common manifestation of CVD in our patients, followed by consolidation and pleural effusion (Table 2). A significant correlation was found between the duration of the symptoms and fibrosis ($p < 0.000$). Fibrosis was diagnosed on HRCT examination in nearly one half of patients with CVD and in one patient with microscopic polyangiitis (Figure 2, Table 3). Fibrosis was predominant in women. Only three out of 27 patients were males with rheumatoid arthritis (RA). Lung consolidations were observed in 1/5 of patients with CVD, most frequently in systemic lupus erythematosus (SLE). All the patients had unilateral consolidation, but one SLE patient with acute bilateral pneumonitis. Pleural effusion frequency distribution is presented in Table 2. In seven cases, pleural effusion appeared prior to the diagnosis of a systemic disease, and in other cases 1–30 years after reaching the diagnosis (Figure 3). There was no correlation between the appearance of pleural effusion and the duration of the systemic disease. Irregular consolidations were the dominant radiologic finding in GPA (Figure 4), followed by nodules. Cavitations were detected in five of eight cases

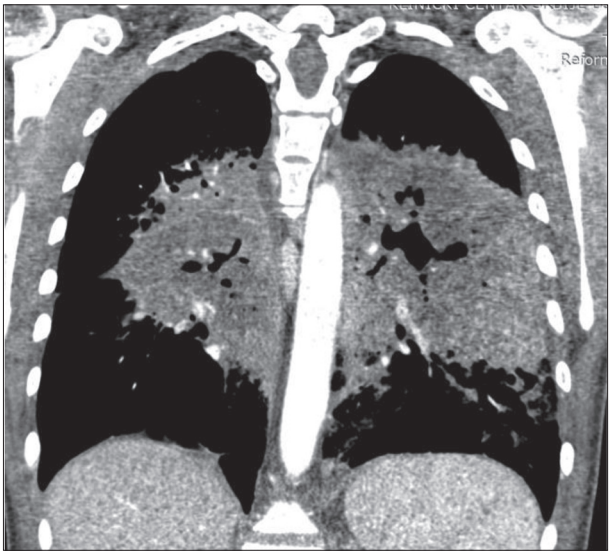

Figure 4. Coronal computed tomography view in a soft tissue window setting in a female patient with granulomatosis with polyangiitis shows irregular parenchymal consolidation with a cavitation in both lungs

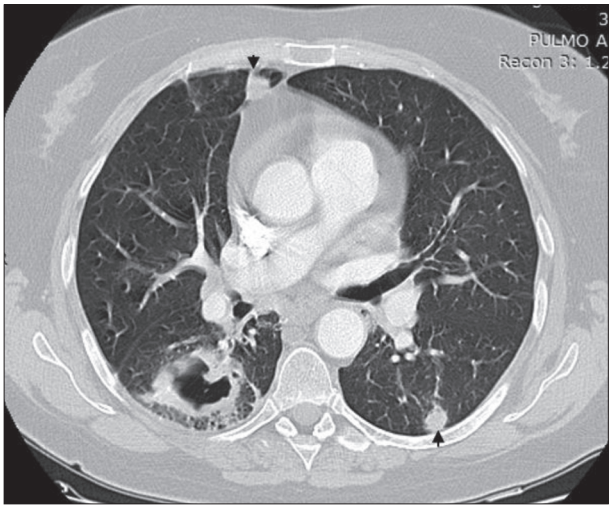

Figure 5. Axial computed tomography view in a lung window setting demonstrates a large cavitary mass with thick irregular borders in the right lower lobe, and the nodules in both lungs (arrows) in granulomatosis with polyangiitis

with nodules (Figure 5) and in three cases with consolidations. The diagnosis was based on a positive serologic test and on clinico-radiological manifestations in 58 patients, and in two cases with ankylosing spondylitis, on clinical and radiological features according to the Roma criteria. Histological verification was achieved in 24 (28.6%) patients from biopsy specimens of the affected organs (lung in 16, kidneys in five, oral mucosa in two, and larynx in one case).

DISCUSSION

Clinical features

In the presented series of our patients with SAD, CVD were more frequent than systemic vasculitis, which corresponds to the literature data. Female patients prevailed in the group with CVD [7]. Contrary to some literature data, the age at onset of CVD and vasculitis were similar in our study, being mostly expressed in the fifth and six decades of life [8]. The dominant symptom was fatigue, slightly more frequent in patients with CVD. Some other studies reported similar frequency of fatigue in SAD that ranged from 70% in Sjögren's syndrome to 80% in systemic sclerosis and RA. The cause of fatigue in SAD is still unclear and some studies explain it by peripheral immune activation and systemic inflammation either directly or indirectly by mitochondrial damage induction [9, 10]. Similarly, according to some other studies, the lung function test abnormalities were found predominantly in patients with CVD [11]. Most of investigated patients had combined pO_2 and pCO_2 disorders and six (16.2%) had hypoxemia without the pCO_2 disturbance. Considerable proportion of our patients had been exposed to tobacco smoke contents through active or passive smoking. It is evidence-based that oxidative and nitrosative stress and exacerbation of chronic inflammation can contribute to the development of autoimmune diseases [12, 13]. Usual peripheral blood laboratory tests were nonspecific and they pointed to an inflammatory syndrome. Concomitant manifestations were frequent in patients with CVD. Cardiovascular events are the major cause of premature death in these patients. Accelerated atherosclerosis is considered the primary cause of cardiovascular diseases and side effects of immunotherapy can also contribute to these diseases [2, 14]. Anemia is a very common abnormality associated with systemic diseases. Recognition of anemia in CVD is very important and correction of anemia is dependent on the correction of underlying CVD [15]. Renal involvement as a concomitant manifestation was present mostly in patients with SLE and in 16 patients with vasculitis, renal failure confirmed generalized form of the disease [16]. Three patients with CVD at the time of analysis had diagnosed carcinoma but none had lung carcinoma. Connective tissue disease represents a large group of diseases which can be associated with carcinoma of different localizations, and most frequently with breast and lung cancers [5, 14]. Risk factors for lung cancer development in connective tissue disease are still the subject of basic research. The effects of immunosuppressive therapy on cancer risk remain controversial [5].

Radiological characteristics

In patients with CVD, lung involvement was manifested dominantly with lung fibrosis followed by consolidations and pleural effusion. In concordance to literature data, all patients with systemic sclerosis, Sjögren's syndrome, mixed connective tissue disease (MCTD), polymyositis, and about a half of the patients with RA had lung fibrosis [17, 18, 19]. Some studies showed 20–80% prevalence of pulmonary fibrosis in patients with scleroderma [3, 11, 18]. The other studies reported ILD in 20–68% of patients with RA [11, 17–20], in up to 65% patients with polymyo-

sitis/dermatomyositis (PM/DM) [11, 21], in 21–66% % cases with MCTD [3], and in 8–38% of Sjögren's syndrome [11, 22]. Pleuropulmonary abnormalities in ankylosing spondylitis are associated with findings such as upper lobe fibrobullous disease, nonspecific interstitial changes, septal and pleural thickening [17, 23]. Although proportions of interstitial pneumonias vary, nonspecific interstitial pneumonia prevailed in our patients with scleroderma and Sjögren's syndrome. This is consistent with the findings of other studies in which reticulations and ground glass opacities were the most common HRCT abnormalities [3, 17, 18, 20]. Similarly to previously reported series, in our patients with rheumatoid arthritis, usual interstitial pneumonia (UIP) was most frequent ILD, but RA-ILD was more common in female patients, which differs from literature data [19, 20, 24]. This can be explained by differences in disease activity and sample size. ILD in CVD have better prognosis than idiopathic ILD, with the exception of RA-related ILD with UIP characteristics [17, 20]. Some studies reported three to four times higher mortality in patients with systemic sclerosis and RA who had ILD than in the general population. Five-year mortality rate is reported to be 35–39% after ILD diagnosis in patients with RA [19]. Consolidations were a less common finding in patients with CVD, being most frequent in SLE, followed by RA. Pneumonia was the most common cause of consolidation [17, 19, 20]. Pleural effusion was diagnosed most commonly in our patients with SLE and RA, with frequencies similar to previously reported results [22, 25, 26, 27]. Pleural involvement has been mentioned as the most common finding in SLE in many studies in the past, but has become far less frequent in the last two decades probably due to the early diagnosis of RA and a more aggressive treatment [19]. Imaging findings of pulmonary vasculitis are diverse and often poorly specific. The most characteristic findings were opacities of different appearance from nodular masses to ill-defined areas of consolidation, both of which cavitated. This finding is highly suggestive of GPA [28, 29]. A series of our patients with GPA showed differences in radiological features of the lung changes when compared with other reported series [29]. In the present study, areas of consolidation were slightly more frequent than nodules, but due to the small number of the patients, the result needs further evaluation on a larger sample size. The spectrum of radiological and clinical findings in our patients with microscopic polyangiitis ranged from interstitial fibrosis to ground glass opacities and pleural effusion. Goodpasture syndrome in one patient manifested with alveolar opacities. Diffuse, bilateral, and low-density patterns in vasculitis corresponded to diffuse hemorrhage and capillaritis on pathologic examinations [28, 30, 31]. Enlarged sample size could examine these findings in the future.

Study limitations

Retrospective design of our study is one of the limitations which is subject to recall bias and possible non-uniformity of the collected data. In addition, we were unable to make any conclusions regarding some of the SAD due to limited sample size. The fact that our study group included SAD patients from the pulmonology referral center is subject to selection bias, which limits the value of the presented findings since the cohort is not representative of all possible autoimmune-disease patients with pleuropulmonary manifestations in the population. Despite the limitations, our study may offer a broad description of a variety of thoracic manifestations of systemic diseases.

CONCLUSION

The SAD can cause a variety of pulmonary abnormalities, predominantly expressed in women in the sixth decade of life. Identification of the pattern-associated antibodies and correlation with clinical findings are necessary for the diagnosis of CTDs. Pulmonary fibrosis is the most common radiologic pattern in CVD, and poorly specific irregular opacities dominate in vasculitis. The pleural cavity is the most affected site in RA and SLE. In order to recognize, diagnose, and manage the SAD in a timely manner, associated efforts and skills of clinicians, radiologists, and pathologists are of the utmost importance.

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Conflict of interest: None declared.

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Плеуропулмонална испољавања системских аутоимуних обољења – анализа серије од 84 случаја

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САЖЕТАК

Увод Системске аутоимуне болести могу узроковати разне плућне и плеуралне абнормалности.

Циљ овог рада је да се прикажу клиничке и радиолошке карактеристике серије болесника са системским аутоимуним болестима хоспитализованих у терцијарној установи.

Методе У овој ретроспективној студији прегледали смо клиничке и радиолошке налазе код болесника са дијагнозом системских аутоимуних болести на Универзитетској болници за плућне болести током деветогодишњег периода.

Резултати Група од 84 болесника (средња старост 53,8 година) састојала се од 64 жене и 20 мушкараца. Педесет осам од 84 болесника (69,04%) боловало је од колагене васкуларне болести (КВБ), а њих 26 је имало системске васкулитисе. Доминантан симптом је био замор (75,8% код КВБ и 69,2% код васкулитиса). Кашаљ, хемоптизије и повишена температура били су чешћи код болесника са васкулитисом. Фиброза је била најчешће радиолошко испољавање КВБ (26/58), затим плеурални изливи (18/58) и консолидације (10/58). Неправилне консолидације су биле доминантан радиолошки налаз код васкулитиса (10/26) и праћене су нодуларним променама (8/26). Хистолошка потврда системске аутоимуне болести је добијена код 28,6% болесника, код 58/84 болесника дијагноза је заснована на позитивним серолошким тестовима и клиничко-радиолошким испољавањима, у два случаја на клиничким и радиолошким карактеристикама према дефинисаним критеријумима.

Закључак Плеуропулмонална испољавања системских аутоимуних болести обично се јављају у шестој деценији, претежно код жена. Клинички налаз и позитивни серолошки тестови указују на системску аутоимуну болест. Фиброза је најчешћи радиолошки налаз, који се налази код скоро половине болесника са колагеним васкуларним болестима, а неправилне консолидације су најчешћи налази у васкулитису.

Кључне речи: аутоимуне болести; васкулитис; плеура; плућа; радиологија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

In-hospital mortality predictors after surgery for Stanford type A aortic dissection – single-center five-year experience

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SUMMARY

Introduction/Objective Stanford type A aortic dissection is a surgical emergency associated with high mortality.

The aim of this study was to determine which group of patients and which characteristics were associated with postoperative, in-hospital mortality.

Methods The retrospective study included 116 patients with type A aortic dissection surgically treated over a five-year period. The association between postoperative, in-hospital mortality and patient characteristics was examined.

Results Total postoperative, in-hospital mortality was 22.4% (26 out of 116 patients). The variables that, after a multivariate analysis, showed a direct correlation with mortality were as follows: admission creatinine value [OR 1.026 (1.006–1.046), p = 0.009], C-reactive protein (CRP) > 10 mg/L [OR 4.764 (1.066–21.283), p = 0.041], and stroke [OR 6.097 (1.399–26.570), p = 0.016]. The receiver operating characteristic (ROC) curve showed that creatinine could be a good predictor of mortality (area under the ROC curve = 0.767; p < 0.0005). The cut-off point was 124.5 μmol/L. The sensitivity was 65% and the specificity was 80%. The cut-off point for CRP was 14.5 mg/L – sensitivity 71.4%, specificity 75% (area under the ROC curve = 0.702, p = 0.021).

Conclusion Surgery for type A aortic dissection is still associated with relatively high mortality. A lower chance of survival may be indicated by elevated admission creatinine and CRP values, as well as stroke.
Keywords: aorta; dissection; mortality; creatinine; CRP; stroke

INTRODUCTION

Aortic dissection is the most common aortic emergency disease, which classically presents with excruciating chest pain, frequently radiating to the back. Type A aortic dissection (TAAD) is a dissection that involves the ascending aorta or the entire aorta down to iliac arteries. It occurs when the intima of the aorta becomes compromised and ruptures (intimal tear or entry) creating a new lumen that fills with blood between the intima and the media. This false lumen is often larger than the true lumen. The incidence of aortic dissection is 3.5 cases per 100,000 person years [1]. With an unknown number of patients dying before hospitalization, the true prevalence is likely greater. In the first 24–48 hours, mortality is estimated to increase by 1–2% per hour from the onset of symptoms [2, 3]. It is of paramount importance to diagnose this condition as soon as possible and to transfer the patient into the facility capable of performing emergent surgical treatment [4, 5]. Despite rapid diagnosis, improvements in surgical technique and better perioperative and postoperative treatment, the mortality of surgically treated patients is still high and varies between 17.4% and 33.4% [3, 5, 6, 7]. However, compared to the previous period, the survival trend is certainly better [3].

The aim of this study was to determine in-hospital mortality in patients who underwent surgery at our institution and identify patient characteristics that could indicate a less favorable patient outcome and thus alert clinicians to high-risk patients.

METHODS

Study population and data collection

This retrospective single-center study included 116 patients with TAAD, who were admitted and operated on at the Institute of Cardiovascular Diseases of Vojvodina in Sremska Kamenica, from January 1, 2014 to December 31, 2018. The study was done in accord with standards of the institutional committee on ethics. Upon initial diagnosis established by echocardiography, the final diagnosis was confirmed by computed tomography (CT) – aortography. TAAD was

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defined, according to the Stanford classification, as involving the ascending aorta and/or aortic arch, progressing distally towards the descending thoracic aorta.

Patients were divided into two groups, depending on the outcome after surgery: the survivors and non-survivors. Postoperative, in-hospital mortality refers to a fatal outcome occurring after the surgery and during hospitalization, regardless of its length. The following patient characteristics and comorbidities were monitored: years of age, sex, body weight, height, body mass index (BMI), hypertension, hyperlipoproteinemia, diabetes, previous cerebrovascular accident, chronic obstructive pulmonary disease, chronic kidney disease, smoking. Of particular importance was the monitoring of preoperative values of the following parameters: systolic arterial pressure, diastolic arterial pressure, heart rate, hemoglobin, white blood cells, neutrophils, lymphocytes, neutrophil to lymphocyte ratio (NLR), eosinophils, platelets, fibrinogen, glycemia, creatinine, and C-reactive protein (CRP). All laboratory analyses were performed immediately upon admission. The values of ejection fraction, the presence of aortic insufficiency, pericardial and pleural effusion, diameter of the ascending aorta, involvement of the supra-aortic branches, presence of stroke, acute kidney injury (AKI), and mesenteric ischemia were monitored. Intraoperative variables were also monitored: cross clamp time and cardiopulmonary bypass (CPB) time. We also compared the type of surgery, the use of deep hypothermic circulatory arrest (DHCA), the incidence of re-exploration for bleeding, the intensive care unit stay, and the total length of hospitalization.

Table 1. Demographic, anthropometric characteristics and comorbidities

Parameter	Total	Survivors	Non-survivors	p
Patients n (%)	116 (100)	90 (77.6)	26 (22.4)	
Male n (%) Female n (%)	66 (56.9) 50 (43.1)	49 (74.2) 41 (82)	17 (25.8) 9 (18)	0.374
Age (years) Mean ± SD Range > 65 years n (%) < 65 years n (%)	 60.8 ± 11.6 25–87 47 (40.5) 69 (59.5)	 58.9 ± 11.5 29 (61.7) 61 (88.4)	 67.5 ± 9.5 18 (38.3) 8 (11.6)	 **0.001** **0.001**
Weight (kg) mean ± SD	80.3 ± 14.4	80 ± 14.4	81.3 ± 14.9	0.700
Height (cm) mean ± SD	173.5 ± 9.4	173.8 ± 9.6	172.6 ± 9.1	0.595
BMI (kg/m^2) mean ± SD	26.6 ± 3.6	26.4 ± 3.6	27.1 ± 3.7	0.383
Hypertension n (%)	79 (68.1)	62 (68.9)	17 (65.4)	0.812
Hyperlipoproteinemia n (%)	9 (7.8)	8 (8.9)	1 (3.8)	0.681
Diabetes mellitus n (%)	6 (5.2)	4 (4.4)	2 (7.7)	0.615
History of cerebrovascular accident n (%)	8 (6.9)	8 (8.9)	0 (0)	0.196
Chronic obstructive pulmonary disease n (%)	4 (3.4)	0 (0)	4 (15.4)	0.002
Chronic kidney disease n (%)	5 (4.3)	4 (4.4)	1 (3.8)	1.000
Smokers n (%)	32 (27.6)	26 (28.9)	6 (23.1)	0.627

BMI – body mass index;
values in bold are statistically significant

Operative procedures

All the patients were operated on in general balanced anesthesia. Perioperative and postoperative monitoring included continuous arterial and central venous pressure measurement, electrocardiography, oxygen saturation (pulse oximetry), body temperature measured in the nasopharynx, diuresis. Arterial blood gas analyses were performed intermittently.

Surgery was performed via median sternotomy, using CPB, in moderate hypothermia or DHCA. CPB was established by arterial cannulation of the femoral or right axillary artery and venous cannulation of the right atrium after systemic heparinization (300 U/kg body weight and maintenance of an activated clotting time of longer than 480 seconds). Antegrade cold crystalloid (St Thomas' Hospital) cardioplegia or cold blood cardioplegia was used for myocardial protection. Depending on the pathological process, we performed tubular graft interposition of the ascending aorta with or without commissural resuspension, tubular graft interposition with aortic valve replacement, interposition of the composite valve graft with implantation of the coronary arteries (Bentall procedure) or hemiarch technique.

Statistical analysis

Descriptive statistics measures were used: arithmetic mean, standard deviation, median, quartiles, frequencies and percentages. A t-test for independent samples and a Mann–Whitney test were used to compare the mean values of the variables of the two populations. The correlation of categorical variables was examined using the χ^2 test for contingency tables or using the Fisher test. The influence of variables on the treatment outcome was determined using univariate and multivariate binary logistic analysis. The predictive quality of the variables on the outcome was evaluated using receiver operating characteristics (ROC) curves. A $p < 0.05$ value was taken for statistical significance of the test. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp., Armonk, NY, USA).

RESULTS

A total of 116 patients who underwent TAAD surgery were included in the study. Total postoperative, in-hospital mortality was 22.4% (26 out of 116). The demographic, anthropometric characteristics and comorbidities of the patients are shown in Table 1. The mean age of the patients was 60.8 ± 11.6 years and 56.9% of patients were male. The youngest patient was 25, while the oldest was 87 years old. Arterial hypertension was presented in 68.1% patients. Other comorbidities were present in a much smaller percentage.

Table 2. Clinical characteristics

Characteristics	Survivors	Non-survivors	p
Hemodynamic parameters at admission			
Systolic arterial pressure (mmHg)	131.6 ± 35.9	127.9 ± 34.2	0.630
Diastolic arterial pressure (mmHg)	73.8 ± 17.8	72.5 ± 17.3	0.739
Heart rate (beats per minute)	77.3 ± 16.8	79.7 ± 23.2	0.560
Laboratory data at admission			
Hemoglobin (g/L)	123.7 ± 22.1	116.9 ± 23.5	0.213
White blood cells (× 10^9/L)	12.2 ± 5.4	11.1 ± 3.7	0.384
Neutrophils (%) median (interquartile range)	76.6 (66.9, 83.9)	81.6 (72.4, 85.2)	0.465
Lymphocytes (%)	14.8 ± 8.7	14.4 ± 11.9	0.840
Neutrophils/lymphocytes	7.5 ± 4.9	8.5 ± 4.8	0.398
Eosinophils (%) median (interquartile range)	1.1 (0.4, 1.6)	0.95 (0.3, 1)	0.369
Platelets (× 10^9/L)	190.5 ± 73.2	189.1 ± 77.9	0.941
Fibrinogen (g/L) median (interquartile range)	2.6 (2, 3.6)	2.4 (1.7, 3.5)	0.490
Glycaemia (mmol/L) median (interquartile range)	6.7 (5.8, 8)	7.3 (6.7, 9.2)	0.128
Creatinine (μmol/L) median (interquartile range)	92.5 (81.5, 110)	148 (114, 164)	**< 0.0005**
Creatinine > 120 μmol/L n (%)	16 (17.8)	13 (50)	**0.001**
Creatinine < 120 μmol/L n (%)	74 (82.2)	13 (50)	
C-reactive protein (mg/L) median (interquartile range)	4.0 (3.5, 7.5)	38 (21, 106)	**0.020**
C-reactive protein > 10 mg/L n (%)	14 (15.6)	10 (38.5)	**0.024**
C-reactive protein < 10 mg/L n (%)	76 (84.4)	16 (61.5)	
Other clinical characteristics			
Ejection fraction (%) mean ± SD	58.5 ± 5.9	57.1 ± 7.4	0.376
Aortic insufficiency n (%)	48 (53.3)	13 (50)	1.000
Ascending aortic diameter (mm) mean ± SD	54 ± 12.1	56.6 ± 8.7	0.339
Involvement of the supra-aortic branches n (%)	35 (38.9)	11 (42.3)	0.285
Pericardial effusion n (%)	35 (38.9)	15 (57.7)	0.151
Pleural effusion n (%)	38 (42.2)	14 (53.8)	0.341
Acute kidney injury n (%)	25 (27.8)	9 (34.6)	0.625
Mesenteric ischemia n (%)	1 (1.1)	2 (7.7)	0.128
Stroke n (%)	17 (18.9)	13 (50)	**0.004**
Re-exploration for bleeding n (%)	21 (23.3)	9 (34.6)	0.309
Intensive care unit stay (days) median (interquartile range)	5 (3, 8)	4.5 (0, 8)	0.234
Hospital stay (days) median (interquartile range)	20 (13, 28)	10 (1, 44)	0.116

Values in bold are statistically significant

Table 3. Type of surgery and intraoperative data

Type of surgery	Survivors	Non-survivors	p
Tubular graft interposition of ascending aorta n (%)	58 (64.5)	9 (34.6)	**0.012**
Tubular graft interposition of ascending aorta and aortic valve replacement n (%)	9 (10)	1 (3.9)	0.453
Tubular graft interposition of ascending aorta with commissural resuspension n (%)	9 (10)	2 (7.7)	1.000
Bentall procedure n (%)	8 (8.9)	5 (19.2)	0.163
Hemiarch n (%)	4 (4.4)	4 (15.4)	0.074
Tubular graft interposition of ascending aorta + CABG n (%)	2 (2.2)	2 (7.7)	0.217
Inability to reconstruct the aorta n (%)	0 (0)	3 (11.5)	**0.010**
Intraoperative data			
Cross clamp time (min.) median (interquartile range)	107 (75, 123)	108 (83, 150)	0.160
CPB time (min) mean ± SD	122.5 ± 42.7	152 ± 57.2	**0.009**
DHCA n (%)	5 (5.6)	5 (19.2)	**0.044**

CABG – coronary artery bypass grafting; CPB – cardiopulmonary bypass;
DHCA – deep hypothermic circulatory arrest;
values in bold are statistically significant

The non-survivors were, on average, older than the survivors (67.5 ± 9.5 *vs.* 58.9 ± 11.5, p = 0.001). There were 47 patients older than 65 years (40.5%) and 18 (38.3%) did not survive in this group, while in 69 patients younger than 65 years (59.5%), 8 (11.6%) did not survive (p = 0.001). Out of 66 male patients, 17 died, while out of 50 women patients, nine died (p = 0.374). The two groups did not differ significantly in weight, height, BMI, and comorbidities, except in the presence of chronic obstructive pulmonary disease, which was higher in the non-survivor group (p = 0.002).

Hemodynamic parameters at admission, blood count parameters, fibrinogen values, and glycaemia did not differ significantly (Table 2). However, the admission creatinine values were significantly higher in non-survivors (148 *vs.* 92.5, p <0.0005) as well as CRP values (38.0 *vs.* 4.0, p = 0.020); 87 patients had creatinine < 120 μmol/L, 13 of whom died, while 29 patients had creatinine > 120 μmol/L, 13 of whom died (p = 0.001). Twenty-four patients had a CRP > 10 mg/L, 10 of whom died (p = 0.024). When comparing the remaining parameters in Table 2, the groups differed significantly in the presence of stroke, which was more present in the non-survivor group (p = 0.004).

Survivors had a significantly higher percentage of tubular graft interposition of ascending aorta (p = 0.012), while non-survivors had a higher percentage of more complicated procedures (Bentall procedure, hemiarch), but did not differ significantly (Table 3). CPB duration was significantly longer in the non-survivor patient group (p = 0.009). Also, surgical work in DHCA was significantly more common in non-survivors (p = 0.044).

Univariate analysis indicated that age > 65 years, admission creatinine and CRP value, CPB time, DHCA, and stroke were associated with in-hospital mortality. These variables were included in the multivariate analysis, which designated the following parameters as

Table 4. Results of univariate and multivariate analysis of predictors of in-hospital mortality

Variable	Univariate		Multivariate	
	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
> 65 years	4.733 (1.843–12.151)	0.001	/	ns
Creatinine	1.021 (1.007–1.034)	0.002	1.026 (1.006–1.046)	0.009
Creatinine > 120 µmol/L	4.625 (1.807–11.836)	0.001	/	ns
CRP > 10 mg/L	3.393 (1.281–8.988)	0.014	4.764 (1.066–21.283)	0.041
CPB time	1.012 (1.002–1.022)	0.014	/	ns
DHCA	4.048 (1.072–15.280)	0.039	/	ns
Stroke	4.235 (1.666–10.766)	0.002	6.097 (1.399–26.570)	0.016

ns – non significant; CRP – C-reactive protein; CPB – cardiopulmonary bypass; DHCA – deep hypothermic circulatory arrest

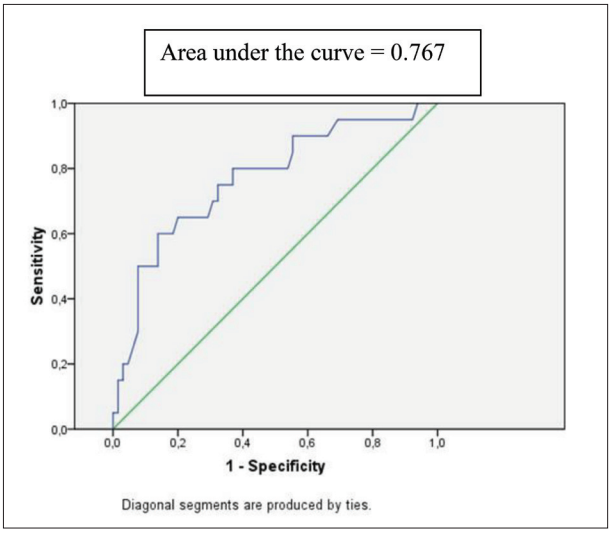

Figure 1. The receiver operating characteristics curve of admission creatinine level

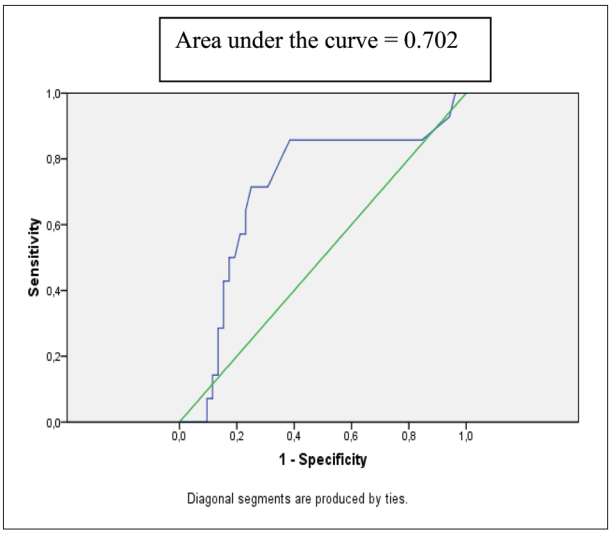

Figure 2. The receiver operating characteristics curve of admission C-reactive protein level

independent in-hospital mortality predictors: creatinine [OR 1.026 (1.006–1.046), p = 0.009], CRP > 10 mg/L [OR 4.764 (1.066–21.283), p = 0.041] and stroke [OR 6.097 (1.399–26.570), p = 0.016] – Table 4.

The ROC curve analysis was performed to detect the best cut-off point for the admission creatinine and CRP values in the prediction of in-hospital mortality. The cut-off point for creatinine was 124.5 µmol/L (area under the ROC curve = 0.767; p <0.0005) (Figure 1). The sensitivity was 65% and the specificity 80%. The cut-off point for CRP was 14.5 mg/L – sensitivity 71.4%, specificity 75% (area under the ROC curve = 0.702, p = 0.021) (Figure 2).

The time distribution and causes of in-hospital mortality are shown in Tables 5 and 6. *Mors in tabula* (30.8%), septic shock / multi-organ dysfunction syndrome (23.1%), and stroke (19.2%) were the most common causes of death.

DISCUSSION

It is well known that TAAD is associated with a high mortality rate. The postoperative, in-hospital mortality in our patient group during a five-year observation period was 22.4%. A study conducted in our country, at another institution, a few years ago, showed that postoperative, in-hospital mortality was almost identical – 23.3% [8]. In large surgical registries, postoperative in-hospital mortality ranges 17.4–33.4% [3, 5, 6, 7]. Considering the fact that the mortality rate is about 57% after medical treatment, we can conclude that surgical emergency is a priority in the treatment of these patients [3].

Although it can occur in young people, especially in patients with connective tissue disorders such as Marfan syndrome, Loyes–Dietz syndrome, Ehler–Danlos syndrome, this disease is typical of the older population. Our study showed that the average age of patients was 60.8 years. Also, mortality is higher in the elderly population because of the higher prevalence of comorbidities in the elderly. Mortality in adults over 65 years was 38.3% *vs.* 11.6% in those under 65 years. According to the worldwide analysis, death more often occurs in the old and the highest mortality occurs at the age of more than 70 years old [3].

A higher percentage of men than women was affected by TAAD, which can be related to the greater prevalence of risk factors in men, such as hypertension, atherosclerosis, smoking. However, the difference in sex mortality has not been shown to be significant. Some previous studies have shown poorer outcome in female patients [3]. Delays in TAAD diagnosis occurring more often in female patients are probably the reason for the higher mortality [3].

The inflammatory mechanism plays an important role in the degeneration and reduction of smooth muscle cells leading to weakened blood vessels [9]. Neutrophils are a key factor in an inflammatory response and their percentage may be an indicator of the severity of the inflammatory response and a predictor of a fatal outcome [10]. Our study did not confirm the correlation between neutrophil percentage and a fatal outcome. Recently, NLR has been used as a predictor of mortality, most commonly in malignancies. It is determined by dividing the absolute neutrophil

Table 5. Time distribution of in-hospital mortality

Time	n (%)
Mors in tabula	8 (30.8)
< 7 days	3 (11.5)
7–30 days	6 (23.1)
> 30 days	9 (34.6)

Table 6. Cause of in-hospital mortality

Cause of death	n (%)
Mors in tabula	8 (30.8)
Septic shock / MODS	6 (23.1)
Stroke	5 (19.2)
Hypovolemic shock	3 (11.6)
Cardiogenic shock	2 (7.7)
Hepatorenal syndrome	1 (3.8)
Respiratory failure	1 (3.8)

MODS – multi-organ dysfunction syndrome

count by the absolute lymphocyte count. While the neutrophil count rapidly increases in conditions with heightened inflammation, the lymphocyte count is reduced and thus the NLR increases significantly. Karakoyun et al. [11] concluded that NLR may be a predictor of fatal outcome in TAAD. Their study was conducted on 37 patients and NLR > 8.51 demonstrated a sensitivity of 77% and specificity of 74% for the prediction of mortality. Our study, which included almost four times as many patients, showed no association between NLR and mortality.

CRP is a non-specific inflammatory marker but may be a predictor of a fatal outcome [6]. Vrsalovic et al. [12] indicated that CRP > 9.8 mg/L is a predictor of poor outcome. In our study, a multivariate analysis showed that admission CRP > 10 mg/L had a direct correlation with in-hospital mortality. Patients with a CRP > 10 mg/L were five times less likely to survive. CRP is produced in the liver, coronary plaques, myocardial infarcts, and aneurysmal tissue [13]. It appears possible that aortic tissue during dissection directly increases the production of CRP, relatively to the severity of the dissection.

In their study on the impact of fibrinogen levels on mortality, Liu et al. [14] found that low fibrinogen concentrations could predict poor outcome. TAAD itself activated the coagulation system before surgery. Excessive fibrinogen consumption leads to a procoagulant state and the formation of thrombus. If this procoagulant condition persists, it can lead to microvascular and macrovascular thrombotic complications, the development of disseminated intravascular coagulation, neurological damage and to an unfavorable outcome [15]. Our results showed no significant correlation between fibrinogen values at admission and mortality, although fibrinogen values were lower in the non-survivors.

AKI is a common complication after thoracic aortic surgery that occurs in up to 44% of patients with TAAD, and significantly increases in-hospital mortality [16, 17, 18]. The pathogenesis of AKI is multifactorial and the most significant are hemodynamic, inflammatory, metabolic factors [2, 19, 20]. Extension of the dissection may involve the renal artery, which may directly impair renal perfusion, thus resulting in AKI [21]. Early detection and prevention of AKI is a key imperative that may help improve patient outcomes [16]. Our study showed that elevated creatinine level at admission may be a good predictor of in-hospital mortality. Wu et al. [22] also concluded that elevated creatinine levels at admission were a good predictor of in-hospital mortality. They found that patients with elevated creatinine had a greater proportion of aortic arch or more extensive aortic involvement, requiring more complicated surgery.

Two more recent studies examined and demonstrated the impact of poorer ejection fraction on postoperative, in-hospital mortality [23, 24]. Our patients did not differ in this parameter. Our study also did not show that aortic insufficiency and the ascending aortic diameter affect mortality, which is correlated with the study by Qiu et al. [25]. The presence of pericardial effusion did not prove to be a predictor, as opposed to a study in which it proved statistically significant [26].

Regarding CPB time, Nozohoor et al. [7] showed that prolonged time directly affects the mortality of patients who underwent TAAD surgery. The duration of CPB is influenced by the type of surgery. Our results show that there were more complex surgical procedures in the group of non-survivors. It logically affects CPB time and in-hospital mortality. Univariate analysis showed that the length of the CPB affected mortality; however, this was not confirmed by multivariate analysis.

Stroke is one of the most common and severe complications of TAAD surgery, with an incidence of up to 30% in multiple studies [27]. This complication significantly affects the morbidity and mortality of patients. In our study, stroke proved to be an independent predictor of postoperative, in-hospital mortality. In-hospital mortality was observed in 43.3% of patients who had a stroke and in 15.1% of those who did not have this complication. Whether these strokes were due to embolic phenomena, dissection of the arch or distal intracranial vessels, or hypoperfusion at the time of surgery is not known. A study conducted by Ghoreishi et al. [27], on 7353 patients from 772 centers, found that stroke is an independent predictor of in-hospital mortality, and the independent risk factors for stroke were the following: femoral arterial cannulation, total arch replacement, longer CPB time, cerebral perfusion time, and total circulatory arrest time. They indicate that all types of hypothermic strategy, including mild, moderate, and deep, result in similar incidence of stroke postoperatively. Improving neuroprotective techniques during circulatory arrest is a leading topic in recent studies. Ghoreishi et al. [27] found that retrograde cerebral perfusion was associated with significantly reduced risk for stroke compared to no cerebral perfusion or antegrade cerebral perfusion. A group of authors from Serbia examined the clinical outcomes of two different surgical techniques: open distal anastomosis in hypothermic circulatory arrest compared to anastomosis with clamped aorta while continuing on extracorporeal circulation [28]. This prospective, randomized study showed that there was no difference in in-hospital mortality between the groups, nor in the *de novo* resulting neurological deficits.

This study had some limitations. It is a single center, retrospective study and the number of patients studied is limited. The etiology of TAAD in most patients wasn't investigated so the presence of pre-existing aortic pathologies was unknown. Due to insufficient data, we were unable to

include in the analysis the time from the onset of symptoms to surgery, which affects mortality.

CONCLUSION

Despite easier and more accessible diagnostics, advanced surgical techniques and better postoperative treatment, TAAD surgery carries a high risk of mortality. Variables suggestive of poor outcome following the surgery are elevated admission creatinine and CRP values, and stroke. These are variables that should alert the clinician to high-risk patients and contribute to lower mortality rates after this serious disease.

Conflict of interest: None declared.

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Претказатељи интрахоспиталне смртности после хируршког лечења аортне дисекције типа Станфорд А – петогодишње искуство једног центра

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САЖЕТАК

Увод/Циљ Аортна дисекција типа Станфорд А хитно је хируршко стање удружено са високом смртношћу.
Циљ ове студије је био да утврди која је група оперисаних болесника повезана са постоперативном, интрахоспиталном смртношћу и које су њене карактеристике.

Методе Ретроспективна студија је обухватила 116 болесника са акутном аортном дисекцијом типа А, оперисаних у петогодишњем периоду. Испитивана је повезаност између постоперативне, интрахоспиталне смртности и карактеристика болесника.

Резултати Укупна интрахоспитална смртност је износила 22,4%. Варијабле које су, после мултиваријантне анализе, показале директну корелацију са смртношћу су: креатинин на пријему (*OP* 1,026 [1,006–1,046], $p = 0,009$), *C*-реактивни протеин (*CRP*) > 10 *mg/L* (*OR* 4,764 [1,066–21,283], $p = 0,041$) и мождани удар (*OR* 6,097 [1,399–26,570], $p = 0,016$). *ROC* крива је показала да креатинин може бити добар претказатељ за смртност (површина испод *ROC* криве = 0,767; $p < 0,0005$). Гранична вредност је 124,5 *μmol/L*. Сензитивност је 65%, а специфичност је 80%. Гранична вредност за *CRP* је 14,5 *mg/L* – сензитивност 71,4%, специфичност 75% (површина испод *ROC* криве = 0,702, $n = 0,021$).

Закључак Хируршко лечење акутне аортне дисекције типа А је и даље повезано са релативно високом смртношћу. На мању шансу за преживљавање могу указати повишене вредности креатинина и *CRP*-а на пријему, као и мождани удар.

Кључне речи: аорта; дисекција; смртност; креатинин; *CRP*; мождани удар

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Comparison of video-assisted thoracoscopic surgery and standard surgical approach in treatment malignant thymus tumor stage I and II – propensity score analysis

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SUMMARY

Introduction/Objective Besides sternotomy, video-assisted thoracoscopic surgery (VATS) is used for thymus tumors treatment.

The objective of our study was to compare oncological and perioperative outcomes in patients with I–II stage of thymic tumors treated with VATS or standard sternotomy procedures.

Method The study included only primary I–II thymoma according to the Masaoka classification, treated between May 2006 and February 2018. Out of 116 treated patients that had pathohistologically verified stage, 100 (86.2%) were matched by propensity score for sex, age, body mass index, myasthenia, tumor size, Masaoka classification stage. Oncological (direct post-operative survival, recurrence) and perioperative outcomes (intraoperative and postoperative complications, length of hospitalization) that affect the efficacy and safety of surgical techniques have been analyzed and compared between the two groups.

Results Among 50 patients operated by VATS, 34 patients (68%) were treated by uniportal approach, 13 (26%) by biportal and three (6%) by threeportal approach. The VATS intervention had shorter intervention time ($p < 0.001$), duration of hospitalization ($p < 0.001$), and usage of thoracic drainage ($p < 0.001$). There was a significant difference in terms of late control ($p < 0.001$). There was no significant difference between the groups regarding visual analogue scale score, as well as in terms of the time of recurrence ($p = 0.305$, $p = 0.268$).

Conclusion Compared to standard sternotomy, VATS thymectomy is an equally effective and significantly safer method with a minimum rate of intra and postoperative complications.

Keywords: thymoma; video assisted thoracoscopy; open thymectomy

INTRODUCTION

Thymus carcinomas belong to the group of epithelial tumors of the thymus, mainly located in the anterior mediastinum [1]. They belong to the group of rare and invasive malignancies and make up to 1.5% of all malignant tumors, and only 0.06% of all tumors of thymus in general [1, 2]. They most commonly occur between the age of 30 and 60, but they can also occur in early childhood and elderly life, without significant predilection by gender [2, 3]. It is important to underline that a few patients have systemic symptoms including autoimmune disease [4]. Approximately 30% of patients with thymoma have myasthenia gravis [5]. Post-thymectomy myasthenia gravis is registered in around 1–3% of the operated patients, and this disorder progresses after extensive thymectomy mostly characterized for open surgery [5, 6, 7].

In surgery, Masaoka Koga staging system is commonly used as the most important determinant of long-term prognosis after surgical resection [8]. Resection/surgery is the first and most important modality for treating tumors of the thymus; the possibility of implementing a complete resection is the most important parameter that defines a long-term prognosis [9, 10]. The rate of relapse ranges from 1–5% for non-invasive to 20% for invasive complete resuscitative tumors [11, 12]. There are controversial attitudes considering surgery, surgical approach, the place of thoracoscopic methods, and the extent of thymoma resection [4].

Nowadays, the majority of thymoma patients have VATS surgery at the Military Medical Academy. Numerous reports show that patients with Masaoka stage I–II thymoma underwent VATS [4, 12]. The minimally invasive approach is the recommended option in the I–II stage of the tumor, while for stage III there are no data on patient's long-term survival, so that open surgery is represented as a therapeutic approach [8, 13–16]. The invasion to the innominate vein, phrenic nerve, or other major vessels should be a contraindication to VATS [13]. It is widely accepted that VATS is technically safe and feasible for thymoma with

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a diameter < 50 mm [14]. VATS mostly includes unipolar technique by one-sided approach (right or left side) with respect to the anatomical localization of the thymus. The right-side approach is more secure because of the relationship with the brachiocephalic vein [4]. Although the definitive guidelines have not yet been established regarding the extent of thymoma resection, it is well known that extensive resection of the thymus may increase the potential risk of the intra and post-operative complications [4].

By developing minimally invasive surgery, video-assisted thoracoscopic surgery (VATS) is imposed as an excellent alternative to sternotomy procedures [1, 16, 17]. However, the safety of VATS and the achievement of a complete stable remission as an efficiency measure and evaluation criterion in assessing the radicalism of resection remain insufficiently examined, since most of the previously cited studies, as well as the studies included in the aforementioned analysis, encompassed a relatively small number of patients.

The study was based on analysis and comparison of oncological outcomes (direct post-operative survival, recurrence), as well as analysis and comparison of the type and frequency of perioperative outcomes (intra and postoperative complications, duration of hospitalization) in patients with I–II stage of thymic tumors treated with VATS and standard sternotomy procedures.

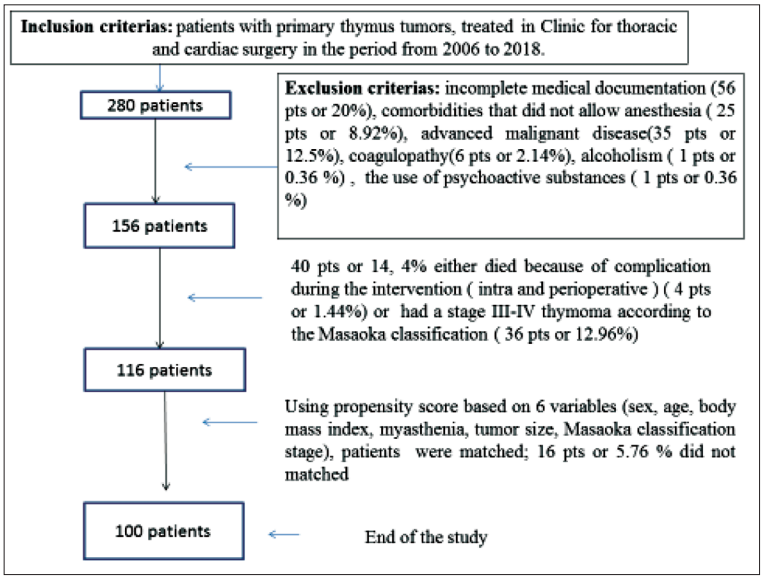

Figure 1. Design of the study

METHODS

The retrospective cohort study included 156 patients with primary thymus tumors, operated at the Clinic for thoracic and cardiac surgery in the period from 2006 to 2018. Criteria for exclusion from the study were incomplete medical documentation (56 patients or 20%), comorbidities that did not allow anesthesia (25 patients or 8.92%), advanced malignant disease (35 patients or 12.5%), coagulopathy (six patients or 2.14%), alcoholism (one patient or 0.36%) and the use of psychoactive substances (one patient or 0.36%). A total of 116 treated patients have the pathohistologically verified stage I–II thymoma according to the Masaoka classification. The remaining 40 patients or 14.4% either died because of complication during the intervention (four patients or 1.44%) or had a stage III–IV thymoma according to the Masaoka classification (36 patients or 12.96%).

Using propensity score based on six variables (sex, age, body mass index, myasthenia, tumor size, Masaoka classification stage) each patient in the VATS-treated group was "matched" with a patient in a group treated with a standard thymectomy with the same propensity score. Of total number of patients included in study (116), 100 patients (86.2%) were matched, resulting in the formation of two identical groups with similar sociodemographic and clinical features (Figure 1).

All epidemiological (sex, age) and anthropometrical data (height, body mass, body mass index derived as (body mass/height^2 (kg/m^2)) were recorded in medical documentation, as well as the number comorbidities and the Charlson Comorbidity Index. In hospital medical abstracts we have also found the data considering comedications (number of drugs and daily doses of drugs) and tumor size.

Among 156 patients underwent surgery, 98 patients (62.8% of the operated) were treated with standard thymectomy. Those from the group who survived the intervention and had the stage I–II thymoma according to the Masaoka classification, were included in matching (58 patients or 59% of all treated with the standard procedure, or 37.5% of all surgery patients).

Since 2012, VATS thymectomy has become a standard surgical technic for thymectomy. VATS technic by one-sided approach (to the right or left side) respects to the anatomical localization of the thymus. All patients treated with VATS were familiar with the surgical approach, potential risks, and complications, and they signed a standardized consent at the Military Medical Academy.

All procedures performed in study involving human participants were in accordance with the ethical standards of the Ethical Commission of Belgrade University of Defense (Ethical Approval from October 30, 2018)

Oncological and perioperative outcomes (intraoperative and postoperative) that affect the efficacy and safety of surgical techniques have been analyzed and compared between the two groups. The following variables were included: duration of the surgical intervention, the length of hospitalization and thoracic drainage, the late control, recurrence.

The first follow-up was one month after the surgery. The patients had the thorax multislice computed tomography done before the control. After six months, they were also checked by the operator and a neurologist. The late control followed after the six-month follow up, regularly planned 12 months after the intervention, but it occurred early if the patient has some late complication of the

intervention (intercostal neuralgia, psychiatric problems linked with the treatment, neurological exacerbation). The time of the late control was expressed in months. Initially, the patient was checked by the operator, who indicated the next procedures and consultations. The visual analogue scale was used for evaluation of post-surgical pain.

Complete statistical analysis of data was made using commercial statistical software SPSS Statistics for Windows, Version 18.0. (SPSS Inc., Chicago, IL, USA). In the case of continuous variables, the data are presented as median, min-max, and interquartile range (IQR) (25–75th percentile). The distribution of data was checked using the Shapiro–Wilk test. Depending on the results of this test, statistical significance between the groups was tested using a t-test for independent groups or alternatively Mann–Whitney test. Some variables are presented in the form of frequencies of particular features (categories) and statistical significance will be determined using the χ^2 test. A statistically significant difference is assessed at the minimum level $p < 0.05$.

RESULTS

Of the total number of patients included in the study (116), 100 patients (86.2%) were matched. There was no statistically significant difference in distribution in terms of sex between two groups ($p = 0.316$). The study results did not show significant difference in distribution and type of comorbidities between the groups. The most frequent associated disease in each group was hypertension (VATS *vs.* thoracotomy: 16% *vs.* 18%, $p = 1.000$) (Tables 1 and 2).

There was no statistically significant difference in age ($p = 0.588$), body mass index ($p = 0.424$), number of comorbidities and Charlson Comorbidity index ($p = 0.735$ and $p = 0.828$ successively), number of drugs ($p = 0.676$) and tumor size ($p = 0.566$) between the group treated with VATS and the group treated with standard technique (Table 2). There was no statistically significant difference in the daily dose of drugs (Pronison®, Imuran®, proton pump inhibitors (IPP), vitamin D (Alpha D3), $CaCO_3$) between the groups (successively $p = 0.597$, $p = 0.111$, $p = 0.832$, $p = 0.664$, $p = 0.664$) (Table 3).

Among the 50 patients treated with VATS, uniportal approach was used in 34 patients (68%), biportal in 13 patients (26%) and threeportal in tree patients (6%).

The duration of VATS intervention was significantly shorter compared to the standard intervention, as well as hospitalization stay and thoracic drainage ($p < 0.001$, $p < 0.001$, $p < 0.001$). All the patients achieved the late control, but the thoracotomy-treated patients came significantly earlier (11 months *vs.* 12 months after the surgery, $p < 0.001$). The patients underwent thoracotomy who came earlier to the late control (five patients or 10% thoracotomy-treated) had the intercostal neuralgia (two patients or 4% thoracotomy-treated), psychiatric problems linked with the treatment (one patient or 2% thoracotomy-treated) and neurological exacerbation (two patients or 4%

Table 1. Gender and comorbidities of the study population

Parameters	VATS (n = 50)	Thoracotomy (n = 50)	p values*
Gender: Male	30 (55.6%)	24 (44.4%)	0.316
Gender: Female	20 (43.5%)	26 (56.5%)	0.316
Comorbidities (no/yes)	40 (80) / 10 (20)	41 (82) / 9 (18)	1.000
Hypertension (no/yes)	42 (84) / 8 (16)	41 (82) / 9 (18)	1.000
Iron deficiency (no/yes)	50 (100) / -	49 (98) / 1 (2)	1.000
Diabetes mellitus (no/yes)	48 (98) / 2 (4)	50 (100) / -	0.495
Ischemic brain disease (no/yes)	49 (98) / 1 (2)	50 (100) / -	1.000
Chronic obstructive pulmonary disease (no/yes)	48 (98) / 2 (4)	50 (100) / -	0.495
Chronic kidney disease (no/yes)	49 (98) / 1 (2)	50 (100) / -	1.000
Hypertrophio prostatae benigna (no/yes)	49 (98) / 1 (2)	49 (98) / 1 (2)	1.000
Other diseases (no/yes)	47 (94) / 3 (6)	50 (100) / -	0.242

* χ^2 test; data are presented as absolute numbers (%)

Table 2. Epidemiological and clinical features of the patients treated with VATS and standard surgery

Epidemiological and clinical characteristics of the patients	VATS (n = 50)	Thoracotomy (n = 50)	p values*
Age (years)	39.5 (27–59.25)	39.5 (33–55.75)	0.588
Body mass index (kg/m^2)	23.9 (22.5–26.4)	24.21 (22.70–26.8)	0.424
Number of comorbidities	0 (0–0)	0 (0–0)	0.735
Charlson Comorbidity Index	0 (0–0)	0 (0–0)	0.828
Number of drugs	0 (0–2.25)	0 (0–2)	0.676
Tumor size (mm)	60 (50–80)	60 (50–95)	0.566

Data are presented as median (IQR – 25–75th percentile);
* Mann–Whitney test

Table 3. Comedication in the group treated with VATS and with the open surgery

Comedication	VATS (n = 50)	Thoracotomy (n = 50)	p values*
Pronison® (mg)	0 (00–20)	0 (0–20)	0.597
Imuran® (mg)	0 (0–0)	0 (0–0)	0.111
Proton pump inhibitors–IPP (mg)	0 (0–40)	0 (0–40)	0.832
Vitamin D–Alpha D3 (mcg)	0 (0–50)	0 (0–50)	0.664
Number of drugs	0 (0–5)	0 (0,00–5)	0.664

Data are presented as median (IQR – 25–75th percentile);
* Mann–Whitney test

Table 4. Surgical outcomes compared between the patients treated with VATS and with open thymectomy

Surgical outcomes	VATS (n = 50)	Thoracotomy (n = 50)	p values*
Duration of operation (min)	50 (45–60)	120 (90–150)	< 0.001
Duration of hospitalisation (days)	4 (3–6)	9 (7–10.25)	< 0.001
Thoracic drainage (days)	2 (1–3)	4 (3–5)	< 0.001
Late control (months)	12 (12–12)	11 (9–12)	< 0.001
Visual analogue scale (0–10)	2 (1–3)	2 (2–3)	0.305
Reccurrence time (months)	0 (0–0)	0 (0–1.50)	0.268

Data are presented as median (IQR – 25–75th percentile);
*Mann–Whitney test

thoracotomy-treated). There was no difference in regard to visual analogue score, as well as in the time of recurrence between the groups (p = 0.305, p = 0.268) (Table 4).

DISCUSSION

Our study results obtained from the analyzing and comparing oncological and perioperative outcomes of the VATS thymectomy and standard thoracotomy support VATS as the recommended approach in the I–II stage of the thymus tumor.

Consistently with our results is the recommendation of the VATS technique as a "gold standard" for the I–II stage of the tumor [9, 14, 15, 16]. For III–IV thymoma there are no data on long-term survival of the patients, so that open surgery is suggested as a therapeutic approach [9, 14, 15, 16]. Based on the previous cited recommendations and ethical principles, our study design did not include stage III–IV thymoma, also excluding other comorbidities' influence upon treatment decision among study patients.

Numerous studies confirm the equal efficacy of VATS thymectomy compared to standard sternotomy, comparable radicalism, and long-term survival, with a better cosmetic effect, lower intensity of postoperative pain and blood loss, reduced hospitalization time, lower early and late postoperative morbidity [7, 16–30].

Besides thymoma, some study data referred the importance of VATS surgery in myasthenia gravis treatment. Thymectomy in patients with myasthenia gravis supported stable clinical course, leading to clinical remission and reduce the dose of comedications used in conservative treatment [7]. A 12-year-long study that monitored the long-term efficacy of VATS thymectomy as part of the treatment of non-myomatosal myasthenia gravis suggests an improvement in 91.6% of surgical cases and a stable remission of 22.2% [18]. There was no measurable difference between the study groups in the daily dose of comedications used in conservative treatment of myasthenia gravis and its side effects (osteoporosis, acute gastritis), supporting previous data considering comparable radicalism. It seems to be important; having in mind that post-thymectomy myasthenia gravis is reported in almost 3% of the operated patients, mostly after standard procedure [5, 6, 7].

Assessing efficacy through the mass of the removed tissue, Lee et al. [19] stated that there is no difference between VATS and open surgery in terms of radicalism of the procedure. Comparable oncological outcomes also refer to Ye et al. [20]. Wang et al. [21] report that there is no difference in terms of a five-year survival between the patients subjected to VATS and open surgery as part of the treatment of thymoma. The same conclusions arise from the studies of Chao et al. [22], as well as Qi et al. [23].

Zahid et al. [24] point out the equivalent postoperative mortality and achieve a stable remission of VATS compared to open surgery. In addition, their study results highlight the superiority of VATS in terms of duration of hospitalization, bleeding, cost of surgery, intensity of pain, and cosmetic effect [24–27]. Ashleigh et al. [25] in meta-analysis underlined the results that are consistent with the referred data. Our study data supported previous cited reports considering duration of VATS procedure and hospitalization, thoracic drainage, with the equal intensity of pain objectified by the visual analogue scale. In addition, recurrences occur with a frequency 0–6.7%, which is comparable to open thymectomy [15, 26, 27]. The recently published meta-analysis, which involved about 1200 patients, points out that VATS is superior in terms of safety (lower incidence of complications and myasthenic crisis) compared to open surgery and equally effective in achieving a complete stable remission [28].

Our results are in accordance with previous referred study findings, especially in the term of late control. The group treated with VATS had the third (late) control later than the group treated with standard thymectomy because they had fewer complications with the comparable time of recurrence.

In Serbia, the first VATS thymectomy was applied in 2012. The Military Medical Academy data referred 70 VATS thymectomies done by three-, two-, and uniportal approach until the end of 2018 [7]. With the improvement of surgical technique, VATS uniportal approach becomes standard and dominant in the Clinic for Thoracic Surgery of the Military Medical Academy in the treatment of stage I–II thymus tumors.

Our study, involving 116 patients, presents a respectable contribution to the further analysis of clinically significant data on VATS thymectomy as an alternative operating pathway for treating I–II patients with thymic tumor compared to standard thymectomy. Results obtained in this study indicate the benefit of VATS thoracoscopy compared to standard thymectomy, which is reflected in greater safety and equal or greater efficacy of VATS, as well as in a lower incidence of postoperative complications and faster recovery. VATS thymectomy is far less invasive and represents an equally effective solution compared to standard sternotomy. Postoperative procedure includes a low-intensity pain, while the scar is small. In addition, the intervention itself can be well presented and documented. Recovery is significantly shorter, and the costs of treatment are lower. The length of home treatment and absence from work is also significantly shorter in patients treated with VATS. Possible complications of VATS thymectomy could include intercostal neuralgia, psychiatric problems linked with the treatment, neurological exacerbation, just like those in alternative methods of thymectomy, but less frequent.

Limitations of our study are retrospective character of the study and great number of excluded patients due to restrictive inclusion criteria.

CONCLUSION

Compared to standard sternotomy, VATS thymectomy is equally as effective and a significantly safer method with a minimum rate of intra and postoperative complications. Our findings support previous study results considering

VATS thymectomy as a gold standard for malignant thymus tumour (stage I and II). Further study on the greater numbers of participants would be necessary to define the effectiveness of VATS surgery for the stage III–IV thymoma according to the Masaoka classification.

Limitation of the study

Limitations of our study are retrospective character of the study and great number of excluded patients due to restrictive inclusion criteria.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethical Commission of the University of Belgrade, Faculty of Medicine, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All procedures performed in study involving human participants were in accordance with the ethical standards of the Ethical Commission of Belgrade University of Defense (Ethical Approval from October 30, 2018) Informed consent was obtained from all individual participants included in the study.

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Поређење видеоасистиране торакоскопске хирургије и стандардног хируршког приступа у лечењу малигних тумора тимуса I и II стадијума – анализа „пропензити скором“

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САЖЕТАК

Увод/Циљ Хируршко лечење тумора тимуса (тимектомије) може се спровести кроз стернотомни приступ или видеасистираном торакоскопском хирургијом (ВАТС).

Циљ студије је да упореди онколошке и периоперативне исходе (интраоперативне и постоперативне компликације, дужину хоспитализације) код болесника са I и II стадијумом тумора тимуса лечених методом ВАТС или стандардном тимектомијом.

Методе Студија је обухватила болеснике са примарним тумором тимуса, I и II патохистолошког стадијума према Масаокиној класификацији, оперисане у периоду између маја 2006. и фебруара 2018. године. Од 116 болесника њих 100 (86,2%) уврштено је у анализу „пропензити скором“ према полу, старости, индексу телесне масе, мијастенији, величини тумора, стадијуму по Масаокиној класификацији. Онколошки и периоперативни исходи који утичу на ефикасност и безбедност хируршке технике су анализирани и упоређени између две групе.

Резултати Од 50 болесника оперисаних ВАТС-ом, код 34 болесника (68%) примењен је унипортални приступ, код 13 болесника (26%) бипортални, а код три болесника (6%) трипортални приступ. ВАТС операција је значајно краће трајала ($p < 0,001$), захтевала је краћу хоспитализацију ($p < 0,001$) и употребу дрена ($p < 0,001$). Оперисани ВАТС-ом су се касније јављали ($p < 0,001$). Није било разлике у погледу ВАС скора, као ни у погледу времена настанка рецидива између испитиваних група ($p = 0,305$, $p = 0,268$).

Закључак У односу на стандардну стернотомију, ВАТС тимектомија је подједнако ефикасна и значајно безбеднија метода са минималном стопом интраоперативних и постоперативних компликација.

Кључне речи: тимом; видеоасистирана торакоскопија; отворена тимектомија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Our results in the treatment of tarsal dislocations

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SUMMARY
Introduction/Objective Tarsal dislocations are rare injuries. Usually, they are caused by high-energy trauma. Depending on the type of dislocation, surgical treatment or closed reduction is used. In this study, 13 patients are presented with the aim to analyze the type of feet dislocations, their treatment, and outcome.
Methods Tarsal dislocation cases treated in the University Hospital in Foča were analyzed during the period 2009–2016. All the cases were clinically and radiographically examined and monitored on control examinations at least three years. The mobility of joints was measured and pain existence was estimated by visual analogue scale.
Results All 13 patients with tarsal dislocation were male. Four patients were treated surgically (two patients with tarsometatarsal and one with cuboid and navicular dislocation) and other patients had non-surgical treatment. In 10 patients, an excellent functional result has been achieved and in two patients with tarsometatarsal dislocation a good functional result. In one patient with cuboidal dislocation satisfactory functional result has been achieved.
Conclusion Out of the 13 reviewed patients with tarsal dislocations, functional results were rated as excellent in 10 dislocations, good in two, and satisfactory in one. Diagnosis and treatment of foot dislocations are demanding, but a favorable functional outcome can be expected with an adequate treatment of these injuries.
Keywords: foot; injuries; outcome; treatment

INTRODUCTION

Tarsal dislocations are rare injuries. There are several types of tarsal dislocations. The most significant are: subtalar dislocations, cuboid bone dislocations, navicular bone dislocations and dislocations of Lisfranc joint.

Subtalar dislocation is defined as simultaneous dislocation of both the talonavicular and the talocalcaneal joints without a major fracture [1].

Subtalar dislocations are classified as medial, lateral, posterior, and anterior based on the displacement of the foot in relationship to the talus. These are uncommon injuries, representing approximately 1% of all traumatic injuries of the foot and 1–2% of all dislocations, being associated with high-energy trauma [2, 3].

Recent studies have emphasized the complex anatomic and kinematic relationship between the talocalcaneal and talonavicular joints and their contributions to hindfoot function [4].

Medial dislocation is the most common and it accounts for 65–85% of all subtalar dislocations. It is the result of forced inversion of the foot when the foot is in the plantar flexion [5].

The cuboid stabilizes the lateral column of the foot. It is the only bone to articulate with both the midtarsal and tarsometatarsal joints. These articulations give the cuboid marked stability, which is reinforced by multiple ligamentous, tendinous and soft tissue attachments. The cuboid dislocations are rare injuries and are frequently overlooked and misdiagnosed on initial presentation. The mechanism of injury is postulated to include a forced inversion and plantar flexion movement of the foot [6].

The navicular is the keystone of the medial longitudinal arch, and is rigidly stabilized by an extensive network of dorsal and plantar ligaments [7]. The navicular bone more often suffers dislocation fracture than pure dislocation [8]. The central third portion is relatively avascular. When devoid of surrounding soft tissues, as in the case of complete dislocation, it is prone to avascular necrosis.

A Lisfranc dislocation or injury typically describes a spectrum of injuries involving the tarsometatarsal joints of the foot. The Lisfranc joint itself is composed of the articulation between the first, second, and third metatarsals bones, and the cuneiform bones [9].

In this study, patients are presented with the aim to analyze the type of tarsal dislocations, their treatment, and outcome.

METHODS

The patients with the tarsal dislocation of the foot, who were treated at the University

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Hospital in Foča (Bosnia and Herzegovina), were analyzed during the period from January 1, 2009 to January 1, 2016. All patients were clinically and radiographically examined and monitored during their hospitalization and then in control examinations in orthopedic ambulance after one and three months, then one and three years after their injury and in case of complications after that period. During every examination, the mobility of joints was measured and pain existence estimated by visual analogue scale with marking 0–10. Zero indicates the absence of pain, while 10 represents the most intense pain possible. X-rays performed at first examination and control examinations were also analyzed. For the patients without pain and those who maintained joint mobility, it was considered they had an excellent functional result. For the patients with the pain which can be classified as 1 or 2 according to the visual analogue scale, or the existence of an easy limitation of joints mobility up to one-third of the arc of motion was considered to be a good functional result of treatment. For the patients with the pain which can be classified as 3 according to visual analogue scale or the existence of an easy limitation of joints mobility more than one-third of the circumference movement was considered to be a satisfactory functional result of treatment.

Table 1. Basic data of patients with tarsal dislocation

Characteristics		Dislocation Type				Total
		Subtalar	Tarsometatarsal	Cuboid	Navicular	
Number of patients		5	5	2	1	13
Age		18–24	19–27	29–48	14	24.5 ± 8.9 (14–48)
Patients surgically treated		0	2	1	1	4
Treatment outcome	Excellent	5	3	1	1	10
	Good	0	2	0	0	2
	Satisfactory	0	0	1	0	1

Table 2. The average time from trauma to treatment

Characteristics	Dislocation Type				Total
	Subtalar	Tarsometatarsal	Cuboid	Navicular	
Number of patients	5	5	2	1	13
Average time from trauma to treatment (minutes)	50	67	55	85	60 ± 19.5
Minimum (minutes)	25	55	45	85	15
Maximum (minutes)	85	80	65	85	80

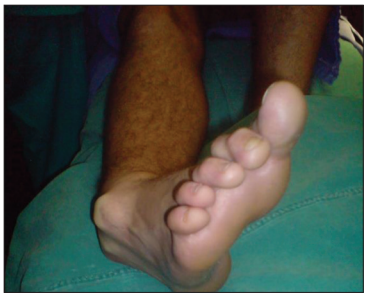

Figure 1. Subtalar dislocation of basketball players

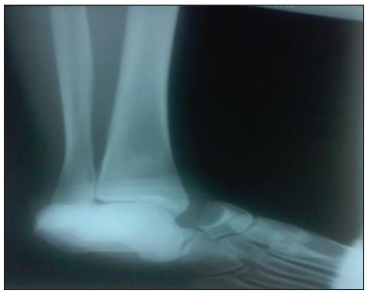

Figure 2. X-ray image of subtalar dislocation

This study protocol was done in accordance with the ethical principles of the Declaration of Helsinki. The data were collected in a setting of usual care, in order to measure the outcome of the treatment. The patients were asked to indicate if they did not allow their anonymous data to be used for scientific studies. The research was approved by the institutional Committee on Ethics of the University Hospital Foča (1/20)

Descriptive statistics were used to calculate central tendency (mean), range, and standard deviation.

RESULTS

Thirteen tarsal dislocations treated between 2009 and 2016 were reported. They were male; the average age was 24 years. The most common was subtalar and tarsometatarsal dislocation (Table 1). Five patients with subtarsal dislocation, five with tarsometatarsal dislocation, two with cuboid dislocation, and one patient with dislocation of the navicular bone were treated (Table 1).

The average time from trauma to treatment was 60 ± 19.5 minutes (subtalar dislocations 25, 30, 45, 65, 85 minutes, tarsometatarsal dislocations 55, 60, 65, 75, 80, cuboid dislocations 45, 65 and navicular dislocation 85 minutes).

Computed tomography scans were performed in most patients with tarsal dislocations (7/13).

The mechanism of injury was different. In cuboid dislocations, one subtalar and two tarsometatarsal, injury occurred in a traffic accident. In dislocation of the navicular bone, the injury was caused by a jump from the height and in three subtalar dislocations, injury was sustained during the sports activities. In four tarsometatarsal dislocations, the injury was sustained by the fall. Four patients were treated surgically.

In all five cases of subtalar dislocation, it was medial dislocation. Closed reduction was performed as urgent and was performed less than two hours after injuries in general anesthesia (Figures 1 and 2, Table 2).

Computed tomography scans were performed in 3/5 subtalar dislocations after closed reduction.

During the follow-up period, three years after the injury, there was no appearance of aseptic necrosis or other complications. At the end of the treatment of all patients, an excellent functional result was achieved. All patients returned to their previous levels of activities with a normal range of motion (Table 3).

We report two patients with dislocation of the cuboid bone. One patient had an open dislocation of the cuboid bone (Figure 3). Open reduction of the cuboid bone was performed through the already present wound, and another patient refused surgery and the cuboid bone remained dislocated.

Table 3. Range of motion ankle and foot joints and return to preinjury levels of activity

Characteristics		Dislocation Type				Total
		Subtalar	Tarsometatarsal	Cuboid	Navicular	
Number of patients		5	5	2	1	13
Range of motion ankle and foot joints	Normal	5	3	1	1	10
	limitation less than 1/3	0	2	0	0	2
	limitation more than 1/3	0	0	1	0	1
Return to preinjury levels of activity		5	5	1	1	14

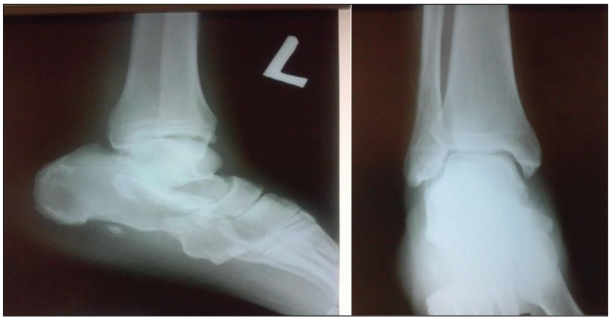

Figure 3. X-ray images after dislocation of the cuboid bone

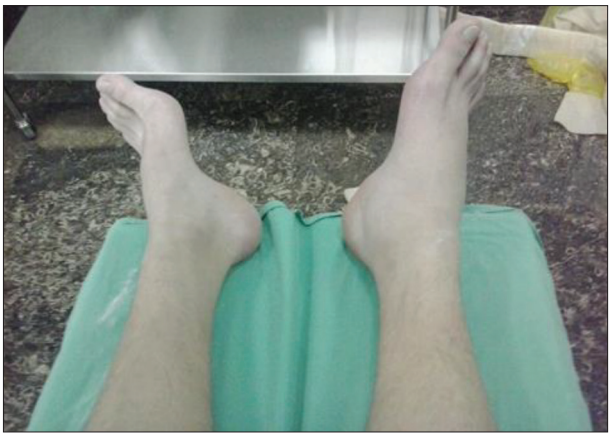

Figure 4. Dislocation of the navicular bone occurred when the student jumped through the school window

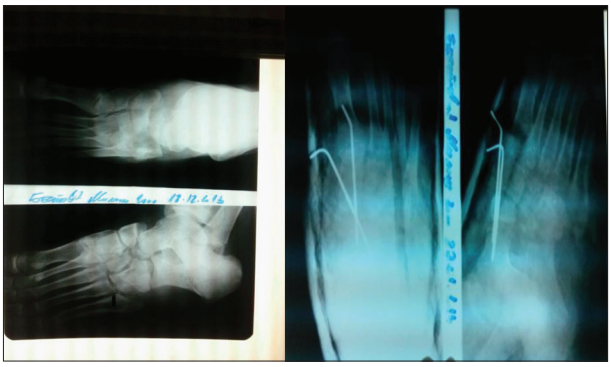

Figure 5. X-ray images of dislocation of the navicular bone before and after the surgery

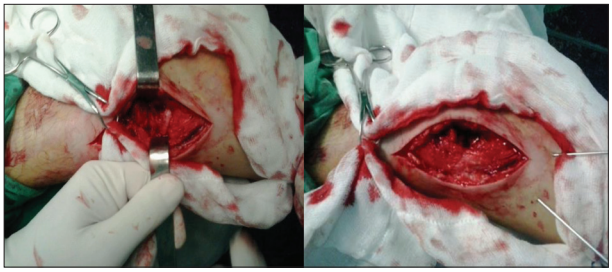

Figure 6. Intraoperative photos of dislocations of the navicular bone and photos after the open reduction and K-wire stabilization

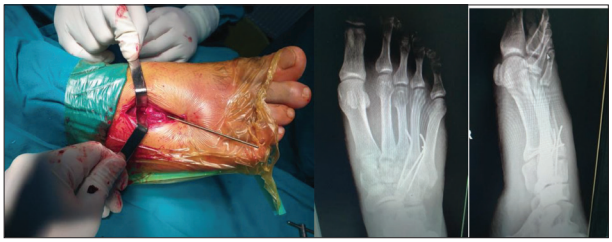

Figure 7. Intraoperative photos tarsometatarsal dislocation after the open reduction and K-wire stabilization and postoperative X-ray image

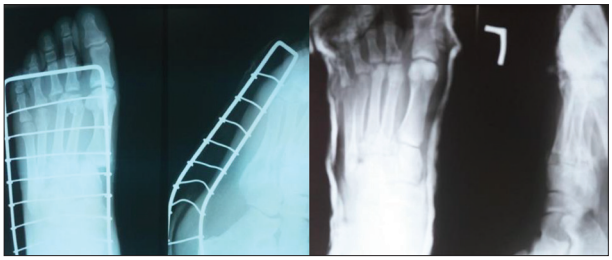

Figure 8. X-ray images before and after the closed reduction of tarsometatarsal dislocation

In the first injury, an excellent functional result was achieved. In the second injury an intensive physiotherapy was applied. Satisfactory functional result was achieved with the presence of the pain. The patient marked the pain with 3 on the visual analogue scale and also experienced limited joint mobility of the ankle and foot more than one third of the movement range (Ankle dorsiflexion/ankle plantarflexion (active) -10/-20, Foot inversion/foot eversion (active) -15/-10). The patient did not return to preinjury levels of activity (Table 3).

One dislocation of the navicular bone was described. The injury was caused by patient jumping from a height. Dislocation was treated surgically with open reduction and K-wire stabilization (Figures 4, 5 and 6).

We report five patients with tarsometatarsal dislocation. Two dislocations were treated surgically (Figure 7) and three dislocations was treated non-surgically by closed reduction (Figure 8).

Computed tomography scans were performed in 4/5 tarsometatarsal dislocations. Functional results were rated excellent in three dislocations, and good in two dislocations. Two patients had limited joint mobility of the ankle and foot less than one third of the movement range (ankle dorsiflexion/ankle plantarflexion (active) -5/-10, -5/-10,

Foot inversion/foot eversion (active) -10/-5, -5/-5), and light intensity pain during higher load (on visual analogue scale 1, 2). All the patients returned to their previous levels of activities.

DISCUSSION

In the seven-year period, at the University Hospital in Foča (Bosnia and Herzegovina), 13 patients with tarsal dislocation were treated. All were male and the mechanism of injury was different. Four patients were treated surgically while others had nonsurgical treatment.

All patients received low-molecular-weight heparin for thromboprophylaxis. The duration of treatment was during the period of immobilization. All surgically treated patients received a single-dose cefazolin (2 g) as an antibiotic prophylaxis. We used the multidisciplinary team approach for pain management.

An excellent joint function was achieved in 10 patients. In two patients the function was good and in one joint function was satisfactory.

Subtalar dislocations are three to ten times more common in male than in female and generally occur in the second or the third decade of life [10]. Medial subtalar dislocation can be a diagnostic problem, because it is a rare injury, and moreover, X-ray of the injury can be confusing due to superposition of bones [11]. The diagnosis of subtalar dislocation is usually made on anteroposterior, lateral, and oblique radiographs of the foot or ankle. The nature of the deformity often limits radiographic positioning. Medial subtalar dislocation results in medial and plantar displacement of the navicular relative to the talar head and medial displacement of the calcaneus relative to the talus. The tibiotalar joint remains congruent. After reduction, standard anteroposterior and lateral radiographs of the foot as well as anteroposterior and mortise views of the ankle should be obtained to confirm optimal results. In the absence of deformity, postreduction radiographs are usually of better quality than those obtained at the time of injury [12]. Closed reduction of these dislocations should be performed as early as possible to avoid further damage to the skin and neurovascular structures. If this is not possible, then open reduction without further delay is recommended [3, 13, 14].

Prognosis of isolated acute traumatic subtalar dislocations is favorable. Emergent closed reduction makes it possible to remove soft tissue injuries [15]. Complications of these injuries include posttraumatic arthrosis of subtalar, talonavicular or tibiotalar joint, aseptic necrosis of the talus and contracture of subtalar joint [16]. The risk of posttraumatic subtalar osteoarthritis is significant, even without an initial subtalar lesion. A postreduction computed tomography scan will enable the diagnosis of osteochondral lesions [15]. Newer evidence supports shorter-term immobilization followed by early range of motion after the initial injury in order to prevent stiffness [17, 18].

All five presented cases with subtalar dislocation had nonsurgical treatment according to the above-mentioned principles. Examination of the foot revealed an obvious deformity, substantial soft-tissue edema, and foot pain. Anteroposterior and lateral radiographs of the ankle was sufficient for the diagnosis subtalar dislocations. Urgent closed reduction of these dislocations was performed under general anesthesia. Computed tomography findings after closed reduction did not alter the treatment plan for any of the patients studied. After reduction, below knee cast was applied. Immobilization was removed after three weeks and the patients were not to bear any weight for the next three weeks. All the patients have undergone physiotherapy.

During the observation period after the injury, which lasted three years, there was no appearance of aseptic necrosis or other complications. At the end of the treatment, an excellent functional result was achieved. All the patients returned to their previous activities with a normal range of motion. An excellent outcome of patients with subtalar dislocation can be expected if: the injury was caused by low energy forces; quick reposition was performed; and the immobilization was not long [19].

We report two patients with dislocation of the cuboid bone. One patient had open dislocation of the cuboid bone. Open reduction of the cuboid bone was performed through the already present wound. After reduction, immobilization was applied. Immobilization was removed after six weeks. He received anti-tetanus prophylaxis.

Other patient refused surgery and the cuboid bone remained dislocated. Both patients have undergone physiotherapy.

An excellent functional result was achieved in one patient and the full mobility feet joints was restored, while in patient who refused surgery the result was satisfactory. He had limited joint mobility of the ankle and foot, pain during higher load (on visual analogue scale 3), and did not return to preinjury levels of activity.

Cuboid dislocations are rare injuries and are frequently overlooked and misdiagnosed on initial presentation [20]. The mechanism of injury is postulated to include a forced inversion and plantar flexion movement of the foot [21]. Important clinical findings include lateral foot pain, a palpable gap at the cuboid level and difficulty to bear weight. Radiographic evaluation of the region is often difficult because of overlap and superimposition of the bones. Anteroposterior, lateral and oblique radiographs should be obtained. Open reduction is usually required [22, 23].

Isolated dislocations of the navicular bone without fracture are rare injuries [24]. Because of the complexity of the midtarsal and tarsometatarsal joint complex, the exact mechanism of injury is often not known, particularly when there are multiple deforming forces present, as in high-energy injuries**.** The clinical symptoms and signs are swelling over the dorsomedial aspect of the foot; tenderness at the "N spot", which is defined as the proximal dorsal portion of the navicular; and pain with active inversion and passive eversion. For all midfoot injuries, standard anteroposterior, lateral and oblique radiographs should be obtained.

The main aim of treatment is early stable anatomical reduction. The fixation method varies from using screws,

plates, Kirschner wires, or external fixators. Complications include prolonged disability due to persistent pain in the navicular; stiffness of the midfoot; nonunion of associated fractures; avascular necrosis of the navicular; deformity of the foot; and post-traumatic arthritis [25, 26].

We report one dislocation of the navicular bone, which was treated surgically by early anatomical open reposition. After K-wire stabilization, below knee cast was applied. Immobilization was removed after four weeks and the patients were non-weight bearing for the next three weeks. All the patients have undergone physiotherapy.

An excellent functional result was achieved. The full mobility feet joints were restored. The patients returned to his previous activities.

Tarsometatarsal joint fracture-dislocation is an easily overlooked injury, which will cause abnormal transduction of the stress from midfoot to forefoot. Therefore, the surgical treatment is essential to obtain anatomical reduction [27].

The following cases are highly suggestive of a Lisfranc lesion:

1) Plantar ecchymosis at the level of the midfoot;

2) Pain on palpation or manipulation of the tarsometatarsal joints;

3) Altered sensitivity in the back of the first inter-metatarsal space;

4) The "piano key test", which consists of moving the head of the affected metatarsal while firmly holding the midfoot and hindfoot;

5) An increase in the distance between the hallux and the second finger, known as a "positive gap", which correlates with inter-cuneiform instability.

In these cases, we must request a radiographic non-weight-bearing study based on three views:

1) Anteroposterior: the alignment between the medial edge of the second metatarsal and the medial edge of the second cuneiform bone should be checked. The distance between the bases of the first and second metatarsals should not exceed 2 millimeters. The "fleck sign," a small bone fragment in the first inter-metatarsal space, indicates the avulsion of the Lisfranc ligament;

2) Internal oblique: the alignment between the medial border of the cuboid bone and the medial border of the fourth metatarsal should be checked;

3) Lateral: the dorsal/plantar displacement of the metatarsals should be assessed.

The definitive surgical intervention must be deferred 10–15 days until the healing of the soft tissues and the appearance of wrinkles on the skin (wrinkle sign). The traditional treatment for Lisfranc lesions is open reduction and internal fixation. However, some authors believe that primary partial arthrodesis offers better results and a lower rate of re-operations [28]. Functional outcomes after Lisfranc fractures are most dependent on the quality of anatomical reduction and not the choice of fixation implant used [29].

Marín-Peña et al. [30] reviewed the patients who had Lisfranc dislocation and showed that after 14 years the score of the American Association of Orthopedic Surgeons for ankle joint and the foot, which included the presence of the pain, function and alignment, was 91.7/100. For the evaluation of long-term outcome of these injuries functional parameters should be the focus of assessment, instead of radiological changes [30].

All patients had pain associated with swelling and inability to walk. Computed tomography scans were performed in 4/5 subtalar dislocations. Three patients with stable fractures were treated with closed reduction and cast immobilization. Two patients with unacceptable closed reduction and risk of soft tissue compromise were treated with open reduction and K-wire stabilization followed by cast immobilization. All patients were informed about the risks, benefits, and alternatives of a given procedure or intervention. They all demanded for less traumatic procedures.

The analysis of long-term data showed three patients with excellent functional results and two with good results. Two patients have limited joint mobility of the ankle and foot less than one third of the movement range and light intensity pain during higher load (on visual analogue scale 1, 2). All the patients returned to their previous levels of activities.

We report 13 tarsal dislocations. Functional result was rated excellent in 10 dislocations, good in two, and satisfactory in one.

After the treatment, there was a minimal limitation range of the movements of the feet joints (in 10 patients there were no restriction of movement, in two patients it was less than one-third of the range of the movement) while in one patient the restriction was more than third of the range of the movement. Two patients had light intensity pain during higher load (on visual analogue scale 1, 2), and one had pain during higher load (on visual analogue scale 3). The results were comparable with the results stated in the literature.

CONCLUSION

Tarsal dislocations are rare injuries. Diagnostics and the treatment of these dislocations are demanding but with adequate treatment a favorable functional outcome can be expected. Out of the 13 reviewed patients with tarsal dislocations, functional result were rated excellent in 10 dislocations, good in two, and satisfactory in one.

Conflicts of interest: None declared.

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Наша искуства у лечењу тарзалних луксација

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САЖЕТАК

Увод/Циљ Тарзалне луксације су ретке повреде. Обично су узроковане траумом високе енергије. Зависно од врсте луксације примењује се оперативно лечење или ортопедска репозиција. У раду је приказано 13 болесника са тарзалним луксацијама са циљем да се анализирају тип луксација, њихово лечење и исход.

Методе Анализирани су случајеви луксација стопала лечени у Универзитетској болници Фоча у периоду 2009–2016. године. Сви су клинички и радиографски обрађени те праћени на контролним прегледима најмање три године. Мерена је покретљивост зглобова и постојање болова процењено је визуелно-аналогном скалом.

Резултати Свих 13 приказаних болесника са тарзалним луксацијама били су мушког пола. Четири болесника лечена су оперативно (два болесника са тарзометатарзалном и по један са кубоидном и навикуларном луксацијом), а остали неоперативно. Код 10 болесника је постигнут одличан функционални резултат, код два болесника са тарзометатарзалном луксацијом добар, а код једног са кубоидном луксацијом задовољавајући резултат.

Закључак Од 13 болесника са тарзалним луксацијама лечених током седам година код десет је постигнут одличан резултат, код два добар, а код једног задовољавајући. Дијагностика и лечење луксација стопала су захтевни али адекватним лечењем ових повреда може се очекивати повољан функционални резултат.

Кључне речи: стопало; повреда; исход; лечење

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The effect of tonsillectomy on voice quality

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SUMMARY

Introduction/Objective Tonsillitis is a very common condition found in the pediatric population but also in adult patients. One of the consequences of such conditions is poor voice quality. Hoarseness, poor voice impostation, interruption, and hypernazalization are just some of the differences in patient voice quality. The objective of this paper was to examine the effects of tonsillectomy on the voice quality.

Methods The sample included 37 patients, 17 female and 20 male, ranging in age 3–39 years. The method involved recording patients one month before and one month after tonsillectomy with a digital sound recorder, with recordings analyzed in the Praat program. The variables monitored in the basic voice were as follows: voice pitch, standard deviation of voice, degree of voice interruption, jitter, shimmer, and signal-to-noise ratio. In the statistical analysis, in addition to standard descriptive analyzes, t-test and ACNOVA were also used.

Results The results showed that there are effects of tonsillectomy on standard deviation of baseline voice ($p = 0.002$), shimmer ($p = 0.002$), baseline voice interruption rate ($p = 0.023$), signal to noise ratio ($p = 0.003$). There were no differences in the effects of tonsillectomy with respect to the sex of the subjects.

Conclusion Based on the conducted research, there were some methodological conclusions that could be considered as a recommendation for future research: increase the number of persons in the sample, introduce a variable of chronological age, type of surgical intervention, and gradation of size of the tonsil and adenoid tissue.

Keywords: tonsillectomy; voice quality; acoustic analysis

INTRODUCTION

Voice, as a significant component of communication, has characteristics that provide some information about the speaker, such as age and sex, but also more subtle information, such as temperament, intention, emotion, or mood. The basic characteristics of voice are pitch, intensity, and color. Depending on the speed of vibration of the vocal cords, a stronger or quieter voice is produced, and higher or lower, depending on their tension and length. A quality, pleasant voice helps listeners focus on what they hear and listen to the speaker with pleasure. An unpleasant voice interferes with communication and can frustrate both the speaker and the listener. Voice quality can be influenced by various factors such as health status, fatigue, hormonal status, stress, articulation disorders, etc. [1].

One of the diseases that often lead to changes in voice quality is tonsillitis. This is due to the most common morphological and structural changes in the oral resonator that occurs in this condition. Morphological changes are associated with changes in the shape of the resonator, and structural changes are associated with changes in the structure of affected tonsil tissue.

Morphological changes most often occur with tonsil hypertrophy leading to a decrease in capacity and a change in the shape of the oral resonator. The phonation current under such conditions does not have a free and proper flow through the oral resonator. The particles of the phonation current encounter mechanical obstacles in the form of hypertrophic tonsils, leading to erroneous oscillations. This causes turbulence in the voice and therefore irregularities in the harmonics. The enlarged tonsils also misdirect the flow of the phonation current, so the phonation current often flows out through the nasal resonator, which leads to hypernasality [2]. In the Serbian language, there are only three voices that are inherently nasal (/m/, /n/, and /ɲ/), while all other voices are oral and any admixture of nasality in these voices is considered an articulatory deviation.

Structural changes need not only be associated with hypertrophic tonsils but also with all other diseases that lead to structural changes in the tissue of the tonsil. Unlike morphological changes, these changes affect the tone, that is, the tension and firmness of the resonator walls, which cause oscillation of the phonation particles and thus affect the voice quality. If the tonus is low or too high or the tissue relief is altered, the voice will surely suffer certain consequences.

Tonsillectomy is one way of treating tonsillitis and is the most common surgery in

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otolaryngology, especially in the pediatric population [3, 4]. One of the indications for tonsillectomy is obstruction, while changes in the voice, although present, do not represent a reason for surgery.

Several studies have shown that tonsillectomy and adenoidectomy have influence on the voice quality [5–8]. After tonsillectomy, a modification of the morphology and structure of the oral resonator occurs, which results in changes in the acoustic characteristics of the voice [9]. Part of the studies suggest that hypertrophic tonsils have hypernasality as a concomitant symptom that decreases postoperatively and thus leads to an improvement of voice quality [10]. However, some studies have shown that after tonsillectomy, only a subjective experience of voice improvement occurs, but this is not confirmed by objective measurements [11, 12].

To date, not many researches have measured voice quality by physical acoustic measures, but mainly using scales for subjective voice assessment. This was one of the reasons for the use of spectral voice analysis in this study. The aim of this study was to investigate the effect of tonsillectomy on voice quality by monitoring basic acoustic parameters before and after surgery.

METHODS

This study was approved by the Ethics Committee of the Faculty of Special Education and Rehabilitation, at the University of Belgrade, Serbia, and the University Hospital in Foča, Republic of Srpska, Bosnia and Herzegovina. All the respondents gave their consent to participate in this research. The study was conducted from August 2014 to September 2015 at the Foča University Hospital, Department of Otolaryngology. The study involved a cohort of participants, 17 female and 20 male, ranging in age 3–39 years (mean age being 11.04 years). All individuals in the sample had clear indications for the operative treatment of adenoid vegetation and palatal tonsils and all operations were performed by the same operating team using the same operating techniques (cold adenotonsillectomy, hemostasis by electrocautery).

All the patients were examined by otolaryngologists. The examination consisted of: taking a detailed medical history, physical examination, and, if necessary, audiological diagnostics. The criteria for inclusion in the study sample were the following: indications for tonsillectomy, adenoidectomy, and tonsilloadenoidectomy.

The criteria for exclusion from the sample were as follows: patients with second-degree voice disorders, neurological diseases, upper and lower respiratory tract infections, and craniofacial malformations affecting speech.

All the patients were recorded with VN-7000 digital voice recorder (Olympus Corporation, Tokyo, Japan) one day before surgery and one month after surgery. The recorded material was then transferred to a computer and processed using the PRAAT program (Paul Boersma and David Weenink, Phonetic Sciences, University of Amsterdam) [13]. Firstly, voice segmentation was made, which allowed the vocal part to be clearly separated from the words and to analyze the basic voice when pronouncing the vocals. The standard Praat bands were used in the analysis, namely the frequency ranges 0–5000 Hz with voice sampling every 0.005 seconds and dynamic range up to 50 dB.

Voice quality was monitored through the following variables: base voice pitch (Hz), standard deviation of baseline voice, voice interruption rate, vocal frequency oscillations (%) – jitter, vocal volume oscillations (dB) – shimmer, and signal-to-noise ratio.

Data were analyzed using the IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY, USA). In the statistical analysis, t-test and ANCOVA were used in addition to standard descriptive analyses.

RESULTS

Table 1 shows the results of the observed variables of voice quality before and after tonsillectomy. It is evident that some changes in voice quality were identified, in some of the analyzed variables. Statistically significant differences were observed in the standard deviation of baseline voice at the level of t = 3.330 and p = 0.002 and it was found that the standard deviation was significantly smaller after the tonsillectomy, which was also seen in mean, which was 66 Hz before the operation, and 31 Hz subsequently.

Table 1. Differences in voice quality before and after tonsillectomy

Variable	Before		After		t	p
	Mean	SD	Mean	SD		
Peak of baseline voice in Hz	248.64	43.76	243.84	59.1	0.596	0.555
SD of baseline voice	66.1	50.08	31.23	27.01	3.330	0.002
Baseline voice interruption rate	23.39	17.31	10.82	13.57	3.408	0.002
Frequency oscillations of vocal cords – jitter (%)	1.29	0.61	1.03	0.56	1.902	0.065
Intensity fluctuations of vocal cords – shimmer (dB)	1.38	0.25	1.23	0.27	2.369	0.023
Signal to noise ratio	7.5	2.46	9.38	2.85	-3.212	0.003

SD – standard deviation;
*statistical significance ($p < 0.05$)

Statistically significant differences were also found in the voice interruption rate (t = 3.408; p = 0.002), in the intensity fluctuations of the vocal cords – shimmer (t = 2.369; p = 0.023) and in the signal-to-noise ratio (t = -3.212; p = 0.003). All analyzed voice parameters showed better values after tonsillectomy. The baseline interruption rate after surgery decreased from 23.39% to 10.82%, the shimmer from 1.38 dB to 1.23 dB, and the signal-to-noise ratio increased from 7.5 to 9.4 dB (Table 1).

Frequency fluctuations of the vocal cords – jitter – also showed a tendency to decrease after tonsillectomy from 1.29% to 1.03%, but this decrease was not statistically

Table 2. Differences in voice quality with respect to sex before and after tonsillectomy

Variable		Men		Women		t	p
		Mean	SD	Mean	SD		
Peak of baseline voice in Hz	Before	254.14	49.39	242.16	36.48	1.480	0.232
	After	241.31	69.27	246.81	46.30	0.878	0.355
SD of baseline voice	Before	74.6	45.06	56.11	55.09	1.806	0.188
	After	33.4	28.05	28.68	26.36	0.832	0.368
Baseline voice interruption rate	Before	19.36	15.83	28.13	18.24	2.326	0.136
	After	13.23	14.29	8	12.48	1.289	0.264
Frequency oscillations of vocal cords – jitter (%)	Before	1.42	0.63	1.13	0.56	0.156	0.031
	After	1.22	0.66	0.81	0.31	2.104	0.088
Intensity fluctuations of vocal cords – shimmer (dB)	Before	1.44	0.178	1.31	0.3	2.373	0.133
	After	1.23	0.25	1.24	0.3	0.000	0.985
Signal to noise ratio	Before	7.06	1.87	8.03	2.98	1.180	0.285
	After	8.93	3.16	9.91	2.42	0.840	0366

SD – standard deviation;
*statistical significance ($p < 0.05$)

significant one month after tonsillectomy. It can be stated that the smallest change was in the pitch. There was a discrete decrease in voice value from 249 Hz to 244 Hz, with no statistical significance (Table 1).

By analyzing the changes in voice quality between men and women through the parameters listed in Table 2, we can see that there is no statistically significant difference that would indicate a different effect of tonsillectomy on male and female sex, respectively. The only statistically significant difference was observed in the frequency oscillation of the vocal cords – jitter, before surgery, which was statistically significantly more pronounced ($t = 5.088$; $p = 0.031$) in men (1.42%) compared to women (1.13%). This difference is lost after tonsillectomy.

DISCUSSION

Dysphonias resulting from diseased tonsils and adenoids can impair person's quality of life in his or her professional, educational, or daily functioning. Therefore, it is important that the principle of monitoring the effects, not only of tonsillectomy but also of other surgical interventions on the day-to-day functioning of operated patients, be established and become part of the therapeutic routine. To our knowledge, no research has been conducted to examine changes in the voice quality of patients after tonsillectomy.

The obtained and analyzed results of our study indicate that there are some changes after tonsillectomy in most of the analyzed acoustic parameters of the voice. Removal of enlarged adenoid tissue and tonsils results in changes in the resonator cavities, especially in the nasopharynx. As a result, the resonator cavities widen, and the soft palate becomes more mobile. These anatomical-morphological changes of the vocal tract resonators that occur after surgery lead to certain changes in the quality of voice, and therefore in the speech of patients [14, 15].

The impact of tonsillectomy on voice pitch has not been determined in this study which confirms the findings of some similar studies [16, 17]. There are also other conclusions that emerged from the research by Mora et al. [7] regarding the changes in pitch. Namely, they found these changes to be statistically highly significant. The results of our research support the fact that no changes in this acoustic parameter of the voice are expected after tonsillectomy because it is an operation that does not directly touch the larynx, and therefore does not affect the rate of vocal cord adduction during phonation [18]. Patients may have subjective observations about changes in their voice after tonsillectomy, as evidenced by research by Behrman et al. [19], who found that one-fifth of patients in their own observation had an improvement, while none had a deterioration of voice after surgery. Similar results have been reported in other studies indicating that there were no changes in the acoustic parameters of the voice and that patients reported a subjective sense of improvement in voice quality [20, 21, 22]. Regardless, the subjective experience of improving voice quality is very important, especially in patients whose general quality of life has been compromised by this disease [23].

One of the variables registered with statistical significance is standard deviation of the voice, indicating that tonsillectomy stabilizes the voice at values predicted for given sex and age. Even though statistical significance was not recorded in the voice peaks, as expected, indirectly – through the standard deviation – the effect of tonsillectomy on this acoustic parameter was observed. This means that tonsillectomy does not affect the abduction of the vocal cords but does affect the stabilization and safer impostation of the voice. This surgical intervention reduces the differences between the minimum and maximum peaks in speech production, which affects the homogeneous grouping of individual voices around assumed standard norms.

Voice interruption is a variable in which a statistically significant difference in the form of percentage reduction after surgery is also found. The presence of intermittent voice in the studied patients is probably due to altered surrounding tissue caused by inflammation, increased secretion, or fatigue. Their attempts to speak with the usual tension of the vocal cords caused the vocal cords not to vibrate at that frequency, which causes spasm. This spasm is perceived in the voice as interruption in phonation.

Removal of altered diseased tissue as well as minimization of post-operative talking leads to functional recovery, as confirmed by the results of our study.

Statistically significant differences were observed in the decrease in the intensity fluctuations of the vocal cords – shimmer, which occurs after surgery. Similar results have been reported in other studies, which show that during the postoperative period, this acoustic parameter normalizes [24, 8]. A decrease in decibels in shimmer indicates an improvement in voice quality because of function of transfer in supraglottic cavities, which was impaired by hypertrophic adenoids and tonsils in the preoperative status [24]. Tarnopolsky et al. [25] point out that there is a difference in shimmer improvements depending on the type of surgical intervention – a greater improvement in this acoustic parameter occurs after adenotonsillectomy than after tonsillectomy. Unlike shimmer, no statistically significant difference was observed in the frequency oscillations of the vocal cords – jitter. However, it should be emphasized that the deviations of voice in jitter prior to surgery were minimal compared to the reference values; the value before surgery was 1.23%, the value after surgery amounted to 1.03%, and the reference value is 1%.

The signal-to-noise ratio in the results of this study showed that most patients had poor voice quality before surgery – their voice contained a substantial amount of noise. After surgery, there was an increase in the difference between signal and noise, indicating a statistically significant improvement in voice quality. The average value of the signal-to-noise ratio did not reach the reference value of 10 dB, but the deviation from this value was minimal and is 9.38 dB. In the research carried by Mora at al. [7], noise-to-harmonic ratio reached the value of orderly voice, which is explained by the changed dynamics of the vocal tract structure.

By analyzing the results of the acoustic parameters of the basal voice before and after tonsillectomy with respect to sex, it was found in our study that there were minimal statistically significant differences. A statistically significant difference appeared in jitter between men and women before surgery, whereas after surgery the difference was present but not statistically significant. This means that the intervention led to a significant improvement in this parameter in men, which caused this difference between them and the women to disappear. There were no statistically significant differences in other acoustic parameters. Other studies have found statistically significant differences in pitch in relation to sex, since it has been found that there is no change in baseline frequency for women, unlike men, where statistically significant change was found [18].

Based on the conducted research, there were some methodological conclusions that could be considered as limitations of our work. Firstly, to be able to make conclusions with high reliability, the number of persons in the sample should be increased; the sample of children and adults should be grouped separately. It would be very important to deepen these studies in the direction of testing with relation to the type of surgical intervention being performed (tonsillectomy, adenoidectomy or tonsilloadenoidectomy). Also, for hypertrophic tonsils, a new variable should be introduced relating to the categorization or gradation of the size of the tonsils and adenoid tissue.

CONCLUSION

The results of our study showed that tonsillectomy affects most of the acoustic parameters of the voice, such as standard deviation of voice peaks, interruption rate of voice, shimmer, and signal-to-noise ratio. The effects of this surgical intervention are not recorded in jitter and the pitch of the baseline voice. Based on this, the general conclusion would be that tonsillectomy has a positive effect on improving voice quality.

Also, the recommendations arising from our research would relate to extending the indications for performing tonsil and adenoid surgery, especially in professions where voice quality is important. The voice is an essential means of work for singers, presenters, or lecturers and it is certainly important for them that their voice is clean, clear, strong, and pleasant. In addition, it would be good to introduce phonopedic therapy and short training on informal exercise programs to be carried out at home in individuals who do not experience improvement in voice quality one month after surgery.

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Утицај тонзилектомије на квалитет гласа

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САЖЕТАК

Увод/Циљ Обољења тонзила су веома честа стања која се срећу у педијатријској популацији али и код одраслих болесника. Једна од последица оваквих стања је лош квалитет гласа. Промуклост, лоша импостација гласа, прекидност и хиперназализација само су нека одступања од нормалног квалитета гласа болесника.

Циљ овог рада је био да се испитају ефекти тонзилектомије на квалитет гласа.

Методе У узорку је било 37 болесника, и то 17 женског и 20 мушког пола, старости 3–39 година. Метод је подразумевао снимање болесника пре и месец дана после тонзилектомије дигиталним снимачем звука; снимак је анализиран у програму *Praat*. Варијабле које су праћене у основном гласу су висина гласа, стандардна девијација гласа, степен прекидности гласа, *jitter, shimmer* и однос сигнал–шум. У статистичкој анализи поред стандардних дескриптивних анализа коришћени су *t*-тест и *ACNOVA*.

Резултати Резултати показују да постоје ефекти тонзилектомије на стандардну девијацију гласа ($p = 0,002$), степен прекидности гласа ($p = 0,002$), *shimmer* ($p = 0,023$) и однос сигнал–шум ($p = 0,003$). Нема разлика у ефектима тонзилектомије у односу на пол испитаника.

Закључак На основу спроведеног истраживања дошло се и до неких методолошких закључака који би се могли посматрати као препорука за будућа истраживања: повећати број особа у узорку, увести варијаблу хронолошког узраста, врсту хируршке интервенције и градацију величине тонзила и аденоидног ткива.

Кључне речи: тонзилектомија; квалитет гласа; акустичка анализа

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Benefits of dexamethasone use in thyroid surgery – a prospective, randomized study

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SUMMARY

Introduction/Objective This study aimed to investigate the effects of preoperative dexamethasone use on the incidence and severity of postoperative nausea and vomiting (PONV), postsurgical pain, and vocal impairment after thyroid surgery.

Methods We performed a prospective, randomized, double-blind study with 50 patients who underwent thyroid surgery. Group A patients (n = 25) received 0.9% NaCl solution (2 ml) before anesthesia, patients in Group B (n = 25) were administered 8 mg of dexamethasone. All the patients preoperatively received 4 mg of ondansetron. During the first 48 hours after surgery, postoperative complications were monitored in defined periods.

Results PONV rate and severity was significantly lower in Group B than in Group A ($p < 0.05$). Patients in Group B reported less pain in resting and in activity ($p < 0.05$) and lower vocal impairment ($p < 0.05$) than patients in Group A in each defined time period.

Conclusion Preoperatively adding dexamethasone to ondansetron is more effective than use of ondansetron alone in the prevention of PONV. Dexamethasone significantly reduces the pain and improves voice function; therefore, we could advise routine single-dose dexamethasone use before thyroid surgery.

Keywords: PONV; postoperative pain; vocal impairment; thyroid surgery; dexamethasone; ondansetron

INTRODUCTION

Common postoperative concerns for patients undergoing thyroid surgery are postoperative nausea and vomiting (usually summarized as PONV), acute postsurgical pain, and vocal impairment. These concerns could, apart from reducing comfort, cause grave local and systemic complications. PONV is defined as nausea and/or vomiting during the first 24 hours after surgery with the incidence among all surgical patients being 20–30% [1]. Patients who undergo thyroid or parathyroid surgery are prone to developing PONV; it occurs in 63–84% of these patients [2, 3].

The etiology of PONV is very complex. Many anesthetic, surgical and individual factors can have a significant impact on the frequency and severity of this complication [4, 5]. Individual risk factors include female sex, young patients, non-smokers, patients with a history of kinetosis and PONV. Apfel et al. [6] developed a simplified risk score as a tool aiming to help the prediction of PONV, according to which there are four main risk factors: female sex, prior history of motion sickness and PONV, non-smoker, and the use of postoperative opioids. According to their results, PONV incidence was 10%, 21%, 39%, 60%, and 78%, in the presence of none, one, two, three, or all four of these risk factors, respectively. Anesthetic risk factors include older volatile anesthetics, nitrous oxide and opioids' use, as well as neostigmine in high doses [7, 8, 9]. Surgical risk factors mainly include duration and the type of surgery [10].

PONV is not caused by a single stimulus or a single cause, so the use of a single antiemetic in PONV prophylaxis is not effective enough. We should use a combination of antiemetic drugs [11–14]. It is not yet known how the combination of dexamethasone and 5-HT_3 receptor antagonists works. Dexamethasone could actually inhibit serotonin central or peripheral production and/or secretion and enhance the antiemetic effects of 5-HT_3 receptor antagonists, or it could sensitize pharmacologic receptors, which leads to potentiating the main effects of other antiemetic drugs [15]. Furthermore, the use of dexamethasone could be effective in prevention of acute postoperative pain and vocal impairment [16, 17, 18].

The objective of this study was to investigate the effects of adding dexamethasone to ondansetron prior to surgery on incidence and severity of PONV, and the effects of dexamethasone on pain and vocal impairment after thyroid surgery.

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METHODS

We performed a prospective, randomized, double-blind clinical study comprising 50 adult patients undergoing elective thyroid surgery (partial or total thyroidectomy) at the Oncology Institute of Vojvodina in Sremska Kamenica, University of Novi Sad, Serbia. Institutional ethics boards approved the study. The inclusion criteria were age ≥ 18 years, patients undergoing thyroid surgery, American Society of Anesthesiologists (ASA) physical status I or II. The exclusion criteria were the use of antiemetic drugs 48 hours before surgery, known contraindication or hypersensitivity to study medications, abnormal levels of serum thyroid hormones, chronic pain, gastrointestinal diseases, BMI < 35, glaucoma, pregnancy, diabetes, and severe cardiovascular, renal, and respiratory diseases.

After admission to the hospital, patients underwent physical examination and were given explanation of the research and the purpose of the study. After the written informed consent had been obtained from the patients, a random division into two groups of patients was done. Randomization was carried out by permuted-block randomization where the block size was six, with sex as the stratification factor.

The enrolling anesthesiologist prepared the group assignment in sealed opaque envelopes. The treating and the enrolling anesthesiologist were different persons. Fifteen minutes before induction of anesthesia the envelopes were opened and an independent nurse, who was not participating in any other part of the study, prepared the drugs.

Ingestion of solid food is discontinued eight hours prior to the scheduled beginning of surgery, and ingestion of clear liquids is discontinued two hours prior to surgery. Both groups of patients received 2.5 mg of midazolam IV 30 minutes prior to anesthesia, and antiemetic drugs 10 minutes before anesthesia. Group A patients (n = 25) received 4 mg of ondansetron and placebo (2 mL of 0.9% NaCl solution), while patients in Group B (n = 25) were administrated 4 mg of ondansetron and 8 mg of dexamethasone (2 mL).

The same team of surgeons performed all the operations. The patients received standardized general anesthesia. For the induction we used propofol 2 mg/kg, fentanyl 2 μg/kg, and atracurium 0.5 mg/kg for tracheal intubation. All the intubations were conducted by experienced anesthesiologists using video laryngoscopy. After intubation, tracheal tube cuff pressure was measured with manometer and then adjusted to 20–30 cm H_2O. Anesthesia was maintained with sevoflurane titrated to achieve minimal alveolar concentration (MAC) 1 and 50% nitrous oxide in oxygen. Ventilation was mechanically controlled and adjusted to maintain the partial pressure of the end-tidal concentration of CO_2 of 35–40 mmHg. Intermittent doses of atracurium were given during anesthesia to maintain adequate muscle relaxation throughout the procedure. Neuromuscular blockade was monitored using train-of-four monitoring and reversion were provided with 0.01 mg/kg of atropine and 0.02 mg/kg of neostigmine. Electrocardiography, heart frequency, blood pressure, blood oxygen saturation, and inspiratory and expiratory concentration of O_2, CO_2, nitrous oxide, and sevoflurane were monitored during anesthesia.

Postoperative pain control was managed with ketorolac 30 mg IV every eight hours. Paracetamole 1 g IV was administered when visual analogue scale (VAS) was ≥ 5. Metoclopramide 10 mg IV was administered to patients who had more than three episodes of vomiting.

During the first 48 hours after surgery, postoperative complications were monitored in defined periods (first, sixth, 12th, 24th, and 48th hour) by the third anesthesiologist. All the data were collected using anesthesia charts, a survey, and observation.

The total PONV rate, incidence, and severity of PONV in Group A and Group B, as well as the incidence of PONV among smokers were the primary end points of this study. The secondary end points were the acute postsurgical pain and vocal impairment. All the data were collected within the first 48 hours following the anesthesia.

A four-point scale was used to assess the presence and severity of PONV: Grade 1 – absence of nausea, Grade 2 – very mild nausea, Grade 3 – moderate nausea and retching (a retroperistalsis of the stomach and esophagus without vomiting), Grade 4 – vomiting (a forceful discharge of stomach contents).

Postsurgical pain was assessed using a 10-point VAS (0 – no pain to 10 – the worst pain imaginable). Pain scores were measured at the state of rest (no coughing) and with activity (coughing).

Analysis of voice quality included the patient's own subjective evaluation of voice according to the Voice Visual Analog Scale (VVAS, 10 – normal voice, 0 – worst voice imaginable).

Statistical evaluation was carried out using SPSS® for Windows®, Version 16.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined for p-value less than 0.05.

RESULTS

Our study involved six (12%) male and 44 (88%) female patients. Statistically significant differences between the groups were not found in patients' demographic characteristics, ASA score, indications for surgery, and type of thyroid surgery (Table 1).

Table 1. Patient characteristics and surgical treatment

Patient characteristics	Group A (n = 25)	Group B (n = 25)	p
Mean age, years	53.3	48.9	0.575
ASA status, No. (%)			
I	0 (0)	3 (12)	0.067
II	25 (100)	22 (88)	0.077
Smokers, No.	11	9	0.564
Type of surgery			
Subtotal thyroidectomy	9	11	0.564
Lobectomy	9	7	0.544
Lobectomy with resection of the isthmus	4	3	0.682
Total thyroidectomy	3	4	0.682

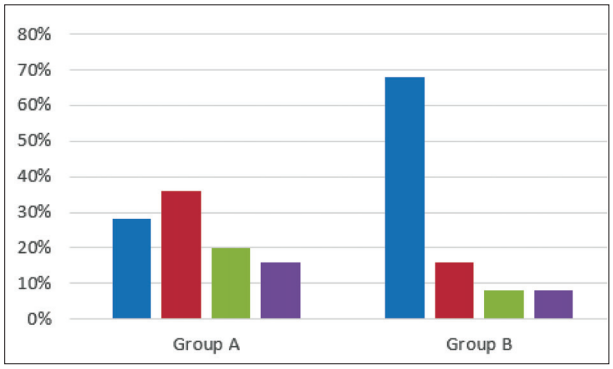

Figure 1. The presence and severity of postoperative nausea and vomiting in Group A and Group B during the period of 48 hours

Table 2. Mean values of postoperative nausea and vomiting (PONV) severity in Group A and Group B; the presence and severity of PONV were assessed using a four-point scale; Grade 1 – no nausea, Grade 2 – very mild nausea, Grade 3 – moderate nausea and retching, Grade 4 – vomiting

Time periods	Group A (n = 25)	Group B (n = 25)	PONV severity p
0–1 h	1.2	0.5	0.034
1–6 h	0.3	0.1	0.214
6–12 h	0.3	0.1	0.18
12–24 h	0.4	0.12	0.138
24–48 h	0.1	0.1	1

The mean duration of anesthesia was 84.5 minutes. No significant difference was found between the groups in the mean duration of anesthesia, which could influence PONV incidence (Group A = 88 minutes, Group B = 81 minutes, p = 0.124). All the patients were hemodynamically stable in the perioperative period.

The total PONV (including very mild nausea) incidence in both groups up to 48 hours after anesthesia was 52% (26/50 patients). In Group A, 72% of patients reported PONV. In Group B, the PONV rate was significantly lower (32% of patients, $p < 0.05$). There were no significant differences in the administered dose of metoclopramide (80 mg in Group A for four patients, 20 mg in Group B for two patients).

PONV severity was also significantly lower in Group B compared to Group A ($p < 0.001$, Figure 1). Very mild nausea (Grade 2) was reported by 16% of patients in Group B and in 36% of patients in Group A. Moderate nausea and retching (Grade 3) were reported by 8% of patients in Group B and in 20% of patients in Group A. Only 8% of patients in Group B had vomiting (Grade 4), compared with 16% of patients in Group A.

During the first hour following surgery, intense vomiting (Grade 4) occurred among 8% (2/25) of patients in Group A, whilst not a single patient in Group B reported intense vomiting, which is statistically lower (p = 0.034). In the following defined periods there was no statistically significant difference between the groups regarding the severity of PONV (Table 2).

Twenty patients were smokers and 30 were nonsmokers. Significant difference in PONV incidence between smokers and nonsmokers was found in the period between the first and the sixth hour (p = 0.004) and the sixth and the 12th hour (p = 0.013), while there was no difference in other defined time intervals.

Regarding the intensity of acute postoperative pain, we found significant difference between the groups in each determined time period following surgery. Patients in Group B reported significantly less pain at the state of rest and on coughing in all the periods than patients in Group A (Table 3). In accordance with this, five patients in Group A received paracetamol (8 g in total), while in Group B paracetamol was administered in only one patient (1 g) ($p < 0.05$).

Our research showed that the development of vocal impairment was significantly lower in Group B compared to Group A ($p < 0.05$) in each defined time period during the first 48 hours after the surgery (Table 3).

DISCUSSION

The most prominent perioperative concerns from the patients' point of view are the ones causing him the biggest discomfort – pain, nausea, and vomiting. PONV happens to be one of the most common causes of dissatisfaction among patients after undergoing anesthesia. Despite the fact that serious complications caused by PONV are rare, nausea and vomiting are still a disagreeable and common complications following the surgery [1]. Fortunately, this unpleasant complication can be effectively managed [4].

In our study, demographic and clinical characteristics, duration of anesthesia, type of surgical intervention, anesthetic and perioperative analgesic use were similar between the two groups. None of the patients in both groups required opioids in the postoperative period. In addition, patients with obesity and previous postoperative emesis and history of sickness while driving had been excluded from the study. There were no difficult intubations. Therefore, the difference in incidence of PONV between the groups could only

Table 3. Mean values of visual analogue scale (VAS) at rest and on coughing and voice visual analog scale (VVRS) in Group A and Group B; VAS – 10-point scale: from 0 – no pain to 10 – the worst pain imaginable; VVAS: from 10 – normal voice to 0 – the worst voice imaginable

Time periods	VAS at rest			VAS on coughing			VVAS		
	Group A (n = 25)	Group B (n = 25)	p	Group A (n = 25)	Group B (n = 25)	p	Group A (n = 25)	Group B (n = 25)	p
0–1 h	3	1.75	**0.002**	4	3	**0.027**	7.5	9	**0.000**
1–6 h	1.75	0.8	**0.002**	3.2	2	**0.001**	8.7	9.3	**0.025**
6–12 h	1.9	0.75	**0.003**	3.5	1.75	**0.000**	8.5	9.5	**0.001**
12–24 h	0.5	1.25	**0.009**	2.6	1.5	**0.002**	8.7	9.8	**0.000**
24–48 h	0.25	0.9	0.001	1.9	0.9	0.001	9	10	0.000

be explained by different antiemetic drugs administered before surgery. One of the main causes of PONV, especially during the early postsurgical recovery period, is certainly the use of inhalational drugs [6, 7, 19]. Nitrous oxide is well known and recognized as the risk factor for PONV. Myles et al. [20] concluded that the use of antiemetic drugs before surgery could eliminate the risk of severe PONV caused by nitrous oxide. We designed our study to show prophylactic effectiveness of dexamethasone and ondansetron on PONV in case of anesthesia with nitrous oxide.

Among 50 patients in the study, 26 had PONV including very mild nausea, moderate nausea, and vomiting, which represents 52% of patients.

We found that the total incidence of PONV after preoperative use of dexamethasone in combination with ondansetron (32% of patients) was significantly lower in comparison to ondansetron alone pretreatment (72% of patients). This is confirmed in some other studies [21–24]. The PONV incidence in our study was higher compared to the mentioned studies, probably because we considered very mild nausea (Grade 2), which patients have described more as an inconvenience. Excluding very mild nausea, the total PONV incidence was 26% (36% in the ondansetron alone group, 16% in the dexamethasone with ondansetron group). Dexamethasone combined with others drugs could significantly reduce the incidence of PONV in postoperative 24 hours [25]. Ahsan et al. [22] and Song et al. [23] compared ondansetron and dexamethasone combination effectiveness with ondansetron alone in preventing postoperative nausea and vomiting. Their results showed that the combination therapy was more effective.

The commonly used dexamethasone doses were 8–10 mg IV. No side effect related to single dose of 8 mg dexamethasone was found in our study and there was no prolonged hospital treatment due to the use of dexamethasone. Our results suggest that the combination of ondansetron and dexamethasone is more effective for control of nausea and vomiting.

Severity of PONV was in our study lower in patients who were pretreated with dexamethasone and ondansetron than with ondansetron only. We found that the difference in the severity of the PONV between the groups is significant only in the first hour following surgery. Although this difference failed to maintain significance during the overall period (0–48 h), the combination of medications is more beneficial than individual ondansetron use according to the trend of 95% confidence intervals.

Although the fact that smoking has antiemetic effect is confirmed by many studies, the etiology of its action is not completely known yet [26, 27]. There is a possibility that people who smoke have a lower incidence of PONV because they are more tolerant to anesthetic gases and other toxins than nonsmokers. According to our results, PONV was more frequent in smokers; we found a marked difference in the incidence of PONV in smokers compared to nonsmokers in the period between the first and the 12th hour after anesthesia. The small number of patients included in the study could be the cause of this result.

Postsurgical pain and PONV are two separate outcomes, but it is known that pain causes anxiety, which could be associated with nausea [16].

The results of meta-analysis conducted by De Oliveira et al. [28] support the fact that steroids have an analgesic effect. Since numerous effects of corticosteroids require gene expression and protein production, it is expected for them to have a delayed onset, which is uncommon for most analgesics. Expectedly, preoperative dosing turned out more effective than intraoperative administration. In the present study we found that patients receiving prophylactic dexamethasone rated postoperative pain significantly lower on the VAS scale at state of rest and on coughing than patients who were not pretreated with dexamethasone throughout the observation period.

Doksrød et al. [24] concluded that the incidence of PONV could be reduced effectively with dexamethasone; there were no differences in effectiveness between the medium (0.15 mg/kg) and the higher dose (0.3 mg/kg). According to their results, dexamethasone had no opioid sparing or analgesic effect after thyroid surgery. The results of a meta-analysis performed by Li et al. [29] were similar.

Worni et al. [17] studied the effects of corticosteroids on voice impairment related to thyroidectomy, and showed improved postoperative voice function, reduced nausea, vomiting, and pain during the first 48 hours after surgery in the group of patients who were pretreated with dexamethasone. Our results also confirmed the benefits from the use of dexamethasone in regard to voice function. We found significantly lower rate of vocal impairment in dexamethasone and ondansetron group in each defined time period within the first 48 hours after surgery.

In a study conducted by Lee et al. [30], effects of ramosetron and dexamethasone were compared with ramosetron alone in patients who undergo thyroid surgery. The PONV incidence, need for additional antiemetics, intensity of postsurgical pain and incidence of shivering were the primary end points of the study. They concluded that combining ramosetron with dexamethasone significantly decreases the incidence of PONV, the need for additional antiemetic treatment, pain intensity immediately after surgery, ketorolac consumption, as well as the incidence of shivering.

In spite of the fact that currently used antiemetics, such as ondansetron and granisetron, have shown their effectiveness, the solution is in better prevention [23, 24].

The second generations of 5-HT_3 antagonists' price is very high, which limits clinical application especially in low-income economies. On the other hand, dexamethasone's clinical use is common due to its low price.

CONCLUSION

According to our findings, preoperatively adding dexamethasone to ondansetron provides much better prevention of PONV than using ondansetron alone. Significant reduction of pain intensity and improvement of the voice function within the first 48 hours after thyroid surgery may

be achieved by applying a single dose of dexamethasone prior to surgery.

Using dexamethasone is a safe and simple method for reducing the incidence and severity of PONV, pain, and vocal impairment; hence, dexamethasone use could reduce the total treatment cost. Therefore, we advise the routine use of a single dexamethasone dose before thyroid surgery.

Conflict of interest: None declared.

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Предности примене дексаметазона код болесника који се подвргавају операцијама штитасте жлезде – проспективно, рандомизовано истраживање

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САЖЕТАК

Увод/Циљ Истраживање је спроведено са циљем да се испита утицај преоперативно примењеног дексаметазона на учесталост и интензитет постоперативне мучнине и повраћања, интензитет постоперативног бола и вокалну дисфункцију после операције штитасте жлезде.

Методе Проспективно, рандомизовано, двоструко слепо истраживање обухватило је 50 болесника код којих је изведена операција штитасте жлезде. Пре увода у анестезију болесници групе А ($n = 25$) примили су 0,9% *NaCl* (2 *ml*), а болесници групе Б ($n = 25$) 8 *mg* дексаметазона (2 *ml*). Сви болесници су преоперативно примили и 4 *mg* ондансетрона. Постоперативне компликације су праћене 48 сати после операције у дефинисаним временским интервалима.

Резултати Постоперативна мучнина и повраћање су били значајно ређи и мањег интензитета ($p < 0,05$) код болесника групе Б у поређењу са болесницима групе А. Болесници групе Б су постоперативно осетили значајно слабији бол у миру и у напору ($p < 0,05$) и имали су мање изражену вокалну дисфункцију ($p < 0,05$) у поређењу са болесницима групе А.

Закључак Преоперативна примена комбинације дексаметазона и ондансетрона је ефикаснија у превенцији постоперативне мучнине и повраћања у поређењу са применом само ондансетрона. С обзиром на то да дексаметазон значајно смањује и интензитет постоперативног бола и унапређује вокалну функцију, можемо предложити рутинску примену појединачне дозе дексаметазона пре операција штитасте жлезде.

Кључне речи: постоперативна мучнина и повраћање; постоперативни бол; вокална дисфункција; хирургија штитасте жлезде; дексаметазон; ондансетрон

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Characteristics and morbidity of prematurely born newborns conceived with assisted reproductive technologies

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SUMMARY

Introduction/Objective The percentage of live-born infants conceived with assisted reproductive technologies (ART) in some European countries reaches 6% and in Serbia over 1%.
The aim of this study was to analyze characteristics and morbidity of prematurely born newborns conceived with ART.

Methods The study included 154 prematurely born newborns from pregnancies conceived with ART and 154 prematurely born newborns conceived naturally, hospitalized at the Institute of Health Care of Children and Adolescents of Vojvodina. Participants from both groups were matched according to gestational age and date of birth.

Results Statistically significantly more newborns with very low birth weight have been in the group of newborns conceived by ART in comparison to newborns conceived naturally (χ^2 test, $p = 0.0001$). Morbidity of newborns conceived with ART is not higher in comparison to newborns of the same gestational age conceived naturally. Bronchopulmonary dysplasia, occurred more frequently in children from ART (χ^2 test, $p = 0.006$) and retinopathy of prematurity occurred more frequently in children conceived spontaneously (χ^2 test, $p = 0.047$). There was no difference in the frequency of birth defects, genetic syndromes, and inborn errors of metabolism between the two groups.

Conclusion Lower birth weight and intrauterine growth restriction are potential risk factors for worse postnatal outcome in newborns from pregnancies conceived with ART.

Keywords: assisted reproductive technologies; prematurely born newborns; morbidity

INTRODUCTION

According to the European Society of Human Reproduction and Embryology, from 1997 to 2014 there have been 1,478,452 newborns reported to be conceived with assisted reproductive technologies (ART) [1] The number of prematurely born infants is significantly higher with assisted conception than the number of infants born from natural conception. To solve this health and, ultimately, the social problem in Serbia, in 2006 the Republic Health Insurance Fund started financing the program of ART conceptions.

Research and identification of short- and long-term effects of ART are very challenging tasks. First and foremost, the reason for this is great heterogeneity in collecting, classifying, analyzing, and interpreting the enormous amount of information gathered so far in various studies. Individual approach to infertility treatment, fast improvement, and constant changes in the methodology of ART, together with previously mentioned problems of data collection and analysis, significantly impede the possibility to accurately comprehend all possible risks and consequences of artificial conception. Despite numerous studies, scientific publications and accumulated evidence, there is still much perplexity in regard to the following questions: 'Does the (artificially) assisted reproduction represent greater risk for inadequate embryo development, poorer perinatal outcome?', 'What are the long-term consequences for the children?', as well as 'Are the risks equal for singleton and multiple pregnancies conceived by ART?' [2–5].

Children born from pregnancies with medically assisted conception have higher risks of intrauterine growth retardation (IUGR), low birth weight (LBW), preterm delivery, and different congenital malformations, all of which could suggest the possibility of disrupted or suboptimal intrauterine growth.

A great deal of the above-mentioned problems have been explained by the fact that the majority of pregnancies achieved by some of the medically assisted reproduction techniques were dominantly multiple pregnancies with additional risks of the mother's age and morbidity, therefore carrying higher risks of suboptimal fetal growth [4]. Nevertheless, this claim is only partially true.

Etiologic factors and pathophysiological mechanisms that influence fetal growth and development can be of intrinsic and extrinsic nature. Intrinsic factors refer to characteristics of the fetus itself and include chromosomal abnormalities, chronic fetal infection, congenital

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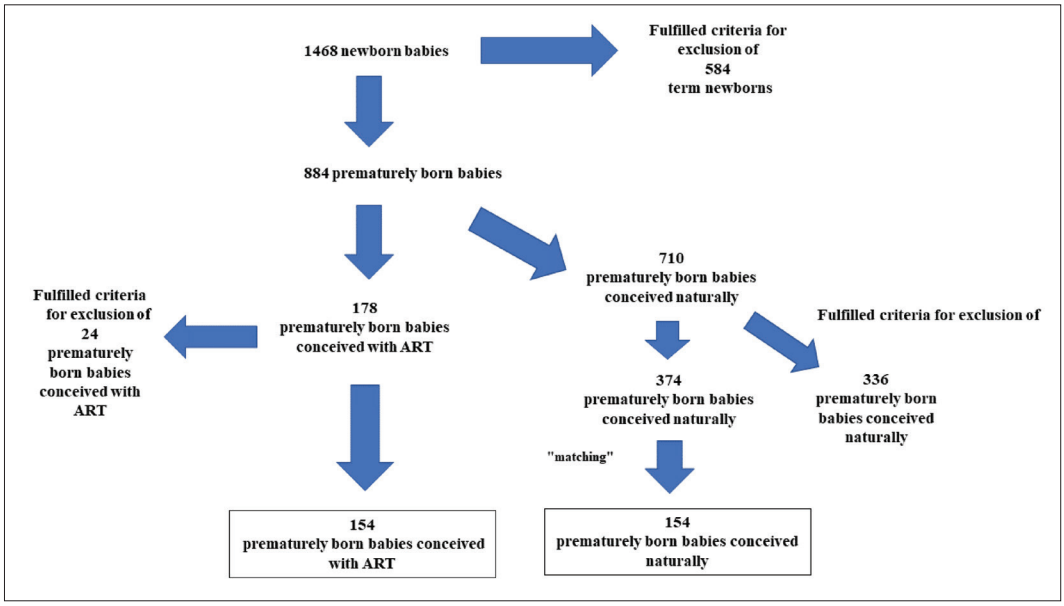

Figure 1. Algorithm for the selection of respondents included in the study

malformations, and genetic variations. Extrinsic factors can be divided into maternal and uteroplacental. Among maternal factors there are mother's periconceptional body weight, height (and age), and periconceptional nutritive status. Maternal pregnancy factors that define fetal growth and development are the existence of the cardiovascular disease, development of pregnancy hypertension syndrome, gestational diabetes, renal diseases, decreased oxygenation, inadequate nutrition during pregnancy, smoking, taking alcohol, medicines, and other chemicals [3, 6]. Uteroplacental factors that negatively affect fetal growth and development are placental insufficiency, disorders of placentation and the occurrence of multiple pregnancies.

Regardless of causes, an infant born with ART is an infant with potentially poorer perinatal outcome mainly because of a higher percentage of multiple pregnancies, higher frequency of preterm deliveries and unwanted outcomes of the ART [7]. In spite of this, in Serbia and in the other regions of the former Yugoslavia, papers on *in vitro* fertilization (IVF) on perinatal and neonatal statistics are very scarce.

The aim of this study was to establish the structure of morbidity of preterm infants conceived with ART (in singleton and multiple pregnancies) treated at the Institute for Health Care of Children and Youth of Vojvodina and to identify perinatal factors that are connected with the occurrence of acute and chronic complications and diseases of prematurely born newborns conceived with ART.

METHODS

The study included preterm infants hospitalized at the Department for Neonatology and Intensive and Semi-Intensive Care and Therapy at the Institute for Health Care of Children and Youth of Vojvodina in Novi Sad. The retrospective study included newborn babies born between January 1, 2011 and December 31 2012, treated at the Department. Data on the patients included in the retrospective part of the study were collected from medical records.

From this cohort, two groups were formed: the experimental group (Group 1) included all the prematurely born babies conceived with ART and hospitalized and treated at the Institute during the given period of time. The control group (Group 2) included all the preterm born babies conceived naturally. Babies in the control group were chosen from the cohort so that their number would correspond to the number of babies in the experimental group. Participants from both groups were matched according to gestational age (GA) and the date of birth. GA of the babies from the control group did not differ more than ± 4 days than that of the babies from the experimental group. Date of birth of the babies from the control group did not differ more than ± 3 months than that of the babies from the experimental group.

The detailed algorithm for the selection of respondents included in the study is given in Figure 1.

At the time of the inclusion in the study, the following data in reference to the babies were considered: intrauterine infection, IUGR, delivery method, Apgar score (AS), anthropometric parameters (body weight, body length, head circumference) at birth, duration of child's initial hospitalization, duration of invasive and/or non-invasive respiratory support and oxygen therapy, hospital discharge diagnosis (the presence of severe consequences of prematurity, which include intracranial hemorrhage of the 3rd and 4th degree (as defined in the International Classification of Diseases – Tenth Revision (ICD-10) under code P52.2), cystic periventricular leukomalacia, retinopathy of prematurity (ROP), bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), sepsis and/or meningitis (microbiologically or clinically diagnosed), presence of congenital anomalies or genetic syndromes and diseases (defined in ICD-10 under codes Q00 to Q99), as well as

the presence of inborn errors of metabolism (defined in ICD-10 under codes E00 to E90).

The subjects' written consent was obtained, according to the Declaration of Helsinki; the study has been approved by the Ethics Committee of the Institute of Health Care of Youth and Adolescents of Vojvodina.

RESULTS

Group 1 consisted of 154 prematurely born newborn babies conceived with ART from 87 mothers. Out of the total, there were 33 newborns from singleton pregnancies, while 121 were born from multiple pregnancies (39 from trigeminal and 82 from twin pregnancies).

Group 2 was formed according to previously described methodology from prematurely born infants of approximately the same GA from naturally conceived pregnancies. This group comprised 154 preterm-born newborn infants from 138 mothers. There were 122 newborns from singleton pregnancies, while 32 newborns were from twin pregnancies (16 twin pregnancies).

The main characteristics of newborns from the groups are given in Table 1.

Table 1. The main characteristics of infants according to the group

Characteristic	Group 1 (n = 154)	Group 2 (n = 154)	p
GA ± SD (weeks)	31.829 ± 2.105	31.167 ± 2.138	0.152
Sex (female/male)	68/86	68/86	/
BW ± SD (g)	1537.516 ± 401.594	1924.6 ± 777.843	**0.049**
BL ± SD (cm)	41.255 ± 3.415	41.25 ± 3.536	0.992
HC ± SD (cm)	29.137 ± 1.686	29.547 ± 2.309	0.130
AS in 1st min. ± SD	5.712 ± 1.750	5.1667 ± 2.133	**0.034**
AS in 5th min. ± SD	7.307 ± 1.210	7.012 ± 0.938	0.054
IUGR (%)	24/154 (15.584)	10/154 (6.493)	**0.011**

GA – gestational age; BW – birth weight; BL – birth length; HC – head circumference, AS – Apgar score; IUGR – intrauterine growth restriction;
values in bold are statistically significant

There has been no statistically significant difference in infants between Group 1 and Group 2 according to GA and sex (Student's t-test, p = 0.152).

There has been a statistically significant difference in birth weight (BW) of newborns from Group 1 and Group 2. Newborns from Group 1 had on average lower body weight on birth (Student's t-test, p = 0.049). The average difference in BW between newborns from Group 1 and those from Group 2 was 59.427 g

The percentages of newborns with BW under 1500 g (very low BW), BW from 1500 g to 2499 g (LBW), and birth weight ≥ 2500 g, in both groups, are shown in Figure 2.

Statistically, significantly there were more newborns with very low BW in Group 1 than in Group 2 (χ^2 test, p = 0.0001). The number of newborns with BW ≥ 2500 g was the same in both groups (χ^2 test, p = 0.702). There was no statistically significant difference in body length at birth and head circumference between newborns of the groups (Student's t-test, p = 0.992, p = 0.13).

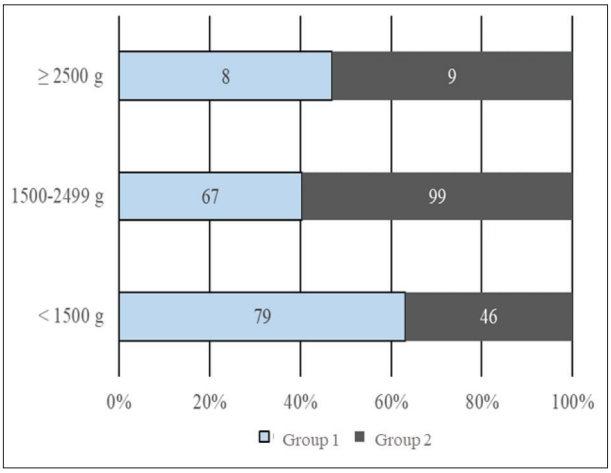

Figure 2. Proportion and absolute frequency of newborns of very low, low, and normal body weight in both groups of newborns

Newborns from Group 1 had a significantly higher AS in the first minute in comparison to newborns from Group 2 (Student's t-test, p = 0.034). The values of AS in the fifth minute have had no statistically significant difference between the two groups (Student's t-test, p = 0.054).

There was no statistically significant difference in frequency of symmetrical and asymmetrical IUGR between the two groups of participants (Fisher's exact test of probability, p = 0.394).

The average duration of hospitalization, the average length of respiratory support and oxygen therapy and morbidity structure (diagnosis at hospital discharge) of children from both groups are given in Table 2. Only the diagnoses listed in the methodology of work was recorded.

Table 2. The average duration of hospitalization, average length on respiratory support and oxygen therapy, and structure of morbidity at discharge from the hospital

Parameter	Group 1 (n = 154)	Group 2 (n = 154)	p
Length of hospitalization ± SD (days)	33.294 ± 15.998	38.351 ± 14.759	**0.012**
MV (days)	2.0719 ± 2.779	6.447 ± 4.872	**< 0.01**
nCPAP (days)	4.0719 ± 2.117	5.512 ± 3.202	0.052
Oxygen therapy (days)	14.046 ± 11.714	13.138 ± 4.391	0.472
ICH (III and IV degrees)	13/154	15/154	0.692
PVL	6/154	5/154	0.759
ROP	24/154	38/154	**0.047**
BPD	24/154	9/154	**0.006**
NEC	16/154	12/154	0.428
Sepsis/meningitis	30/154	28/154	0.771
Congenital anomalies and genetic syndromes (ICD-10 codes from Q00 to Q99)	22/154	28/154	0.354
Inborn errors of metabolism (ICD-10 codes from E00 to E90)	0/154	0/154	/

MV – mechanical venitlatory support; nCPAP – nasal continuous positive airway pressure; ICH – intracranial haemorrhage; PVL – periventicular leukomalacia; ROP – retinopathy of prematurity; BPD – bronchopulmonary dysplasia; NEC – necrotizing enterocolitis; ICD-10 – International Classification of Diseases 10th revision;
values in bold are statistically significant

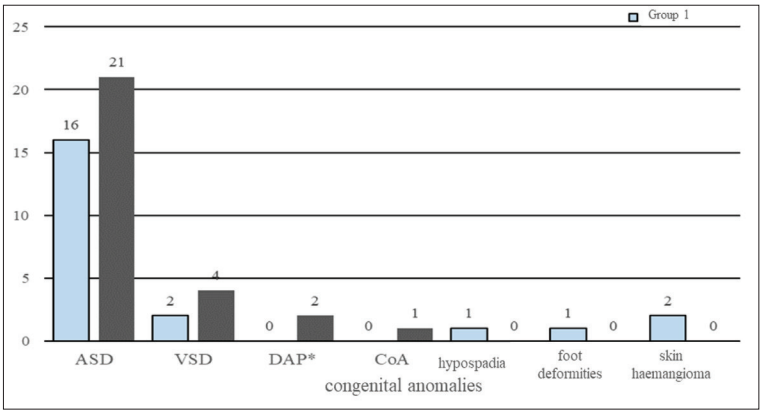

Figure 3. Structure of congenital anomalies and distribution of their absolute frequencies according to the groups of participants;

ASD – atrial septal defect; VSD – ventricular septal defect; DAP – persistent arterial duct after the age of six months; CoA – coarctation of the aorta

The average duration of hospitalization was statistically significantly shorter with newborns of Group 1 in comparison to those of Group 2. (Student's t-test, p = 0.012). The average duration of use of mechanical respiratory support was shorter in newborns of Group 1. The difference was statistically significant (Student's t-test, p < 0.01) (Table 2).

Duration of non-invasive respiratory support and oxygen therapy was on average slightly shorter in newborns of Group 1 in comparison to newborns of Group 2, but the difference was not statistically significant (Student's t-test, p = 0.052, p = 0.472).

The frequency of ROP was statistically significantly lower in newborns of Group 1 than in those of Group 2 (χ^2 test, p = 0.047). Newborns of Group 1 had a lower relative risk for ROP development (RR = 0.6316; CI 0.399–1.00) in comparison to newborns of Group 2.

The frequency of BPD was statistically significantly higher in newborns of Group 1 (RR = 2.823; CI 1.355–5.879) than in newborns of Group 2.

The incidence of higher-grade intracranial hemorrhage, periventricular leukomalacia, NEC, sepsis/meningitis was similar in both groups (χ^2 test, p = 0.692, p = 0.759, p = 0.428, p = 0.771).

There were no participants with diagnosed inborn errors of metabolism in either of the groups in the given period of time.

The overall frequency of congenital anomalies and genetic syndromes (defined under the 10th revision of the International Classification of Disease starting from Q00 to Q99) did not differ significantly between the groups (χ^2 test, p = 0.354).

The structure of congenital malformations and the distribution of their absolute frequencies according to the groups is given in Figure 3.

In most cases, there were simple heart defects that were registered in participants of both groups. In Group 1 there were 16 newborns with registered atrial septal defect, while there were 21 of them in Group 2. The difference was not statistically significant (χ^2 test, p = 0.381). Ventricular septal defect (small and medium) was registered in two cases with newborns of Group 2. This difference was not statistically significant (Fisher's test of exact probability, p = 0.684).The other listed/mentioned congenital anomalies occurred occasionally.

DISCUSSION

According to anthropometric parameters at birth and the presence of IUGR, the study results show that prematurely born infants conceived by ART in comparison to prematurely born newborns conceived naturally are statistically significantly different in terms of BW and incidence of IUGR. In the group of newborns who were conceived by ART, there were significantly more newborns with very low BW. The average difference between the body weight of newborns conceived by IVF and those conceived naturally was -59.472 ± 426.34 g.

Most of the studies carried out so far confirm these results. Results from a study by Lei et al. [8] showed that artificial conception increases the risk of LBW.

In a review article, Šljivančanin and Kontić-Vučinić [9] state that different studies' conclusion showed that infants from ART have significantly worse perinatal outcome (LBW, VLBW, SGA) compared with natural conception. This fact has also been confirmed in our research.

In a sample of our participants (Group 1), the value of AS in the first minute of life was statistically significantly higher than the value of AS in Group 2. In the studies available to us, lower values of AS in the first and fifth minute of life were most often reported for newborns conceived with ART [10, 11, 12]. The difference in our findings can be mostly explained by the fact that pregnancies conceived by ART in Serbia are more frequently and more patiently monitored and, therefore, the likelihood of early delivery is anticipated better and a better strategy for premature birth has been developed. On the other hand, premature births in spontaneously conceived pregnancies are usually caused by unexpected events related to the health situation of the fetal mother; they were sudden and "unplanned," which significantly influenced the delivery, immediate prenatal treatment of the pregnant woman and the fetus, and accordingly influenced the "condition" of the child immediately after birth. The most common cause of premature birth in the control group was premature contractions, with no significant previous medical history, and the cesarean section was more often indicated in pregnancies conceived with IVF. The value of AS in the 5th minute did not differ significantly between the groups, but newborns that were spontaneously conceived had a higher AS (increase), which could point to the possibility that spontaneously conceived infants had a slightly more prompt reaction after initial stabilization and a slightly better capacity to adapt to extrauterine conditions of life.

As indicators of neonatal morbidity, in this study, we observed the total length/duration of hospitalization, the number of days on mechanical respiratory support, the number of days on non-invasive respiratory support,

the duration of oxygen therapy and significant diagnosis when discharged from hospital (high intracranial hemorrhage, periventricular leukomalacia, ROP, BPD, NEC, sepsis/meningitis, and congenital malformations, genetic syndromes and inborn errors of metabolism. From all the observed parameters/categories, in this study, statistically significantly different among the groups were the following: length of hospitalization, duration of mechanical respiratory support, and the frequency of BPD and ROP. Infants conceived with ART had a spent less time on mechanical respiratory support and were discharged earlier from hospital (shorter hospitalization), and more often had BPD diagnosed. Children from the control group were more often diagnosed with ROP.

Taking into consideration controversial discussions among professionals about the connection of IVF procedures and congenital malformations, we emphasize as a significant data, that in our sample of prematurely born newborns, there was no difference in the frequency of birth defects, genetic syndromes, and inborn errors of metabolism between newborns conceived naturally and those conceived by ART. This is most likely the result of well-organized and comprehensive monitoring of ART-initiated pregnancies (regular examinations, expert ultrasound, etc.). In contrast to our results, Giorgione et al. [13] concluded that fetuses conceived with IVF/ICSI methods are at an increased risk of developing congenital heart defects compared with those conceived spontaneously.

Generally, the observations mentioned in this study are in agreement with the results of other studies that dealt with immediate and short-term outcomes in prematurely born newborns conceived by ART [3, 14, 15]. Disagreement exists in the results that refer to the frequency of BPD and ROP. In a study conducted by Corchia et al. [16], the results indicate that the assisted conception represents a protective factor in relation to BPD, which is in collision with the findings of our study. Also, unlike our study, the study by Corchia et al. [16] has shown that there is no significant difference in the incidence of ROP between prematurely born newborns conceived with ART and those who were spontaneously conceived. By contrast, other studies found an increased incidence of both BPD and ROP in babies who were conceived by IVF [15, 17].

In the light of recent events due to COVID-19 pandemic, the major scientific societies have provided recommendations to suspend IVF treatments in order to support healthcare systems by avoiding putting them under additional risk [17, 18].

Although there is no evidence that the virus causing COVID-19 might have negative effects on IVF outcomes, the possibility of the virus affecting sperm function and egg performance cannot be excluded [17]. However, the prolonged lockdown of health services providing fertility treatments might be detrimental for society as a whole, and infertility patients in particular [19].

These are new challenges in the field of reproductive medicine, which leads to further research regarding characteristics and morbidity of newborns conceived with ART during the COVID-19 pandemic.

CONCLUSION

Morbidity of prematurely born newborns conceived with ART is not higher in comparison to prematurely born newborns of the same gestational age conceived naturally. In the morbidity structure of newborns conceived with ART, the same diseases and complications are present as among prematurely born newborns of the same gestational age conceived naturally. Frequency of some diseases is similar, with the exception of BPD, which occurs more often among prematurely born newborns conceived with ART, and ROP, which occurs more often in prematurely born newborns conceived naturally. Lower BW and IUGR are potential risk factors for poorer postnatal outcome in newborns from pregnancies conceived with ART. AS in the first minute of prematurely born newborns conceived with ART is higher in comparison to AS of prematurely born newborns conceived naturally.

NOTE

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Карактеристике и морбидитет превремено рођене новорођенчади зачете вантелесном оплодњом

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САЖЕТАК

Увод/Циљ Проценат живорођене новорођенчади зачете неком од метода вантелесне оплодње (асистиране репродуктивне технологије) у сталном је порасту. У неким европским земљама досеже и 6%. У Србији је он нешто виши од 1%. Циљ рада је био да се анализирају карактеристике и морбидитет превремено рођене новорођенчади зачете асистираном репродуктивном технологијом.

Методе Студија је обухватила 154 превремено рођена новорођенчета зачета вантелесном оплодњом и 154 превремено рођена новорођенчета зачета природним путем, која су била хоспитализована у Институту за здравствену заштиту деце и омладине Војводине. Испитаници из обе групе су уједначени према гестацијској старости и датуму рођења.

Резултати У овом истраживању било је статистички значајно више новорођенчади са врло малом порођајном масом у групи новорођенчади зачете асистираном репродуктивном технологијом у односу на новорођенчад из спонтано зачетих трудноћа (χ^2 тест, $p = 0{,}0001$). Стопа морбидитета превремено рођене деце зачете вантелесном оплодњом није већа у односу на превремено рођену децу зачету природним путем. Бронхопулмонална дисплазија (χ^2 тест, $p = 0{,}006$) јавља се чешће код деце зачете вантелесном оплодњом, а ретинопатија прематуритета (χ^2 тест, $p = 0{,}047$) јавља се чешће код деце зачете природним путем исте гестацијске старости. Није било разлике у учесталости конгениталних аномалија, генетских синдрома и метаболичких поремећаја између група.

Закључак Мала порођајна маса и интраутерини застој раста су могући фактори ризика за лошији постнатални исход код новорођенчади зачете асистираном репродуктивном технологијом.

Кључне речи: асистиране репродуктивне технологије; превремено рођена новорођенчад; морбидитет

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Trends in bone mineral density among nutritional status categories of Vojvodina elderly population

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SUMMARY

Introduction/Objective Low bone mineral density (BMD) is commonly associated with alterations of nutritional status.

The aims of the present study were to evaluate the prevalence of low BMD and its associated nutritional risk factors in Vojvodina population and to use linear regression equations to predict the BMD by using a simple marker of nutritional status, body mass index (BMI).

Methods In this retrospective, cross-sectional study, the study population included subjects who were undergoing assessment of BMD between January and December 2017, and who have met the study inclusion criteria. A total of 1974 patients (1866 women and 108 men) were included in this analysis of nutritional status according to anthropometry and BMI index, and dual-energy X-ray absorptiometry (DEXA) measurements of BMD of the femoral neck and lumbar spine. The relationship between BMI and BMD was analyzed by linear regression equation.

Results Median age was 63 (56–70) years. Considering nutritional status category, there were 40% overweight, 31% obese and 29% normal weight subjects. In most of the sample, the subjects had low BMD, 37% had osteopenia, and 25% had osteoporosis. In both bone areas we observed trends of lowering BMD as the subjects BMI decreased. Subjects with osteoporosis are more prone to BMI depended BMD changes, concerning subjects with osteopenia and normal BMD. In addition, normal weight subjects compared to overweight and obese had the highest prediction coefficients of BMI-depended changes on BMD.

Conclusion High prevalence of low BMD coexists with overweight and obese elderly females in Vojvodina. Prediction equations for the calculation of BMD can be used to evaluate the effect of BMI changes on BMD in clinical settings.

Keywords: bone mineral density; body mass index; osteoporosis; osteopenia; linear regression

INTRODUCTION

The world population is about 7.6 billion people at this moment and it is expected to increase by one billion in the next ten years and to reach approximately 10 billion by 2050. Due to the simultaneous ageing trend of the population at the global level, the number of elderly people over 60 years of age, which was 962 million in 2017, is expected to increase more than double by 2050 [1]. In Serbia, almost one fifth of the female population and 15 % of males are older than 65 years. In addition, current demographic trends of the population in Vojvodina indicate regressive type of age structure characterized by 40.2% of people over 50 years [2].

Population ageing results in the increased incidence of osteoporosis in elderly women [3]. Osteoporosis is a disease characterized with low bone mineral density (BMD) and compromised bone microarchitecture, both leading to the more expressed bone fragility and increased risk of fracture. According to the estimation done in 2010, 22 million women and 5.5 million men in Europe suffer from osteoporosis. About 40% of elderly women and 15–30% of elderly men are likely to have osteoporotic fracture over the course of life [4, 5].

Low BMD and impaired bone quality are commonly associated with nutritional status. Altered nutritional status, mostly underweight category is associated with low BMD and compromised bone microarchitecture. Even though overweight and obesity are generally associated with higher BMD, recent studies imply that overweight and obese patients also have serious negative impact on bone metabolism [6, 7, 8]. Obesity is heterogenous, multifactorial, and a complex disease, which is positively associated to many chronic disorders. Its diagnosis is based on the evaluation of nutrition status or body mass index (BMI), distribution of excessive fat deposits and determination of body composition [9]. Rates of nutritional abnormalities, overweight and obesity are rising rapidly. The results of 2006 research showed that more than a half of adult population of Serbia (55.7%) was overweight and obese. In Serbia, Vojvodina has the highest total prevalence of overweight and obesity, which is as high as 58.5% of the population [10].

Previous analysis focused on the subjects in Vojvodina shown high prevalence of osteopenia

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and significant positive correlation between T score and BMI in older women [11]. Additionally, nutritional status of the subjects was mostly disturbed; high prevalence of overweight (43%), and obese subjects (20%) was reported. Considering the increasing trend of risk factors for low BMD in our population, ageing coexisted with nutritional status abnormalities, this study aimed to use linear regression equations to predict the BMD by using a simple marker of nutritional status, body mass index (BMI), on sample population subjects from the general population of Vojvodina.

METHODS

The study, a retrospective cross-sectional survey, was carried out at the Clinical Center of Vojvodina, Novi Sad. The study population included subjects who were undergoing assessment of BMD between January and December 2017, and who have met the study inclusion criteria. The study sample consisted of 1974 adults (1866 women and 108 men). The inclusion criteria of this study required all subjects to be aged 50 years and above, with complete medical documentation. Exclusion criteria was clinical evidence of existing secondary causes of BMD disorders (endocrine, gastrointestinal, hematologic, or rheumatic diseases, drug-induced osteoporosis) [12]. This study was approved by the Ethics Committee of the Clinical Center of Vojvodina.

Anthropometric measurements analyzed were body weight (medical weighing scale with precision of 0.1 kilograms), body height (Martin anthropometer, centimeters), and BMI derived from Quetelet's equation. The subject's nutritional status was defined based on their BMI as normal weight (BMI 18.5–24.99 kg/m^2), overweight (BMI 25–29.99 kg/m^2), and obesity (BMI ≥ 30kg/m^2) [9].

BMD (g/cm^2) was measured with GE Lunar equipment (GE Healthcare, Chicago, IL, USA) by applying the method of dual-energy X-ray absorptiometry (DEXA) in the region of lumbar spine (calculated values were means of four measured values L1–L4) and femoral neck. According to the World Health Organization standards, subjects were classified into subgroups: osteoporosis (T ≤ −2.5), osteopenia (−2.5 < T < −1.0), normal finding (T ≥ −1.0) [13].

Statistical Analysis

The obtained results were analyzed in the MATLAB 8 (MathWorks, Inc., Natick, MA, USA) computing environment. Normality was examined with Shapiro–Wilk test, which showed that the analyzed continuous parameters did not have a normal distribution and therefore they were represented in the form of median (Q1–Q3). Statistical significance was examined by applying Kruskal–Wallis test with post hoc testing on the defined subgroups (normal finding, osteopenia and osteoporosis), as well as on the subgroups according to the nutrition status of subjects (normal weight, overweight, and obesity). Finally, we have used linear regression to analyze trends of considered parameters in relation with BMI changes.

RESULTS

Table 1 shows general characteristics of the study group. The majority of study sample subjects were elderly women, within nutritional status category of overweight and with osteopenia in the region of femoral neck and lumbar spine.

Table 1. General characteristics of the study sample subjects

Characteristics (n = 1974)	
Female (n/N, %)	1866/1974 (95%)
Age in years	63 (56–70)
BMI (kg/m^2)	27.4 (24.5–30.9)
FN – BMD (g/cm^2)	0.9 (0.7–1)
FN – T Score	-1.1 (/-1.9/ -0.3)
FN – Z Score	-0.3 (/-1/ -0.4)
LS – BMD – (g/cm^2)	1 (0.9–1.1)
LS – T score	-1.5 (/-2.5/ -0.4)
LS – Z score	-0.3 (/-1/ -0.7)

BMI (kg/m^2) – body mass index;
BMD – bone mineral density; FN – femoral neck,
LS – lumbar spine

Clinical characteristics of the subjects by BMD categories are given in Table 2. Observed subjects differ significantly according to their age, osteoporotic subjects were significantly older compared to osteopenic and those with

Table 2. Clinical characteristics and osteodensitometry measurements of the study sample subjects by categories

Parameters	Osteoporosis (n = 494)	Osteopenia (n = 745)	Normal finding (n = 735)	Kruskal–Wallis test	Post hoc testing
Age (years)	65 (59–76)	62 (58–71)	60 (54–66)	p < 0.001	p < 0.001*
BMI (kg/m^2)	25.5 (21.7–27.3)	27.3 (23.9–30)	28.9 (25.9–32.4)	p < 0.001	p < 0.001*
Femoral neck BMD measurements					
BMD (g/cm^2)	0.8 (0.6–0.7)	0.9 (0.76–0.84)	1 (0.9–1)	p < 0.001	p < 0.001*
T Score	-2 (/-3.3/ – /-2.6/)	-1.1 (/-2.0/ – /-1.4/)	-0.4 (/-0.7/ -0.3)	p < 0.001	p < 0.001*
Z Score	-0.9 (/-2.2/ – /-1.2/)	-0.4 (/-1.2/ – /-0.4/)	0.1 (/-0.1/ – /-0.9/)	p < 0.001	p < 0.001*
Lumbar spine BMD measurements					
BMD (g/cm^2)	0.8 (0.7–0.9)	0.9 (0.8–1)	1.1 (1–1.2)	p < 0.001	p < 0.001*
T Score	-3 (/-3.7/ – /-2.2/)	-1.8 (/-2.8/ – /-1.3/)	0.0 (/-1.6/ -0.3)	p < 0.001	p < 0.001*
Z score	-1.4 (/-2/ – /-0.5/)	-0.4 (/-1.2/ – /-0.1/)	1 (/-0.5/ -1.3)	p < 0.001	p < 0.001*

BMI (kg/m^2) – body mass index; BMD (g/cm^2) – bone mineral density; * – post hoc testing between groups osteoporosis *vs.* osteopenia, osteoporosis *vs.* normal finding, osteopenia *vs.* normal finding

Table 3. Comparisons of regional BMD measurements in the region of femoral neck and lumbar spine by the nutritional status of the patients

Parameters	Normal weight (N = 579) 23.1 (21.6–24.03) kg/m²	Overweight (N = 790) 27.3 (26.3–28.6) kg/m²	Obesity (N = 605) 32.8 (31.2–35.3) kg/m²	Kruskal–Wallis test	Post hoc testing
Femoral neck BMD measurements					
BMD (g/cm²)	0.8 (0.7–0.9)	0.9 (0.8–1)	0.9 (0.8–1)	p < 0.001	p < 0.001*
T Score	-1.6 (/-2.3/ – /-0.9/)	-1.1 (/-1.9/ – /-0.3/)	-0.6 (/-1.4/ – /-0.2/)	p < 0.001	p < 0.001*
Z Score	-0.7 (/-1.3/ -0)	-0.3 (/-1.1/ -0.4)	0 (/-0.7/ -0.6)	p < 0.001	p < 0.001*
Lumbar spine BMD measurements					
BMD (g/cm²)	1 (0.8–1.1)	1 (0.9–1.1)	1.1 (1–1)	p < 0.001	p < 0.001*
T Score	-1.9 (/-2.9/ – /-1/)	-1.6 (/-2.5/ – /-0.5/)	-1 (/-2.5/ – /-0.5/)	p < 0.001	p < 0.001*
Z Score	-0.5 (/-1.3/ -0.3)	-0.2 (/-1/ -0.7)	-0.1 (/-0.9/ -1.1)	p < 0.001	p < 0.001*

BMD – bone mineral density; T-score – number of standard deviations by which bone mineral density in an individual differs from the mean value expected in young healthy women; Z-score – the number of standard deviations by which bone mineral density in an individual differs from the mean value expected for age and sex

Table 4. Regression equations of BMD of femoral neck and lumbar spine in relation to BMI in all subjects

Formulae	Trend
Femoral neck BMD measurements	
BMD = 0.011 × BMI + 0.581	↑
T-Score = 0.091 × BMI – 3.621	↑
Z-Score = 0.057 × BMI – 1.906	↑
Lumbar spine BMD measurements	
BMD = 0.011 × BMI + 0.698	↑
T-Score = 0.094 × BMI – 4.012	↑
Z-Score = 0.052 × BMI – 1.589	↑

BMI (kg/m²) – body mass index; BMD (g/cm²) – bone mineral density

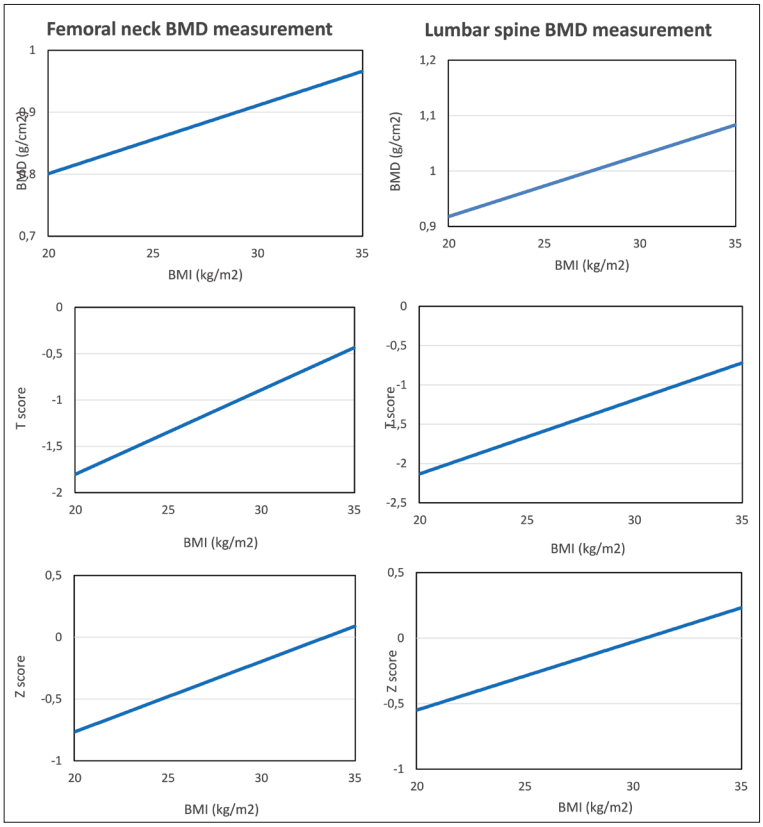

Figure 1. Trend lines of bone mineral density of femoral neck and lumbar spine in relation to body mass index in all subjects

normal bone mass [65 (59–76) *vs.* 62 (58–71) *vs.* 60 (54–66), p < 0.001]. The subjects with osteoporosis had significantly lower BMI values compared to subjects with osteopenia and subjects with normal BMD in the both observed bone region [25.5 (21.7–27.3) *vs.* 27.3 (23.9–30) *vs.* 28.9 (25.9–32.4) kg/m², p < 0.001]

Table 3 shows regional BMD measurements (BMD, T-score, and Z-score) in the region of femoral neck and lumbar spine by the nutritional status of the patients (p < 0.001). Obese patients had significantly higher values of BMD, T-score, and Z-score compared to overweight and normal weight subjects (p < 0.001). Overweight subjects had significantly higher values of BMD, T-score, and Z-score compared to normal weight subjects (p < 0.001).

The method of linear regression was applied on the entire dataset to determine the associations between BMI and regional BMD measurements (BMD, T-score, and Z-score) in the region of femoral neck and lumbar spine, and the obtained results are given in Table 4. Trend analyses based on regression approaches indicate the tendency of BMD increase with increasing BMI, as shown in Figure 1.

The association between BMI and regional BMD measurements (BMD, T-score, and Z-score) in the region of femoral neck and lumbar spine was determined in the groups of osteoporosis, osteopenia, and normal finding and the results obtained by linear regression are given in Tables 5a, 5b, and 5c. In regression equation, the prediction coefficients between BMI and the osteodensitometry measurements were the highest in the group with osteoporosis as compared with the other two groups, which means that the observed parameters change most rapidly with the change of BMI in that group.

The graphs are given in Figure 2. The estimations can be done by means of the obtained formulae and graphs. For example, if a person is in the group with osteoporosis and has BMI = 22 kg/m², the observed parameter values are expected to be as follows:

Table 5a. Regression equations of BMD of femoral neck and lumbar spine in relation to BMI in subjects with osteoporosis

Formulae	Trend
Femoral neck BMD measurements	
BMD = 0.01 × BMI + 0.509	↑
T-Score = 0.081 × BMI – 4.128	↑
Z-Score = 0.047 × BMI – 2.171	↑
Lumbar spine BMD measurements	
BMD = 0.004 × BMI + 0.7	↑
T-Score = 0.031 × BMI – 4.007	↑
Z-Score = /-0.014/ × BMI – 1.159	↓

BMI (kg/m²) – body mass index,
BMD (g/cm²) – bone mineral density

Table 5b. Regression equations of BMD of femoral neck and lumbar spine in relation to BMI in subjects with osteopenia

Formulae	Trend
Femoral neck BMD measurements	
BMD = 0.006 × BMI + 0.691	↑
T-Score = 0.061 × BMI – 2.884	↑
Z-Score = 0.036 × BMI – 1.399	↑
Lumbar spine BMD measurements	
BMD = 0.002 × BMI + 0.92	↑
T-Score = 0.013 × BMI – 2.144	↑
Z-Score = /-0.016/ × BMI – 0.013	↓

BMI (kg/m²) – body mass index;
BMD (g/cm²) – bone mineral density

Table 5c. Regression equations of BMD of femoral neck and lumbar spine in relation to BMI in subjects with normal BMD measurements

Formulae	Trend
Femoral neck BMD measurements	
BMD = 0.006 × BMI + 0.79	↑
T-Score = 0.053 × BMI – 1.912	↑
Z-Score = 0.032 × BMI – 0.075	↑
Lumbar spine BMD measurements	
BMD = 0.004 × BMI + 1.075	↑
T-Score = 0.035 × BMI – 0.811	↑
Z-Score = /-0.015/ × BMI – 0.701	↓

BMI (kg/m²) – body mass index;
BMD (g/cm²) – bone mineral density

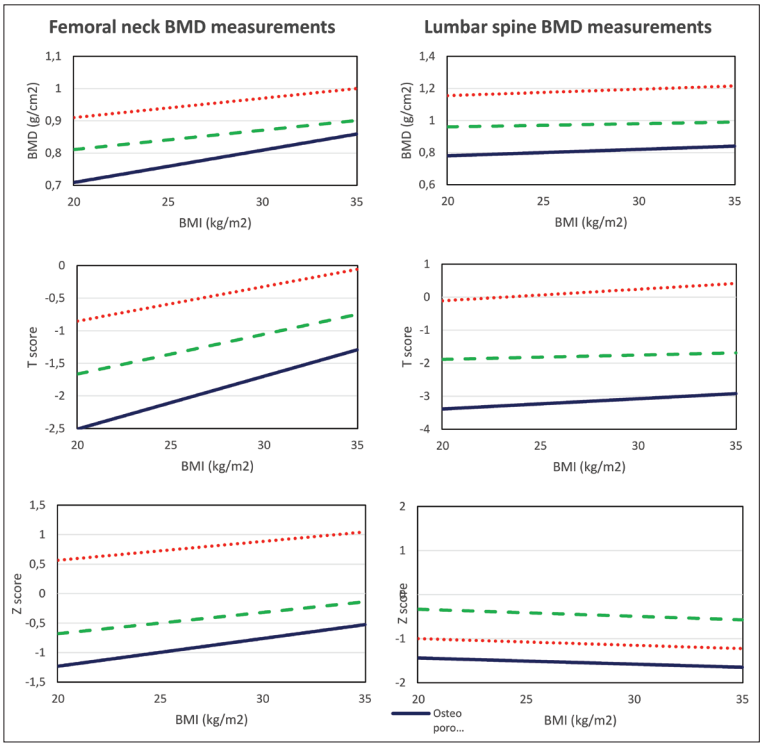

Figure 2. Trend lines of bone mineral density of femoral neck and lumbar spine for groups Osteoporosis, Osteopenia, and Normal finding in relation to body mass index in all subjects

Femoral neck BMD measurements
BMD = 0.01 × 22 + 0.509 = 0.729
T-score = 0.081 × 22 – 4.128 = -2.346
Z-score = 0.047 × 22 – 2.171 = -1.137
Lumbar spine BMD measurements
BMD = 0.004 × 22 + 0.7 = 0.788
T-score = 0.031 × 22 – 4.007 = -3.325
Z-score = -0.014 × 22 – 1.159 = -1.467

The association between BMI and both bone site measurements was determined in a similar way in the groups of normal weight, overweight and obesity, and the results obtained by linear regression are given in Table 6a, 6b, and 6c. Prediction coefficients of change in BMD dependent on BMI were the highest in the group of subjects with normal weight in regard to the other two groups, which means that the observed parameters change most rapidly with the change of BMI in that group. The graphs are given in Figure 3. The estimations can be done by means of the obtained formulae and graphs. For example, if a subject in the group with normal weight has BMI = 22 kg/m², the observed parameter values are expected to be:

Femoral neck BMD measurements
BMD = 0.021 × 22 + 0.349 = 0.811
T-score = 0.175 × 22 – 5.521 = -1.671
Z-score = 0.161 × 22– 4.299 = -0.757
Lumbar spine BMD measurements
BMD = 0.012 × 22 + 0.671 = 0.935
T-score = 0.103 × 22 – 4.253 = -1.987
Z-score = 0.099 × 22 – 2.698 = -0.52

DISCUSSION

Osteoporosis is the most common type of metabolic bone disease in developed countries. The progressive course of the disease could lead to severe complications and it represents an important social and economic problem [5]. Results from our study have shown that the majority of studied elderly subjects in Vojvodina have relatively high prevalence of bone structural deterioration due to loss of bone mass, as well as nutritional status abnormalities.

In this study, subjects were mostly women (95%), mean age 63 (56–70) years. Considering bone abnormalities, majority of the subjects had low bone mass, 37% had osteopenia, and 25% had osteoporosis. The study results are like those of other surveys in the Europe with 21% of women aged ≥ 50 years estimated to have osteoporosis [4]. Our

Table 6a. Regression equations of BMD of femoral neck and lumbar spine in relation to BMI in normal weight subjects

Formulae	Trend
Femoral neck BMD measurements	
BMD = 0.021 × BMI + 0.349	↑
T-Score = 0.175 × BMI – 5.521	↑
Z-Score = 0.161 × BMI – 4.299	↑
Lumbar spine BMD measurements	
BMD = 0.012 × BMI + 0.671	↑
T-Score = 0.103 × BMI – 4.253	↑
Z-Score = 0.099 × BMI – 2.698	↑

BMI (kg/m^2) – body mass index;
BMD (g/cm^2) – bone mineral density

Table 6b. Regression equations of BMD of femoral neck and lumbar spine in relation to BMI in overweight subjects

Formulae	Trend
Femoral neck BMD measurements	
BMD = 0.012 × BMI + 0.555	↑
T-Score = 0.097 × BMI – 3.737	↑
Z-Score = 0.057 × BMI – 1.848	↑
Lumbar spine BMD measurements	
BMD = 0.015 × BMI + 0.597	↑
T-Score = 0.118 × BMI – 4.643	↑
Z-Score = 0.067 × BMI – 1.909	↑

BMI (kg/m^2) – body mass index;
BMD (g/cm^2) – bone mineral density

Table 6c. Regression equations of BMD of femoral neck and lumbar spine in relation to BMI in obese subjects

Formulae	Trend
Femoral neck BMD measurements	
BMD = 0.008 × BMI + 0.661	↑
T-Score = 0.075 × BMI – 3.094	↑
Z-Score = 0.023 × BMI – 0.789	↑
Lumbar spine BMD measurements	
BMD = 0.011 × BMI + 0.719	↑
T-Score = 0.089 × BMI – 3.876	↑
Z-Score = 0.027 × BMI – 0.756	↑

BMI (kg/m^2) – body mass index;
BMD (g/cm^2) – bone mineral density

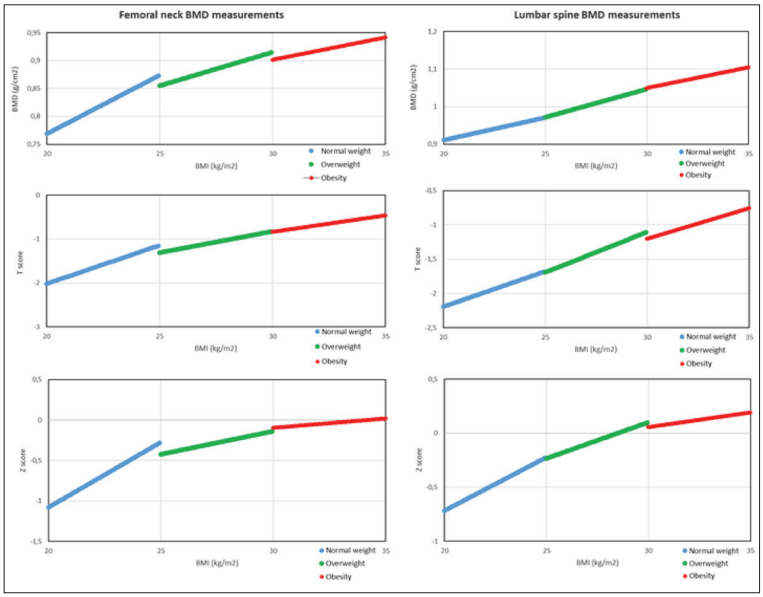

Figure 3. Trend lines of bone mineral density of femoral neck and lumbar spine for groups Normal weight, Overweight and Obesity in relation to body mass index in all subjects

observed results are in line with physiological process of age-related bone remodeling, considering that the peak of bone mass is reached in the middle of third decade in the life, and afterwards, the gradual physiological involution of bone mass follows with ageing. In addition, known effects of estrogen deficiency on cortical bone mineralization and loss of bone strength are present in the elderly population [14]. During the ageing continuum, the imbalance between bone formation and bone resorption with consequent bone mass loss could be exacerbated by several pathophysiological factors. Extrinsic pathophysiological factors, alternations in nutrition and physical inactivity, could promote the decline in bone mass and osteoporosis [15].

Regarding nutritional status in our studied subjects aged ≥ 50 years, there were 40% overweight, 31% obese, and 29% normal weight subjects. Obese subjects from our sample had considerably higher values of BMD in the region of femoral neck and lumbar spine compared to overweight and normal weight subjects. In both bone areas, we observed trends of lowering BMD as the subjects BMI decrease.

Age-related changes of the body composition and physical inactivity could also have complex effect on bone health. Despite the generally positive effects of weight on bone health in the elderly, alterations of nutritional status associated with greater fat mass may be potentially harmful [16, 17]. Some studies have suggested that being overweight and obese results in a detrimental effect on bone health. Obesity is primarily associated with a certain type of osteoporotic fractures in aging individuals, regardless of greater BMD. The data obtained by the Global Longitudinal Osteoporosis in Women study have shown that the general prevalence and incidence of fractures did not significantly differ between obese and normal weight subjects, but obese subjects were more prone to the ankle and upper leg fractures [18]. Leslie et al. [19] performed a large prospective study of 40,050 women and 3600 men aged over 50, to assess the relationship between skeletal health and estimated total body lean and fat mass. Study showed that increased lean mass is protective to skeletal health and positively associated with BMD, while excessive fat mass had no effect on BMD. In addition, higher fat mass was not independent risk factor of fractures over the study period [19]. Further, some studies reported that complications of osteoporosis usually occur in obese subjects with coexisting comorbid conditions requiring corticosteroid therapy, asthma, and emphysema [20].

Our results have demonstrated that subjects with osteoporosis were mostly within overweight nutritional category. In inactive elderly individuals, overweight is usually associated with abdominal obesity [21]. The common approach that the excessive body mass has a protective role in osteoporosis prevention has been doubted due to results

of studies on the negative effect exerted by the abdominal-visceral adipose tissue (AT) on the BMD. In addition to the AT effects to bone by mechanical burden and conversion of gonadal steroids, increased bone marrow adipogenesis, secretion of proinflammatory cytokines and adipokines could exert negative effects of adipocytes in the bone tissue [22].

Furthermore, regression equations and prediction coefficients in our study showed that subjects with osteoporosis are more prone to BMI-related BMD changes, regarding subjects with osteopenia and normal BMD. In addition, normal weight subjects compared to overweight and obese, had highest prediction coefficients of changes in BMD. These observations are in accordance with results obtained from studies by other researchers [23, 24]. In this study the higher BMI had a more significant correlation with the femoral neck BMD than with BMD of lumbar spine. The femoral neck has a higher percentage of cortical bones as compared with the vertebrae, which can have a stronger effect on a cortical than on trabecular bone [25]. Elderly population and obesity is associated with an inadequate status of micronutrients or hidden hunger, thus indirectly affecting bone status [26, 27].

Limitations of this study include its cross-sectional design and setting, thus preventing causal relationships and generalization. Further details on specific aspects of the body composition, data considering physical activity, and predictors of bone status such as diet and nutrients are also needed.

CONCLUSION

High prevalence of low bone mass coexists with overweight and obesity in the elderly age category of females in Vojvodina. Prediction equations for the calculation of BMD can be used to evaluate the effect of BMI changes on BMD in clinical settings.

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Conflict of interest: None declared.

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Трендови минералне коштане густине у односу на нутритивни статус старије популације Војводине

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САЖЕТАК

Увод/Циљ Смањена минерална коштана густина (МКГ) често се повезује са поремећајима нутритивног статуса.

Циљеви ове студије су били да се утврде преваленција смањене коштане густине и повезаност са нутритивним факторима ризика у узорку популације Војводине, и да се примене модели предикције МКГ коришћењем једноставног маркера нутритивног статуса, индекса телесне масе (ИТМ).

Методе У ретроспективној студији пресека испитивану популацију су чинили болесници који су у периоду од јануара до децембра 2017. године урадили мерење МКГ и испуњавали критеријуме за укључење у испитивање. У узорку од 1974 испитаника (1866 жена и 108 мушкараца) анализирани су нутритивни статус према антропометријским параметрима и ИТМ, као и двоенергетска рендгенска апсорпциона мерења МКГ у регији врата бутне кости и лумбалне кичме. Повезаност између БМИ и МКГ је испитивана линеарним регресионим једначинама.

Резултати Медијана година живота испитаника је била 63 (56–70 година). Нутритивни статус је код 40% испитаника био прекомерна ухрањеност, код 31% испитаника гојазност и код 29% испитаника нормална ухрањеност. Већина испитаника је имала смањену МКГ, 37% њих је имало остеопенију, а 25% остеопорозу. У посматраним регијама кости уочили смо тренд снижавања МКГ како се смањује ИТМ испитаника. Испитаници са остеопорозом склонији су променама МКГ које су зависне од ИТМ, у односу на испитанике са остеопенијом и нормалном МКГ. Нормално ухрањени, у поређењу са испитаницима других нутритивних категорија, имају најповољније коефицијенте раста МКГ према регресионим једначинама.

Закључак Висока преваленција смањене МКГ је удружена са поремећајима нутритивног статуса, прекомерном ухрањеношћу и гојазношћу код старијих жена у Војводини. Једначине предвиђања за израчунавање МКГ се могу користити за процену ефеката промене у ИТМ на МКГ у клиничким условима.

Кључне речи: минерална коштана густина; индекс телесне масе; остеопороза; остеопенија; линеарна регресија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The connection between the family's socioeconomic status and the consumption of cigarettes, alcohol and marijuana in adolescents of the Brčko District of Bosnia and Herzegovina

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SUMMARY

Introduction/Objective The objective of this paper was to determine the connection between the socioeconomic status (SES) of the respondents and cigarette smoking and the use of alcohol and marijuana. Is there a connection between the SES respondents and their gender and place of residence?

Methods A total of 4188 primary and secondary school respondents from Brčko District of Bosna and Herzegovina participated in a cross-sectional study based on the European School Survey Project on Alcohol and Other Drugs questionnaire, adapted to this research. The data was collected using the questionnaire prepared for each respondent. Data on gender, marital status, occupation, and professional qualifications of parents were used to determine a family's SES according to the Hollingshead methodology.

Results Alcohol and marijuana use are in relation to SES respondents ($p < 0.001$ or $p = 0.008$): respondents living in low-SES families use alcohol or marijuana at a lower percentage than respondents from middle-SES or high-SES families. Smoking habits are not in relation to SES respondents ($p = 0.678$). The place of residence is connected to SES respondents ($p < 0.001$): more respondents from low-SES families live in rural areas, while those from medium-SES and high-SES families predominantly live in urban areas.

Conclusion The SES of the respondents is in relation to their place of residence, alcohol and marijuana use, but it is not related to cigarette smoking.

Keywords: SES; alcohol; cigarettes; marijuana, rural; urban

INTRODUCTION

The role of the family in the upbringing of children is of great importance in adolescence, because it is a period characterized by intense physiological changes of adolescents, manifested by behavioral changes, as well as by their propensity to experiment with cigarette smoking, alcohol drinking, and drug use [1].

The impact of the family's socioeconomic status (SES) on the health habits of adolescents is the subject of ongoing research by scientists and health policy makers, as they have a major impact on their future psychophysical development [1–5]. According to research available in literature, adolescents living in low-SES families tend to be prone to risky and unhealthy behaviors, including the cigarettes, alcohol, and marijuana use [3, 6]. A frequent link is between the adolescent's family SES and cigarette smoking, which shows that adolescents from a low-SES families more often smoke cigarettes [4, 7, 8]. Drinking alcohol is in pronounced correlation with the adolescent's family SES, according to which adolescents from high-SES families drink alcohol more often [9, 10]. It is enough for an adolescent from a low-SES family to move in the company of his/her peers who come from high-SES families to start frequently overusing alcohol [9, 10].

Most researches show a strong link between adolescents' marijuana use and school dropout, taking into account that low SES also strongly influences it [4, 11, 12]. The prevailing standpoint of experts is that the SES plays an important role in the development of adolescents, and, therefore, on the adolescents' decision to start consuming some of the dangerous and illicit substances [13, 14]. Contrary to those attitudes, there are allegations that the link between the SES and cigarettes, alcohol, and illicit drug use has not been fully clarified and that a family's SES and behavior of their members cannot be verified with certainty [5, 6].

The objective of this paper is to determine the connection between the SES respondents and cigarette, alcohol, and marijuana use. Is there a connection between the SES respondents and their gender and place of residence?

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METHODS

Research area

The Brčko District of Bosnia and Herzegovina (Figure 1) covers an area of 493.3 km², where a total of 83,516 inhabitants lived in 2013, with an average population density of 169 inhabitants per square kilometer [15].

Figure 1. The area of the Brčko District of Bosnia and Herzegovina, colored red

Respondents

It was planned for this research to include all 4676 pupils from nine grades of elementary schools and all secondary school students in the Brčko District. Out of that number, 1016 were 9th grade students from 12 elementary schools and 3660 students from four high schools; 4188 students (89.6%), were surveyed.

METHODS

The research was designed as a cross-sectional study and was carried out using the European School Survey Project on Alcohol and Other Drugs questionnaire (ESPAD) adapted for this research and translated into the official languages of BiH [16]. This questionnaire was created by a group of experts from the European Monitoring Center for Drugs and Drug Addiction and the Pompidou Group, set up by the Council of Europe with the aim of comparing and analyzing the results of research on the consumption of cigarettes and substance abuse in different countries. The questionnaire contains 45 questions, which are divided into appropriate thematic units.

Demographic data for each adolescent that contained gender, place of residence, marital status of parents, professional qualification, and parents' occupation were collected using a unique questionnaire prepared for this research. Gender, marital status, occupation, and professional qualification were used to determine the family's SES according to the methodology prescribed by Hollingshead [17]. According to this methodology, the minimum number of points determined by the SES is 8, and the maximum number is 66. According to the number of points, a family's SES is classified into a low SES (8–27), medium SES (28–47), and high SES (46–66).

After the prior approval of the school directors, the examination was conducted in the school year 2011/2012 in one school class in the period from October 20 to November 28, 2011. The students themselves filled out the questionnaires with a previous explanation by a person who was specially trained to fill in the questionnaire. The person who supervised the examination provided assistance to students in completing the questionnaire in the sense of explaining the question without affecting the final answer. After completing their questionnaires, students placed them in an envelope they pasted and handed over to the person who supervised the examination. The adolescents' answer to the question, "Have you ever smoked cigarettes, drank alcohol or used marijuana?" was utilized for this article. The information on their place of residence, education of their parents, and their occupation and marital status was also used.

Prior to the research, parents of the respondents had been given informed consent where the manner and the purpose of the research were described by the ESPAD methodology. This methodology does not provide imperative approval of the Ethics Committee. This research was done in accordance with the Helsinki declaration.

Statistical analysis

The frequency of individual answers to the questions posed in the questionnaire was presented as absolute and relative frequencies. The difference between the observed and the expected frequencies was assessed with a χ^2 test. Statistical significance was confirmed at $p < 0.05$. We used statistical software IBM SPSS Statistics, Version 20.0. (IBM Corp., Armonk, NY, USA) for data processing.

Respondents volunteered to anonymously fill out the questionnaires after being informed that the results obtained will be used exclusively for scientific purposes. The respondents did not enter their first and last names when filling out the questionnaire.

RESULTS

Of the 4188 respondents who were surveyed, 4084 filled out gender data, of which 2013 (49.3%) were boys and 2071 (50.7%) were girls. A family's SES in relation to gender was calculated on a sample of 4078 respondents, and a family's SES regarding the place of residence was calculated on a sample of 4056 respondents. The SES of the respondents' families in the Brčko District in relation to their gender and place of residence is shown in Table 1.

The results showed that the greatest number of adolescents live in low-SES families, i.e. 1991 (48.8%), 1749

Table 1. The SES of the respondents' families of in the Brčko District according to their gender and place of residence

Variables	SES			
	Total n (%)	Low n (%)	Medium n (%)	High n (%)
Gender[1]				
Male	2009 (49.3)	993 (49.4)	851 (42.4)	165 (8.2)
Female	2069 (50.7)	998 (48.2)	898 (43.4)	173 (8.4)
Total n (%)	4078 (100)	1991 (48.8)	1749 (42.9)	338 (8.3)
Place of residence[2]				
Rural	2232 (55)	1355 (60.7)	799 (35.8)	78 (3.5)
Urban	1824 (45)	618 (33.9)	946 (51.9)	260 (14.3)
Total n (%)	4056 (100)	1973 (48.7)	1745 (43)	338 (8.3)

SES – socioeconomic status;
[1]p = 0.747 (SES by gender);
[2]p < 0.001 (SES by place of residence)

Table 2. The SES of the families of adolescent smokers and non-smoker

Smoking[1]	Total n (%)	SES		
		Low n (%)	Medium n (%)	High n (%)
Yes	1740 (42.8)	860 (49.4)	741 (42.6)	139 (8)
No	2325 (57.2)	1119 (48.1)	1009 (43.4)	197 (8.5)
Total n (%)	4065 (100)	1979 (48.7)	1750 (43)	336 (8.3)

SES – socioeconomic status;
[1]p = 0.678 (SES by smoking)

Table 3. The SES of the families of adolescent smokers in relation to their gender and place of residence

Variables	Total n (%)	SES		
		Low n (%)	Medium n (%)	High n (%)
Sex[1]				
Male	885 (51.4)	436 (49.3)	372 (42)	77 (8.7)
Female	836 (48.6)	411 (49.2)	364 (43.5)	62 (7.3)
Total n (%)	1721 (100)	847 (49.2)	737 (42.8)	138 (8)
Place of residence[2]				
Rural	999 (58.1)	592 (59.3)	374 (37.4)	33 (3.3)
Urban	717 (41.9)	251 (35)	361 (50.4)	105 (14.6)
Total n (%)	1716 (100)	843 (49.1)	735 (42.8)	138 (8)

SES – socioeconomic status;
[1]p = 0.575 (SES by gender);
[2]p < 0.001 (SES by place of residence)

adolescents (42.9%) live in medium-SES families, and the least number of adolescents, 338 (8.3%), live in high-SES families. The difference in the distribution of male adolescents and female adolescents in relation to their family's SES categoriy is not statistically significant, which means that we cannot confirm the connection between the gender of the respondents and their families' SES (p = 0.747). The adolescent's residence is statistically significantly related to the SES of the respondent's family (p < 0.001) and the results show that low-SES respondents most often live in the rural areas (68.7%, 1355/1973) compared to respondents from medium- and high-SES families, who most often live in the urban (54.2%, 946/1745, p < 0.001 and 76.9%, 260/338, p < 0.001). The SES of adolescent smokers' and non-smokers' families is shown in Table 2.

The answer to the question about smoking was given by 4065 respondents, taking into account that 57.2% respondents stated that they never smoked cigarettes, while 42.8% answered that they did smoke. Comparing the results of the SES of adolescent smokers' and non-smokers' families, we established that the SES of adolescents' families is not related to their smoking habits (p = 0.678). The SES of adolescent smokers' families in relation to their gender and place of residence are shown in Table 3.

Table 4. The SES of the families of adolescents who drink alcohol in relation to those who do not drink

Alcohol drinking[1]	Total n (%)	SES		
		Low n (%)	Medium n (%)	High n (%)
Yes	2353 (59)	1071 (45.5)	1072 (45.6)	210 (8.9)
No	1637 (41)	869 (53.1)	645 (39.4)	123 (7.5)
Total n (%)	3990 (100)	1940 (48.6)	1717 (43)	333 (8.3)

SES – socioeconomic status;
[1]p < 0.001 (SES by alcohol drinking)

Table 5. The SES of the families of adolescents who consume alcohol in relation to their gender and place of residence

Variables	Total n (%)	SES		
		Low, n (%)	Medium, n (%)	High, n (%)
Sex[1]				
Male	1240 (53.3)	565 (45.6)	565 (45.6)	110 (8.9)
Female	1086 (46.7)	491 (45.2)	496 (45.7)	99 (9.1)
Total n (%)	2326 (100)	1056 (45.4)	1061 (45.6)	209 (9)
Place of residence[2]				
Rural	1275 (55)	723 (56.7)	500 (39.2)	52 (4.1)
Urban	1040 (45)	323 (31.1)	560 (53.8)	157 (15.1)
Total n (%)	2315 (100)	1046 (45.2)	1060 (45.8)	209 (9)

SES – socioeconomic status;
[1]p = 0.972 (SES by sex);
[2]p < 0.001 (SES by place of residence)

Analyzing the influence of the SES of male and female adolescent respondents' families on smoking habits, we found that the SES is not related to the gender of the smokers (p = 0.575). The smokers' families' SES is statistically significantly related to the place of residence (p < 0.001). The results obtained show that smokers from low-SES families are more likely to live in rural areas (70.2%, 592/843) than their peers from medium-SES families (50.9%, 374/735, p < 0.001) or their peers from high-SES families that most often live in urban areas (76.1%, 105/138, p < 0.001). The correlation between the SES of families of adolescents who drank alcohol in relation to those who did not is shown in Table 4.

The answer to the question about alcohol consumption was given by 3990 respondents, according to which 59% of the respondents drank and 41% did not. There is a statistically significant connection between the familyies SES and alcohol consumption (p < 0.001). Lower percentage of respondents coming from low-SES families drank alcohol (55.2%, 1071/1940) than of respondents from medium-SES (62.4%, 1072/1717, p < 0.001) and high-SES families (63.1%, 210/333, p = 0.009). The difference in the frequency of alcohol consumption between medium-SES family respondents and high-SES family respondents was not statistically significant (62.4% and 63.1%, respectively, p = 0.877). The SES of families of adolescents who consume alcohol in relation to their gender and place of residence are shown in Table 5.

Comparing the SES of alcohol-drinking respondents' families and the respondents' gender, we found that male

Table 6. The SES of the families of adolescents who use marijuana in relation to those who do not

Marijuana use[1]	Total n (%)	SES		
		Low n (%)	Medium n (%)	High n (%)
Yes	267 (6.6)	112 (41.9)	121 (45.3)	34 (12.7)
No	3791 (93.4)	1862 (49.1)	1626 (42.9)	303 (8)
Total n (%)	4058 (100)	1974 (48.6)	1747 (43.1)	337 (8.3)

SES – socioeconomic status;
[1]p = 0.008 (SES by marijuana smoking)

Table 7. The SES of the families of adolescents who use marijuana in relation to their gender and place of residence

Variables	Total n (%)	SES		
		Low n (%)	Medium n (%)	High n (%)
Gender[1]				
Male	177 (67.6)	77 (43.5)	77 (43.5)	23 (13)
Female	85 (32.4)	32 (37.6)	42 (49.4)	11(12.9)
Total n (%)	262 (100)	109 (41.6)	119 (45.4)	34 (13)
Place of residence[2]				
Rural	122 (46.6)	65 (53.3)	50 (41)	7 (5.7)
Urban	140 (53.4)	44 (31.4)	70 (50)	26 (18.6)
Total n (%)	262 (100)	109 (41.6)	120 (45.8)	33 (12.6)

SES – socioeconomic status;
[1]p = 0.633 (SES by gender);
[2]p < 0.001 (SES by place of residence)

and female adolescents drink alcohol regardless of their families' SES (p = 0.972). Analyzing the SES of alcohol-drinking respondents' families and the respondents' place of residence, we found that the SES and the place of residence were statistically significantly related (p < 0.001). The results show that alcohol-drinking respondents from low-SES families more often live in rural areas (69.1%, 723/1046) compared to respondents who come from the medium-SES and high-SES families, who live in urban areas more often (52.8%, 560/1060, p < 0.001, or 75.1%, 157/209, p < 0.001, respectively). The SES of families of adolescents who use marijuana in relation to those who do not smoke is shown in Table 6.

The question whether they smoke marijuana or not was answered by 4058 respondents, of which the vast majority never smoked marijuana 3791 (93.4%), while only 267 (6.6%) said they did. The results show that SES of the respondents' families is statistically significantly related to marijuana smoking (p = 0.008). The majority of those who smoke marijuana live in medium-SES families (45.3%), 41.9% live in low-SES families, and 12.7% live in high-SES families; 5.7% (112/1974) of respondents coming from low-SES families have smoked marijuana, which is a smaller percentage compared to marijuana-smoking respondents coming from middle-SES (6.9%, 121/1747) or high-SES families (10.2%, 34/337, p = 0.028). The SES of families of adolescents who consume marijuana in relation to the respondents' gender and place of residence is shown in Table 7.

Analyzing the results of marijuana use, we found that, regardless of the fact that men use marijuana more often (p < 0.001), the SES of families of those who consume marijuana is not related to gender (p = 0.633). The results of marijuana use, taking into account the SES of the respondents' families in relation to the respondents' place of residence, show that the SES is statistically significantly related to the place of residence (p < 0.001). We found that respondents who use marijuana and are coming from low-SES families most often live in the rural areas (59.6%, 65/109); in contrast, their peers who come from medium- and high-SES families mostly live in urban areas (58.3%, 70/120, p = 0.010, and 78.8%, 26/33, p < 0.001, respectively).

DISCUSSION

The results obtained by this research show the connection between families' SES and alcohol and marijuana use, but not the connection between families' SES and smoking cigarettes.

Connection between SES and cigarette smoking in developed countries according to Doku et al. [18] is exclusively related to low SES, while in developing countries there is not enough relevant research to indicate the connection of SES and cigarette smoking [8, 13]. By reviewing the available literature, we found no results that would coincide with the results of our research. The reason for this huge difference between our results and those from the literature regarding the connection between cigarette smoking and SES can be explained by different methods of determining the SES. In our research, for the assessment of a family's SES, we used the Hollingshead methodology, which includes the factor of education, gender, occupation, and marital status of parents, while other authors in the literature, in addition to these, also used psychosocial factors such as psychological factors, cultural factors, peer influence on smoking habits, parents' relationship, smoking cigarettes tolerance in the family, and the attitude of society towards smoking cigarettes [17, 19, 20].

Cigarette smoking is often a symbol of maturity and growth, so the tolerance to this phenomenon is very high, and as a result, we have a great deal of cigarette availability at every step. Due to the attitude of society towards cigarette smoking, there is no social awareness of the harmful effects of smoking on the population's health and there is no social condemnation of such behavior [20]. All of the above can be an important cause of the lack of connection between smoking and SES, which should be scientifically determined by a new research.

Our results regarding the connection of alcohol and marijuana use with high SES corresponds with the results of most authors [9, 11, 21]. The reason alcohol drinking and marijuana use are associated with adolescents from richer families lies in the fact that these substances are expensive and require more money to be purchased, which can only be afforded by adolescents from high-SES families [6, 22]. Alcohol and marijuana cannot be consumed as widely as compared to cigarettes; instead, the users have to go to cafes or night clubs, for which it is necessary to have more money. Access of adolescents to these places is a sign of insufficient parents' care for their children's leisure activities in social circles prone to risky behavior.

Investigating the connection between risky behavior and consumption of illicit substances in relation to the place of residence and SES, we identified a difference in behavior in adolescents from rural areas compared to those from the urban ones. Namely, adolescents from low-SES families who smoke, drink and consume marijuana live in rural areas at a higher percentage, while respondents from medium- and high-SES families who consume these substances predominantly live in urban areas. Our results on the connection of the use of psychoactive substances with the SES of families living in rural areas are consistent with the results of the authors from the United States, according to which respondents from low-SES families living in rural areas smoke, drink, and use marijuana to a much larger extent than their peers living in medium- and high-SES families [23]. The reasons for this behavior of the rural population have not been clarified, but the literature published in the United States offers reasons that can influence this connection of SES and risk behavior [23]. The reasons explaining this phenomenon in the rural population, which we can apply to our research, is high unemployment of the young population, while those who are employed work difficult low-paid jobs, causing the majority of the population to live in poverty. Due to the isolated nature of the rural areas there is poorly organized education, poor health care, as well as conservative living standards, with a slow change in life habits [23, 24, 25]. The traditional brandy production in the rural areas District of Brčko allows adolescents to drink alcohol without sanctions. These are the reasons why residents of the rural areas find it difficult to stop smoking cigarettes and drinking alcohol, and a large percentage of those who do try to stop give up their intention. Poor success in achieving a complete abstinence of smoking and drinking alcohol lies in the fact that cigarettes and alcohol are widely available at home, as they are part of everyday rural cultural rituals. Marijuana use among the rural population is a "logical sequence" of searching for a stronger psychoactive substance by which the adolescent will "kill the boredom" [25]. The reasons why adolescents from rural areas coming from medium- and high-SES families do not consume these substances at a significant percentage are not explained, which should be the subject of further research.

However, good socioeconomic opportunities are a risk factor, and will cause adolescents from urban areas to consume cigarettes, alcohol, and marijuana. The model elaborated by Hollingshead is not sufficient to give an adequate response to this behavior of adolescents from urban areas, but it is also necessary to include consideration of other factors [8]. This means that in addition to the basic parameters, it is necessary to include parameters of a sociological nature, such as a busy way of life, a lack of free time for parents, a constant search for additional income to meet the needs of urban life [25]. A busy way of life and a great deal of demands from people to spend most of their time at work cause adolescents to remain without parental control and care. Because of the alienation of parents and children, the latter lose self-confidence, they withdraw into themselves, and communicate with their parents only when they need money. These are the possible reasons why adolescents from urban areas who come from middle- and high-SES families smoke more often than those who come from low-SES families.

It is characteristic for the urban environment that adolescents who come from low-SES families and have friends who come from high-SES families consume cigarettes, alcohol, and marijuana as much as their friends from high-SES families do or are involved in other illicit activities with the friends.

The disadvantage of this research is that the data on which SES calculation was made was obtained by a statement from adolescents without verifying its truthfulness. One of the limiting factors is that there are no pre-defined rules for the rural/urban definition, but the respondents themselves decided whether the place they lived in was rural or urban. In future research it is necessary to expand the parameters for determining the SES, i.e. in addition to the parameters used in the Hollingshead methodology, psychosocial parameters should also be included.

CONCLUSION

The SES of adolescents' families in the Brčko District is connected with the consumption of alcohol and marijuana but is not connected with cigarette smoking. The gender of the respondents and cigarette, alcohol, and marijuana use are not related to the SES of the respondents' families. The place of residence and cigarette, alcohol, and marijuana use are connected to the SES of the respondents' families – respondents from rural environments who consume these substances more frequently are from low-SES families; urban adolescents who consume these substances are more frequently from middle- or high-SES families.

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Повезаност социоекономског статуса породице и конзумације дувана, алкохола и марихуане код адолесцената Брчко Дистрикта Босне и Херцеговине

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САЖЕТАК

Увод/Циљ Циљ рада је био утврдити повезаност социоекономског статуса (СЕС) испитаника и пушења цигарета дувана, пијења алкохола и конзумације марихуане, као и утврдити да ли постоји повезаност СЕС испитаника и његовог пола и места становања.

Методе У пресечној студији, заснованој на упитнику Европског школског истраживања о употреби алкохола и других дрога прилагођеном овом истраживању, учествовало је 4188 испитаника основних и средњих школа. Подаци су прикупљени помоћу упитника припремљених за сваког испитаника. Подаци о полу, брачном статусу, занимању и стручној спреми родитеља су коришћени за одређивање СЕС по Холингсхедовој методологији.

Резултати Пијење алкохола и конзумација марихуане је у релацији са СЕС испитаника ($p < 0,001$, односно $p = 0,008$): испитаници који живе у породицама са ниским СЕС конзумирају алкохол односно марихуану у мањем проценту него испитаници из породица са средњим или високим СЕС. Пушачке навике нису у релацији са СЕС испитаника ($p = 0,678$). Место становања је повезано са СЕС испитаника који пуше цигарете дувана, пију алкохол и конзумирају марихуану ($p < 0,001$): више испитаника из породица са ниским СЕС живи на селу, док испитаници са средњим и високим СЕС претежно живе у граду ($p < 0,001$).

Закључак СЕС испитаника је у релацији са местом становања, пијењем алкохола и конзумацијом марихуане, али није у релацији са пушењем цигарета дувана.

Кључне речи: социоекономски статус; алкохол; цигарете; марихуана; село; град

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PRELIMINARY AND SHORT COMMUNICATION / ПРЕТХОДНО И КРАТКО САОПШТЕЊЕ

ECHOS survey on echocardiography in Serbia during the COVID-19 pandemic

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SUMMARY

Introduction/Objective The purpose of the current Echocardiographic Society of Serbia (ECHOS) survey was to assess echocardiography practice in Serbia during the Coronavirus disease 2019 (COVID-19) pandemic.

Methods An online survey consisting of 12 questions about the usa of echocardiography, the availability of portable ultrasound devices and personal protective equipment (PPE) was sent to all ECHOS members.

Results Overall, 126 ECHOS members (43%) answered the survey. One-third of respondents (36%) were physicians from specialized COVID-19 centers. During the pandemic, indications for echocardiographic examination were restricted in both COVID-19 and non-COVID-19 centers. In COVID-19 centers, 41% of respondents performed lung ultrasound to each patient versus 26% in non-COVID-19 centers. Transesophageal echocardiography was not performed in suspected or confirmed COVID-19 cases in any center. Portable ultrasound devices were available to 66% of respondents from COVID-19 versus 44% of respondents from non-COVID-19 centers ($p = 0.018$). The respondents reported regular use of PPE, regardless of the patient's COVID-19 status and found their personal knowledge about protective measures and use of PPE satisfactory.

Conclusion During the COVID-19 pandemic in Serbia, indications for echocardiography were restricted to clinical scenarios in which the results of examination were expected to alter patient management. In both COVID-19 and non-COVID-19 centers, the use of PPE was in line with national and international recommendations. A wider availability of portable ultrasound devices and application of lung ultrasound could improve patient management in similar situations in the future.

Keywords: echocardiography; survey; COVID-19; Serbia

INTRODUCTION

The novel coronavirus 2019 or severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) that results in COVID-19 has reached pandemic level in March 2020 [1]. During the pandemic in Serbia, several hospitals were turned into specialized COVID-19 centers and have been providing care only to confirmed COVID-19 patients, while the remaining centers continued providing health services, including echocardiography, to presumably COVID-19-negative patients.

Apart from causing pneumonia, SARS-CoV-2 may also affect the cardiovascular system, resulting in poorer prognosis [2]. Consequently, in COVID-19 centers, a clinical suspicion of cardiovascular involvement in patients with severe COVID-19 disease is likely to trigger cardiac diagnostic work-up that typically includes echocardiography, as it was the case with other respiratory viruses in the past [3].

Cardiologists and other health care personnel performing echocardiography at both COVID and non-COVID-19 centers were at risk of getting infected, and the availability of personal protective equipment (PPE) and the training on its proper use were of paramount importance to minimize the risk of infection [4, 5, 6]. The aim of the current Echocardiographic Society of Serbia (ECHOS) survey was to assess the use of echocardiography and the availability of PPE during the pandemic in Serbia, in both COVID and non-COVID-19 centers.

METHODS

The survey was conducted from April 22 to April 30, 2020. All ECHOS members (293 at

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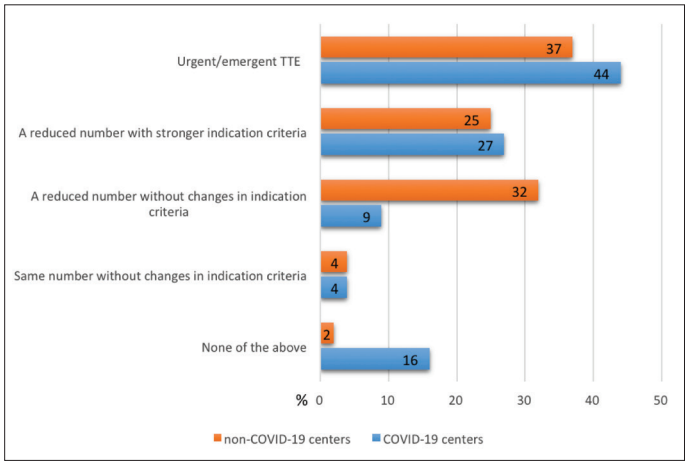

Figure 1. Transthoracic echocardiographic examinations in Serbia during the COVID-19 pandemic

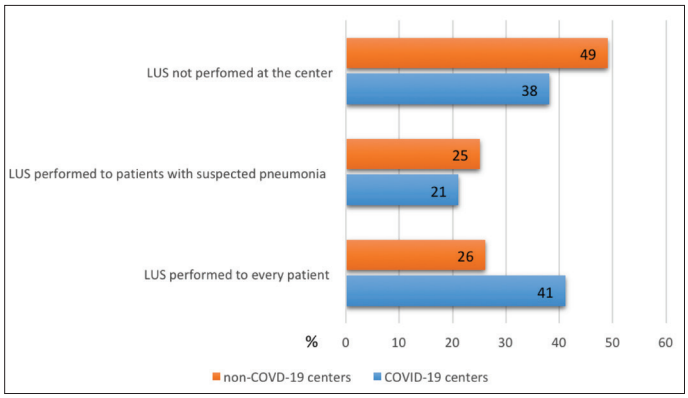

Figure 2. Lung ultrasound during the COVID-19 pandemic

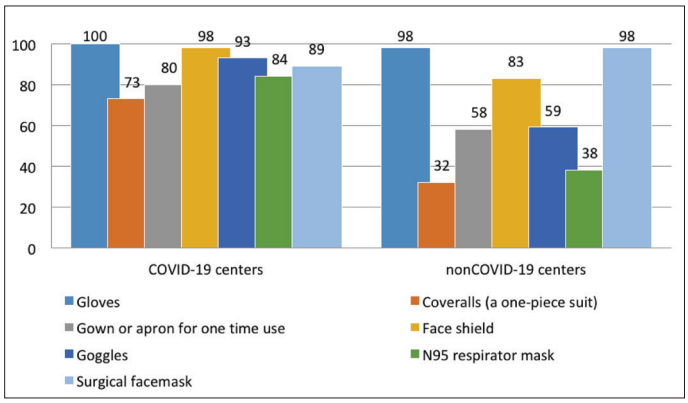

Figure 3. Summary of available personal protection equipment

the time of the survey) were invited to anonymously complete an online questionnaire consisting of 12 questions about the use of echocardiography during the COVID-19 pandemic, the availability of portable echocardiographic devices, PPE, and education regarding the use of PPE. The data was collected and analyzed using commercially available software (PASW Statistics 18, version 18, SPSS, Inc., Chicago, IL, USA). Categorical data was summarized by proportions and compared using a Fisher's exact test. The test was two-tailed, and a p-value < 0.05 was considered significant.

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.

RESULTS

Overall, 126 ECHOS members (43%) from all regions of Serbia, answered the survey. Approximately one-third of respondents (36%) were from the COVID-19 centers. After the outbreak of the pandemic in Serbia, indications for echocardiographic examinations were restricted in COVID-19 as well as in non-COVID-19 centers, as shown in Figure 1.

Transesophageal echocardiography (TEE) was not performed in suspected or confirmed cases of COVID-19 at any center – in patients in whom COVID-19 was not suspected, TEE was performed in 2% in COVID-19 and in 4% in non-COVID-19 centers. In COVID-19 centers, lung ultrasound (LUS) was performed in every patient by 41% respondents, only when pneumonia was suspected by 21% respondents, while 38% of respondents did not perform LUS at all. In non-COVID-19 centers these percentages were 26%, 25%, and 49%, respectively (Figure 2).

Small, portable echocardiographic machines or hand-held ultrasound devices were available to 52% of respondents (66% from COVID-19 centers *vs.* 44% from non-COVID-19 centers, p = 0.018). Available PPE in both types of centers is summarized in Figure 3. N95 respirator mask was more frequently available at COVID-19 compared to non-COVID-19 centers (84% *vs.* 38%, p < 0.00001). The protocols of cleaning and disinfection of echocardiographic machines and probes were affected by the pandemic in both COVID and non-COVID-19 centers. A thorough disinfection of echocardiographic equipment regardless of COVID-19 status was performed in 35% of COVID-19 and 46% of non-COVID-19 centers (p = 0.25). Respondents from both types of centers found their personal knowledge about protective measures and the use of PPE satisfactory but the majority stated that they could benefit from additional education, as shown in Table 1.

Table 1. Summary of personal educational preferences regarding personal protection equipment during echocardiographic examinations

Personal educational stand	COVID-19 centers	Non-COVID-19 centers
My knowledge is complete	29%	20%
My knowledge is satisfactory but I need further education	60%	69%
My knowledge is insufficient	11%	11%

DISCUSSION

This survey was carried out by ECHOS around the peak of the pandemic in Serbia. At the time of the survey, there were a few national and global recommendations on cardiac imaging during the pandemic based on expert opinion, national guidelines, and available evidence [4–12].

After the outbreak of the corona virus pandemic in Serbia, indications for echocardiographic examinations were restricted in COVID-19 and non-COVID-19 centers alike. This is in accordance with the cardiac imaging societies' recommendations, which advised that only essential echocardiographic studies should be performed, focusing solely on the acquisition of images needed to answer the clinical question that is likely to change the management strategy [5, 12]. The avoidance of performing TTE, and particularly TEE in patients in which the test results are unlikely to change the management strategy is recommended [5, 12]. The TEE increases the risk of spread of COVID-19 due to the exposure of health care personnel to aerosolization of large viral load [6, 12]. Therefore, it should not be performed if an alternative imaging modality is available [12]. In line with this, TEE was not performed in suspected or confirmed cases of COVID-19 at any center, but it was still performed when needed in selected COVID-19 negative cases.

Small, laptop-sized, portable machines and hand-held ultrasound devices were at disposal to 52% of respondents. This data, as the measure of quality of echocardiography practice in critically ill patients, at the time being, is not at the satisfactory level in Serbia. The "point of care" ultrasound, focus cardiac ultrasound, and critical care echocardiography could be preferred bedside imaging options and effective alternatives for initial assessment and treatment guidance of cardiovascular complications of COVID19 infection [5, 8, 12].

In COVID-19 centers, LUS has been done by 62% of respondents, while 38% did not perform LUS at all, suggesting that the usage of the LUS is not at a desirable level in Serbia. The current clinical evidence suggests that LUS may be useful for the diagnosis and prognosis of COVID-19 pneumonia [8]. However, limited evidence exists for the use of LUS to differentiate acute respiratory distress syndrome from heart failure [8].

During echocardiographic examinations, the N95 respirator mask was more often available at COVID-19 than non-COVID-19 centers. Worldwide, the level of PPE depended on the risk level of the patient with regard to COVID-19 status [13].

The Institute of Public Health of Serbia issued a series of recommendations for health care personnel providing care to suspected or confirmed COVID-19 patients as well as non-COVID-19 patients [14]. Over time, these national recommendations were updated according to the new data. Thus, in COVID-19 centers, health care personnel should be protected by wearing a N95 respirator mask, coveralls or impermeable coat with cap, gloves, and goggles, or a face shield [14, 15]. In non-COVID-19 centers, PPE consisting of surgical facemask, a single use gown, gloves, and a face shield was considered sufficient [14, 15]. In our survey, a lower degree of protection was present in non-COVID-19 centers, which was in accordance with these recommendations.

It should also be underlined that the risk of infection remains in the examination rooms and therefore the equipment should be frequently sanitized [12, 16]. However, the cleaning and disinfection of echocardiographic machines and probes were performed slightly less frequently at COVID centers than in non-COVID-19 centers, which was probably due to the impression that the risk of cross infection at COVID-19 centers was lower. Local standards vary, but echocardiogram machines and probes should be thoroughly cleaned, ideally in the patient's room and again in the hallway [12, 16]. Respondents from both types of centers found their personal knowledge of protective measures and the use of PPE satisfactory but needed additional education.

Although less than 50% of ECHOS members participated in the survey, this response rate is comparable to our previous and similar international surveys [17, 18, 19]. In addition, our survey was conducted several weeks after the outbreak of the epidemic is Serbia – it is, therefore, possible that the initial shortages of PPE, which was a global phenomenon occurring even in more performant health care systems, were not captured by the current survey. It would be worthwhile to repeat the current survey at the end of the pandemic and to include a larger number of participants.

CONCLUSION

This survey revealed that the usage of echocardiography during COVID-19 pandemic in Serbia was in line with international standards. In both COVID-19 and non-COVID-19 centers, the use of PPE was in line with national recommendations. A wider availability of portable ultrasound devices and usage of LUS could facilitate patient management in similar situations in the future.

ACKNOWLEDGMENT

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Conflict of interest: None declared.

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Анализа спроведене анкете EXOC у Србији током пандемије *COVID*-19

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САЖЕТАК

Увод/Циљ Национална анкета Ехокардиографског удружења Србије (EXOC) спроведена је са циљем да се процени примена ехокардиографије у Србији током пандемије вируса корона 2019.

Методе Анкета која се састојала од 12 питања о примени ехокардиографије, доступности преносивих ехокардиографских уређаја и личне заштитне опреме (ЛЗО) послата је електронским путем свим члановима EXOC-а.

Резултати Укупно је 126 чланова EXOC-а (43%) одговорило на анкету. Око трећина испитаника (36%) били су лекари из специјализованих центара COVID-19. Током пандемије, индикације за ехокардиографски преглед биле су редуковане и у центрима COVID-19 и у центрима не-*COVID*-19. У центрима *COVID*-19 41% испитаника је ултразвук плућа радило сваком болеснику, док је тај проценат у центрима не-*COVID*-19 износио 26%. Трансезофагеална ехокардиографија није рађена сумњивим или потврђеним случајевима заразе вирусом корона ни у једном центру. Доступност преносивих ултразвучних апарата пријавило је 66% испитаника у центрима COVID-19 наспрам 44% испитаника у центрима не-*COVID*-19 ($p = 0,018$). Испитаници су пријавили редовну употребу ЛЗО, без обзира на статус болесника у вези са вирусом корона и сматрали су да је њихово знање о мерама заштите и употреби ЛЗО задовољавајуће.

Закључак Током пандемије *COVID*-19 у Србији, индикације за ехокардиографију биле су редуковане и ограничене на случајеве где се очекивало да ће резултати прегледа утицати на ток лечења болесника. Како у центрима *COVID*-19 тако и у центрима не-*COVID*-19 употреба ЛЗО била је у складу са националним и међународним препорукама. Шира доступност преносивих и ручних ехокардиографских апарата и употреба ултразвука плућа могу бити од великог значаја за успешно превазилажење сличних ситуација у будућности.

Кључне речи: ехокардиографија; анкета; COVID-19; Србија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Recurrent aphthous stomatitis as the only clinical sign of celiac disease in an obese adolescent – case report and literature review

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SUMMARY

Introduction Recurrent aphthous stomatitis (RAS) is a relatively common oral mucosal lesion of unclear etiology. It occurs in otherwise healthy people, but also in various infectious and non-infectious diseases, including celiac disease (CD). We present an obese adolescent with RAS as the only clinical sign of CD.

Case outline An adolescent aged 15 2/12 years come with very pronounced RAS in previous five months. He had no other difficulties. The patient was obese from the age of 12. Other data were without peculiarities. On admission he was 165 cm tall (P25), obese (BMI 27 kg/m^2), in the final stage of puberty, with stretch marks in the distal areas of the abdomen, thighs and gluteus and very pronounced pain-sensitive aphthae in the buccal and labial mucosa accompanied by swelling of the lips and perioral region. Except for lower serum iron levels (8 μmol/l), routine laboratory blood tests were within the reference range. The serological test for CD was positive (antibodies to tissue transglutaminase IgA 78.5 U/ml, anti-endomysial antibodies IgA positive). Endoscopy revealed reflux esophagitis, without any other pathological findings. Stereomicroscopic and pathohistological analysis of the duodenal mucosa samples showed mild destructive enteropathy (Marsh IIIa). Pathohistological examination of the gastric mucosa revealed grade I-II lymphocytic gastritis. The urease test for Helicobacter pylori was negative. A gluten-free diet resulted in the withdrawal of aphthous stomatitis and no recurrence later.

Conclusion Within the differential diagnostic analysis of the RAS causes, CD should also be considered. Additionally, obesity does not exclude the presence of CD.

Keywords: recurrent aphthous stomatitis; celiac disease; obesity

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is a relatively common oral mucosal lesion [1–5]. It occurs in children as well as in adults and the elderly [1, 2]. The most common age of onset is the second and third decade of life, becoming less common with advancing age [1]. It is slightly more common in females than in males [3]. In the United States, it is found in 0.89–1.64% of the general population, and in some countries even more often [1]. The cause of RAS is not clear [1, 3]. It is seen in otherwise healthy people, but also in various infectious and non-infectious diseases, including celiac disease (CD) [1, 6–15]. In addition, RAS is associated with genetic predisposition, iron and vitamin B12 deficiency, local mechanical injuries, stress, and hormonal imbalance [16, 17, 18]. We present an obese adolescent with RAS as the only clinical manifestation that indicated CD.

CASE REPORT

A boy aged 15 2/12 years referred for examination and treatment due to very pronounced RAS in previous five months (Figures 1 and 2). He had no other difficulties. Oral aphthous eruptions were not associated with infection, local trauma, stress, or any other factor. Personal and family history in terms of allergic diathesis was negative. Standard local therapeutic measures did not give the desired effect. From the age of 12, he began to gain weight. Also, he complained of occasional episodes of postprandial heartburn. Other data from personal and family history without peculiarities. On admission, the patient was 165 cm tall (P25), obese (BMI 27 kg/m^2), in the final stage of puberty, with stretch marks in the distal areas of the abdomen, thighs, and gluteus, and very pronounced pain-sensitive aphthae in the buccal and labial mucosa accompanied by swelling of the lips and perioral region. Erythrocyte sedimentation rate, C-reactive protein, blood count, bilirubin, serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, creatinine, lipid profile, creatinine, and other laboratory analyses, except lower serum iron levels (8 μmol/l), were within their reference ranges. IgA antibodies to tissue transglutaminase (AtTG) were elevated (78.5 U/ml) and anti-endomysial antibodies IgA positive.

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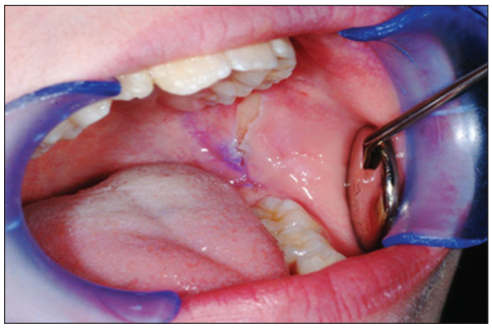

Figure 1. Deep aphthous change treated with gentian violet

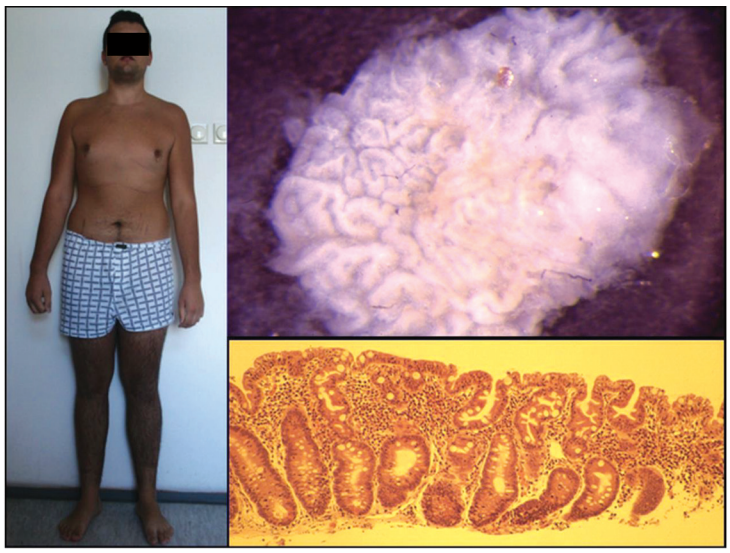

Figure 2. Our patient with steromicroscopic (top) and pathohistological (bottom) appearance of the small intestinal mucosa ▶

Esophagogastroduodenoscopy revealed reflux esophagitis, without any other pathological findings. Stereomicroscopic and pathohistological analysis of the duodenal mucosal samples showed mild destructive enteropathy (Marsh IIIa) (Figure 2). Pathohistological examination of the gastric mucosa revealed grade I-II lymphocytic gastritis. The urease test for *Helicobacter pylori* was negative. A gluten-free diet resulted in the withdrawal of aphthous stomatitis and no recurrence later. In addition, he received instructions related to the correction of diet and the inclusion of appropriate physical activity in order to normalize body weight. At the control examination after three months, normal values of serum iron and ferritin were registered, which was also the case with AtTG after six months. The degree of obesity, however, remained unchanged.

DISCUSSION

CD is a systemic autoimmune disease induced by gluten and related prolamins of wheat, rye, and barley [19]. It occurs as a result of a polygenic predisposition in a set of *HLA DQ2* and *HLA DQ8* genes that play the central role [19]. Although present in all population groups, it is most common in the white population (~1%) [20]. The basis of the disease and the key finding in its diagnostics is symptomatic or asymptomatic gluten-sensitive enteropathy, a nonspecific inflammation of the small intestinal mucosa that disappears on a gluten-free diet [19]. In addition to enteropathy, the disease is also characterized by a full spectrum of extraintestinal manifestations, including RAS [19, 21–25]. What makes our patient unusual is the fact that RAS was the only sign to indicate CD. In addition, he was obese, which is also atypical for CDs [19]. According to the data obtained from the father and the boy himself, the eruptions to the standard local therapy-resistant five-month RAS were not related to intercurrent infections, local mechanical injuries, and stressful situations [10, 13, 14, 16]. Also, he did not show a tendency to allergic manifestations [13]. Having in mind this fact, regardless of the boy's obesity, serological screening on CD was performed. Since AtTG IgA were elevated (78.5 U/ml) and anti-endomysial antibodies IgA positive, in accordance with the criteria of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition for the diagnosis of CD, enterobiopsy was performed [19]. The morphological appearance of the small intestinal mucosa, both stereomicroscopically and pathohistologically, was consistent with the diagnosis of CD. A gluten-free diet resulted in the complete withdrawal of RAS, as found by other authors [26, 27]. In the further course with a strict gluten-free diet, the patient did not have recurrences of aphthous stomatitis. At the control examination after three months, normal values of serum iron and ferritin were registered, which was also the case with AtTG after six months.

In conclusion, the combination of RAS and obesity in clinical presentation with CD is extremely rare. Hence, in our experience, CD should be kept in mind, even in obese patients, as a cause of RAS.

Ethical standards: Written consent for the publication of this article was obtained from the patient's parents.

Conflict of interest: None declared.

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Рекурентни афтозни стоматитис као једини клинички знак целијачне болести код обезног адолесцента – приказ болесника и преглед литературе

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САЖЕТАК

Увод Рекурентни афтозни стоматитис (РАС) представља релативно честу оралну мукозну лезију нејасне етиологије. Јавља се код иначе здравих особа, али и у склопу различитих инфективних и неинфективних обољења, укључујући и целијачну болест (ЦБ). Приказујемо обезног адолесцента са РАС као јединим клиничким знаком ЦБ.

Приказ болесника Адолесцент узраста 15 2/12 година долази са веома израженим РАС последњих пет месеци. Друге сметње није имао. Гојазан је од 12. године. Остали подаци били су без особености. На пријему је висок 165 *cm* (П25), гојазан (БМИ 27 *kg/m*^{2}), у завршној фази пубертета, са стријама у подручју дисталних подручја абдомена, бутина и глутеуса и веома израженим болно осетљивим афтама у подручју букалне и лабијалне слузокоже праћеним отоком усана и периоралног региона. Сем нижег нивоа серумског гвожђа (8 *μmol/l*), рутинске лабораторијске анализе крви су биле у референтном оквиру. Серолошки тест на ЦБ је био позитиван (антитела на ткивну трансглутаминазу *IgA* 78,5 *U/ml*, антиендомизијумска антитела *IgA* класе позитивна). Ендоскопијом је констатован рефлукс езофагитис, без другог патолошког налаза. Стереомикроскопска и патохистолошка анализа узорака слузокоже дуоденума су показале лакшу деструктивну ентеропатију (*Marsh IIIa*). Патохистолошким прегледом слузокоже желуца установљен је лимфоцитни гастритис I-II степена. Уреазни тест на *Helicobacter pylori* је био негативан. Дијета без глутена резултирала је повлачењу афтозног стоматитиса и није било рецидива у каснијем току.

Закључак У оквиру дифенцијално-дијагностикоr размашрања узрока РАС треба узети у обзир и ЦБ. Додатно, гојазност не искључује присуство ЦБ.

Кључне речи: рекурентни афтозни стоматитис; целијачна болест; обезитет

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Cementoblastoma – an unusual radiographic presentation

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SUMMARY
Introduction Cementoblastoma is an uncommon tumor of the jaws that originates from odontogenic ectomesenchyme, characterized by proliferating cementum-like tissue.
Case outline We present the case of a cementoblastoma in the mandible with atypical radiographic image: no well-defined borders and no radiolucent rim. Apart from that, taking into account data from the literature review, different clinicopathological, and radiographic presentations of tumors and lesions that may resemble cementoblastoma are discussed.
Conclusion Cementoblastoma must be removed as soon as possible, together with the associated tooth. Recurrence rate is a relevant phenomenon and is estimated to 11.8%, so the long-term follow-up is mandatory.
Keywords: cementoblastoma; odontogenic tumours; maxillofacial tumours

INTRODUCTION

Cementoblastoma was first documented by Dewey in 1927 [1]. Cementoblastoma is an uncommon tumor of the jaws that originates from the odontogenic ectomesenchyme, characterized by proliferating cementum-like tissue. It represents only 1–6.2% of all odontogenic tumors. The World Health Organization classified benign cementoblastoma and cementifying fibroma as the only true neoplasms [2, 3, 4]. The growth potential of the tumor is unlimited and there are several of the cases reporting the aggressive behavior of the cementoblastoma. Typical radiographic presentation of cementoblastoma is well-defined oval radiopacity with a thin radiolucent periphery.

CASE REPORT

A 19-year-old female without contributory medical history was complaining about the pain in the mandible molar area. Intraoral examination revealed a large cavity in the distal part of the first lower left molar. The pulp vitality test was negative. The radiographic examination showed a highly radiopaque mass attached between the mesial and distal roots. The mass was oval (15 × 20 mm), was positioned toward the base of the lower jaw, and was causing the resorption of the mesial root. Both retroalveolar and panoramic X-rays gave the impression that the mass was fused to the surrounding bone, without clear borders (Figure 1).

Clinical symptoms and findings implied to a chronic pulpal infection. On the other hand, radiological presentations of the lesion suggested to several differentials: hypercementosis, cemento-osseus dysplasia, condensing osteitis, idiopathic osteosclerosis, cementoblastoma, odontoma, osteoblastoma, fibrous dysplasia. In order to get more precise information concerning the lesion, a cone bream computer tomography was performed. The scans confirmed unclear borders of radiopaque mass that was pushing down the mandibular canal to the base of the lower jaw (Figure 2).

A provisional diagnosis of chronic low-grade infection was made and it was decided to perform a root canal treatment at first. The patient gave her informed consent. Although the endodontic treatment relived the pain, the patient was anxious about the unknown mass inside the bone and the biopsy was scheduled. The bony specimen taken during the biopsy was fixed in 4% buffered formalin and together with the X-rays sent for histopathology (Figure 3).

Histopathological examination revealed that the tumor was composed of sheets of dens, irregular lamellated, and cementum-like tissue. Cementum-like structures with broad trabeculae were presented as well as sheets of irregularly placed tumor cells within lacunae. Cementoblasts were plump with moderate amount of cytoplasm, hyperchromatic nuclei, but no mitotic activity. Although many authors describe the presence of osteoclast like giant cells, in our case giant cells were not seen. Diagnosis of cementoblastoma was made (Figure 4).

Surgical removal of the tumor, along with the involving tooth and peripheral osteotomy were performed. Preservation of the lower mandibular nerve was obtained. Postoperative

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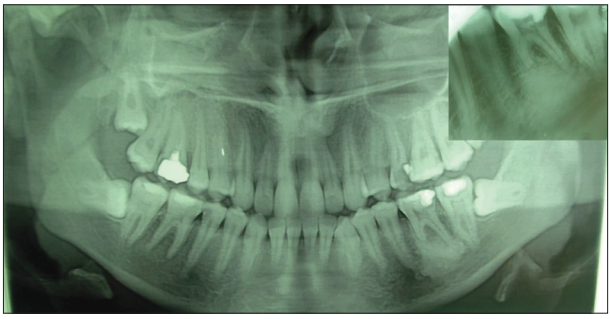

Figure 1. Retroalveolar and panoramic radiography: highly radiopaque mass is attached between the roots of tooth number 36

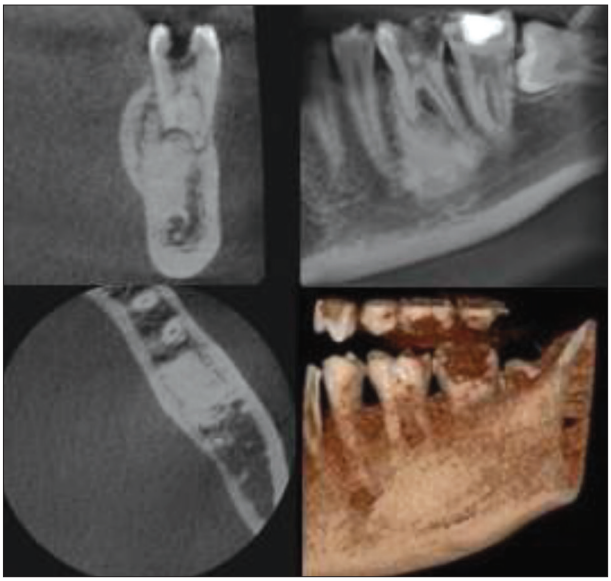

Figure 2. Cone beam computed tomography scans: unclear border of radiopaque mass is pushing down mandibular canal to the base of the lower jaw and causing the resorption of the mesial root

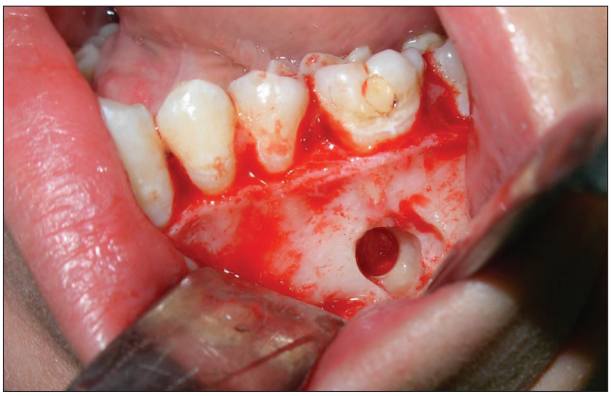

Figure 3. Intraoperative insight in biopsy: It was very difficult to identify tumour and its borders. The biopsy is performed according to preoperative radiography planning

period was uneventful and complete patient recovery was accomplished. Three years follow-up acknowledged the absence of the tumour (Figure 5).

DISCUSSION

Cementoblastoma, classified as odontogenic ectomesenchymal tumor, arises mostly in the permanent dentition

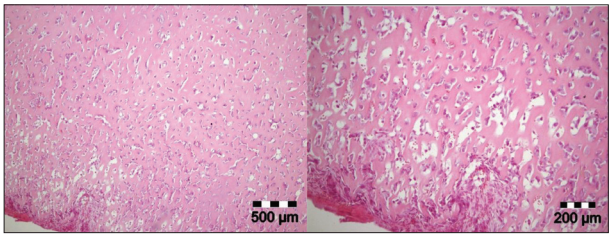

Figure 4a and 4b. Histologic findings: a – tumor consists cementum-like tissue (HE, 10×); b – prominent cementoblasts and trabeculae of uncalcified cemental matrix perpendicular to the surface (HE, 20×)

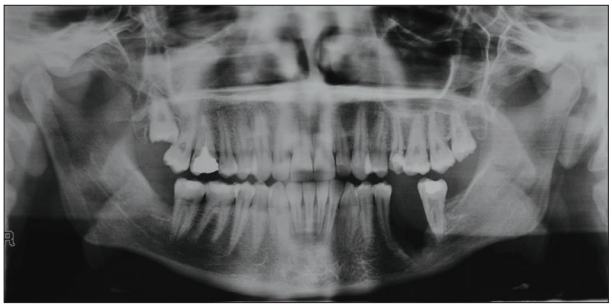

Figure 5. Follow-up radiography: There are no signs of tumor recurrence

with several incidences reported in primary or unerupted teeth [5–9]. Slow growing mass of cementum or cementum-like tissue is usually located in the posterior area of lower jaw (80%), and associated with permanent first molar. The tumor generally occurs among young population and has equal sex distribution [10, 11, 12]. Associated tooth is usually vital and if the pathological changes of tooth are presented they are coincidental [13]. Cementoblastoma has a pathognomonic radiographic appearance as a well-defined solitary ovoid radiopacity with a thin radiolucent periphery. The tumor is frequently fused to partly resorbed root/roots of the associated tooth [14, 15]. In the case when associated tooth was extracted prior to diagnosis of cementoblastoma, patient pre-extraction X-rays are of great importance [16]. In our case, the resorption of the adjacent root was present, there were no bony expansion and characteristic radiographic appearance was missing. Cone beam computed tomography showed that tumorous mass was more radiopaque than surrounding bone but there were no clear borders and radiolucent rim.

There are several differentials that should be considered: hypercementosis, focal cement osseous dysplasia, condensing osteitis, idiopathic osteosclerosis, odontoma, osteoblastoma, osteoid osteoma and fibrous dysplasia (Table 1).

Hypercementosis is a non-neoplastic condition in which excessive cementum is deposited in continuation with regular radicular cementum. It is widely accepted as an age-related phenomenon involving mostly the older population. Premolars are the most affected teeth, bilateral involvement is not uncommon and is usually presented without clinical symptoms. Apart from the idiopathic nature of hypercementosis, this condition is associated with several local, more commonly periapical pathosis, or systemic factors. Radiographically, hypercementosis is an occasional finding. The radiolucent shadow of the periodontal membrane

Table 1. Clinical, radiographic, and histopathological features of radiopaque lesions of the jaws

Lesions	Age / Sex	Clinical	Tooth involvement	Radiographic	Histopathology
Hypercementosis	Both / over 40 years old	No symptoms; mandibular premolar area;	Yes (vital, no root resorption)	Well-defined radiopacity with radiolucent halo	Cellular/acellular cementum
Condensing osteitis	Both / younger population	Discrete or no symptoms; dental inflammatory stimulus with chronic pulpal involvement; mandibular jaw; no root resorption;	Yes (non-vital, no root resorption)	Well-defined radiopacity without radiolucent halo	Cancellous/compact bone
Idiopathic osteosclerosis	Both / younger population	No symptoms; mandibular jaw;	No	Well-defined radiopacity without radiolucent halo	Thickened trabeculae; reduced marrow fibrovascular spaces
Cementoblastoma	Both / younger population	Discrete or no symptoms; mandibular molar area;	Yes (usually vital; can cause root resorption)	Well-defined radiopacity with radiolucent halo	Cementicles fused to form a mass and fibrovascular stroma
Odontoma	Both / younger population	No symptoms; frontal parts of maxilla and posterior parts of mandible; main cause of delayed teeth eruption;	No	Well-defined tooth shape radiopacity with a radiolucent halo	Dental hard tissues; dentin and enamel
Osteoblastoma	Male / younger population	Presence of a mild pain during the night, not relieved with salicylates; unlimited growth potential; facial asymmetry, swelling;	No	Well-defined radiopacity correlated with the amount of tissue calcification	Anastomosing trabeculae of woven bone rimmed by single layer of benign activated osteoblasts and numerous osteoclasts
Osteoma	Male / 20–50 years old	Presence of a mild pain during the night, relieved with salicylates; limited growth potential;	No	Well-defined radiopacity correlated with the amount of tissue calcification	Dense, compact mature bone
Fibrous dysplasia	Female / younger population	Asymptomatic; facial asymmetry, swelling;	No	"Ground-glass" radiographic appearance; loss of lamina dura	Fibroblastic proliferation with irregular shaped trabeculae (Chinese letters)
Osteosarcoma	Both / no prediction	Symptomatic; pain; fast volume increase; presence of malignant features;	No	May be lytic, sclerotic or both; presence of radiopacity resembling sunrays	Atypical mesenchymal cells with osteoblastic differentiation and new lamellar bone production

and the radiopaque lamina dura are always seen as the outer border of hypercementosis [17].

Cemento-osseous dysplasia is reactive or dysplastic process. Clinically is usually asymptomatic and appears in the apical region of vital teeth as frequent coincidental X-ray founding [18].

Condensing osteitis is characterized by presence of a low grade, chronical, dental inflammatory stimulus of the adjacent tooth. Radiographically is seen as localized bony sclerotic area associated to the apex of the tooth but without radiolucent halo [19]. In addition to this, calcifications in condensing osteitis represent necrotic irregularly mineralized bone, contrary to cementum calcifications in cementoblastoma. Therapy is primarily focused to endodontic treatment of the involved tooth.

Idiopathic osteosclerosis is similar to condensing osteitis but without tooth involvement. The cause is unknown, usually affects younger population and the therapy is not required. Radiographical finding is the same as focal sclerosing osteomyelitis but the sclerotic area is not connected to the adjacent teeth [20].

Odontoma is odontogenic tumor composed of various dental tissues. It is slow growing, non-aggressive, true neoplasm found usually in younger population. Usually, odontoma is asymptomatic or can cause delayed teeth eruption. Radiographically is easy to differentiate to cementoblastoma since odontoma is not fused to the adjacent tooth and has tooth shape structure [21].

Osteoblastoma is benign bone forming tumor. It is very similar to cementoblastoma but with few differences. Instead of cemetoblasts and cementoclasts, it is characterized by woven bone production and proliferation of numerous plump activated osteoblasts, many osteoclasts, and fibrovascular stroma. Clinically, there is evident night pain that cannot be relieved by salicylate intake. Radiographical finding is the same as cementoblastoma. The degree of opacification on the X-ray correlates to the amount of calcification, but the lesion is not attached to the tooth [22].

Osteoid osteoma is similar to osteoblastoma but with reduced growing potential and sclerotic surrounding bone. Usually, it does not exceed 10 mm in diameter and is not related to the teeth [22].

Fibrous dysplasia is a rare non-neoplastic fibro-osseous lesion of cranial bones. Fibroblastic proliferation with irregular shaped trabeculae and no osteoblastic rimming are histological criteria for diagnosis. It usually involves younger population and is asymptomatic until causes facial asymmetry, enlargement etc. Radiographical finding shows typical "ground-glass" appearance and the absence of lamina dura [23, 24].

Histologically, cementoblastoma is composed of broad trabeculae of sparsely cellular cementum merged with areas of cemental islands in vascular stroma. The peripheral zone shows radiating columns of cementum running perpendicular to the surface of the lesion [15]. Microscopic specimen of our case had the same characteristics as previously mentioned. Resembling microscopical image can be found in osteoid osteoma, osteoblastoma, and osteosarcoma. Major difference of osteosarcoma is the presence of atypical mesenchymal cells and sharp circumscription with no permeation of surrounding bone [17].

Recent studies involving the expression of cementum protein (CEMP-1) could help better understanding of cementoblastoma. CEMP-1 has been isolated from human cementoblastoma and is considered to be a specific marker of cementoblasts, periodontal progenitor cells, and mineralization process. The expression of CEMP-1 was positive in subpopulation of cementoblasts and mineralized tissues. It could help identify and standardize tumoral lesions, and should be considered as a useful diagnostic tool [25].

As seen in our case and from literature data, clinical manifestations of cementoblastoma may vary. In this case, there was not radiolucent rim around tumor, although the aggressive nature of tumor was demonstrated by root resorption. Radiographic aspects of cementoblastoma are correlated with the amount of calcification. Immature lesions are usually radiolucent and with the maturation, radiopacity increases [15]. Histopathologically, cementum is similar to bone and cementoblastoma may be easily misinterpreted as different pathology. That is why the diagnosis cannot be made on examination of the biopsy specimen alone. The pathologist may misdiagnose such lesions if the clinical and radiographic findings are not considered [15].

The treatment of choice is surgical extirpation on tumour. Cementoblastomas must be removed as soon as possible, together with the associated tooth. Recurrence rate is a relevant phenomenon and is estimated to 11.8% [10]. Appropriate treatment should consist of surgical removal of the lesion with the affected tooth, followed by through curettage or peripheral osteotomy. Sometimes, *en block* resection is not sufficient and marginal or even segmental resection of the jaw is required [26]. In our case, tumour was fused to the surrounding bone so additional peripheral osteotomy was necessary. Luckily, the tumour did not cause bone expansion or cortical bone perforation associated with the higher recurrence rates [10]. Nevertheless, long-term follow-up of the patient is mandatory.

Conflict of interest: None declared.

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Цементобластом – необична радиографска манифестација

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САЖЕТАК

Увод Цементобластом је тумор виличних костију који води порекло од одонтогеног ектомезенхима, а карактерише га пролиферишуће ткиво налик на цемент.

Приказ болесника У раду је приказан цементобластом доње вилице, атипичне радиографске манифестације: без јасно дефинисане границе и без зоне периферног расветљења. Прегледом доступне литературе евалуирали смо различите туморе/лезије који клиничко-патолошки или радиолошки могу личити на цементобластом.

Закључак Цементобластом захтева што ранији хируршки третман, при чему је потребно уклонити и захваћени зуб. Рецидиви су релативно чести (око 11,8%), па су због тога неопходне дугорочне контроле болесника.

Кључне речи: цементобластом, одонтогени тумори, тумори максилофацијалне регије

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Blastic plasmacytoid dendritic cell neoplasm of the uterus

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SUMMARY
Introduction Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare and very aggressive hematological malignancy derived from precursor of the plasmacytoid dendritic cell. We present a case with cervix uteri involvement without skin lesions, which is, to the best of our knowledge, the first case of BPDCN localized in the cervix.
Case outline A 66-year-old previously healthy women initially presented with a four-week history of vaginal bleeding. Gynecologic examination revealed a tumorous bleeding formation on cervix uteri. Except paleness of the skin, physical examination results were normal. Complete blood counts showed anemia and thrombocytopenia. Computed tomography scans showed an expansive tumorous formation at the level of the isthmus and cervix uteri, 60 × 42 mm in size. Cervical biopsy was done and final pathohistological diagnosis was BPDCN. Karyotype analysis results from the bone marrow aspiration specimen demonstrated tetrasomy of chromosome 2 and monosomy of chromosome 16. The patient did not accept treatment and died two months after the initial diagnosis was established.
Conclusion Attributes such as aggressive clinical course of BPDCN, demonstrated unusual localization, infrequency, and the absence of consensus about standard treatment options, demand constructive clinical reasoning and tight cooperation between medical professionals of various fields.
Keywords: BPDCN; hematologic malignancy; aggressive

INTRODUCTION

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is rare and very aggressive hematological malignancy derived from precursor of the plasmocytoid dendritic cell (pDC) [1]. First it was described in mid-1990s and formerly was known as hematodermic neoplasm and blastic natural killer lymphoma [2, 3, 4]. In 2008, in WHO classification for hematopoietic tumors it was categorized under "acute myeloid leukemia (AML) and related precursor neoplasm" [5]. However, in 2016 WHO myeloid neoplasm and acute leukemia classification, BPDCN is distinguished as a separate entity, in contrast to the previous classification [6]. BPDCN is characterized by high frequency of cutaneous involvement at diagnosis, which can be the only clinical manifestation at the beginning [7]. Bone marrow and lymph nodes involvement is observed in about 50% of cases [8]. A minority of cases initially present with acute leukemia, but leukemia is more often a presentation of the advanced disease [9]. Other infrequent sites of BPDCN localization are the spleen, liver, central nervous system, tonsils, lungs, kidneys, and muscles [7]. We present a case with cervix uteri involvement without skin lesions, which is, to the best of our knowledge, the first case of BPDCN localized in the cervix.

CASE REPORT

A 66-year-old previously healthy women initially presented with a four-week history of vaginal bleeding. Gynecologic examination showed a tumorous bleeding formation on cervix uteri. Except paleness of the skin, physical examination results were normal. Complete blood counts showed bicytopenia (hemoglobin 10 g/dL, platelet count 29,000/mm^3, and white blood cell count 6500/mm^3). Routine hemostasis screening test results were normal (international normalized ratio of 1.17, fibrinogen 2.03 g/l, activated partial thromboplastic time 34 seconds, D-dimer 299 μg/l). Lactate dehydrogenase was elevated to 1777 U/L, while other components of the biochemical panel were in reference ranges. Computed tomography (CT) scans revealed expansive tumorous formation in the level of the isthmus and cervix uteri 60 × 42 mm in size, which invaded all the layers of uterus and partly propagated by periuterine adipose tissue (Figure 1). CT also revealed multiple enlargements of iliac,

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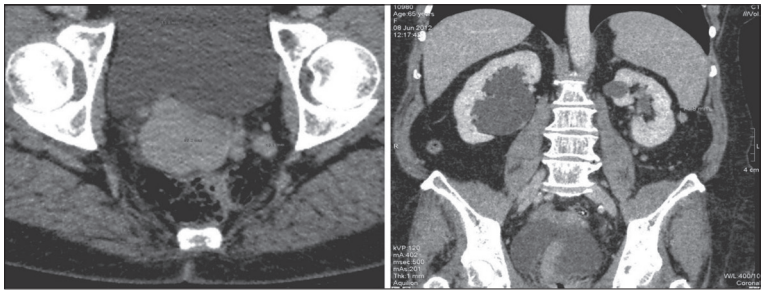

Figure 1. Pelvic computed tomography scans showed a 60 mm mass at the level of the isthmus and cervix uteri, which invaded all the layers of the uterus and partly propagated by periuterine adipose tissue

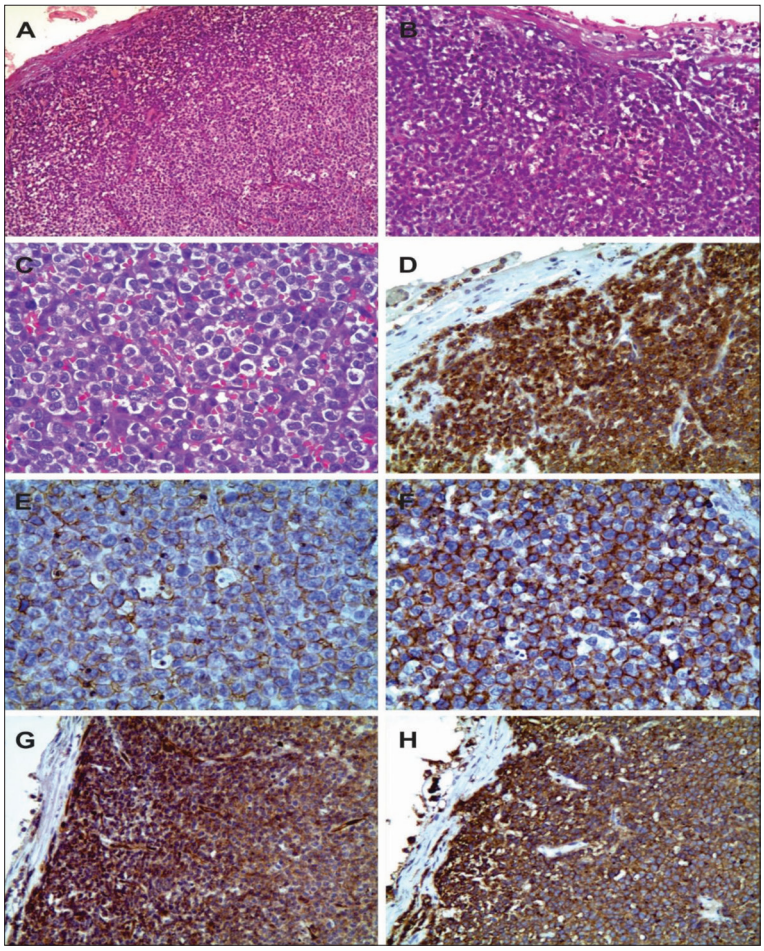

Figure 2. The patient's cervix pathohistology and immunohistochemistry; hematoxylin and eosin staining showed small- to medium-sized blastoid cells diffusely infiltrating predominantly cervical stroma, sparing the epithelium (A, B, C); immunohistochemically, tumor cells were positive for CD4 (D), CD43 (E), CD 56 (F), CD 123 (G), CD45 (H)

retroperitoneal, mediastinal lymph nodes with peritoneal nodular formations.

Cervical biopsy was made and pathohistological examination of specimen showed diffuse infiltration of mucosa with uniform small to medium size cells with blast-like morphology. Tumor cells predominantly occupy the cervical stroma sparing the squamous epithelium. The cells showed large, irregular, oval nuclei with finely granulating chromatin, one or more nucleoli and scant and agranular cytoplasm (Figure 2: A, B, C). Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue. Tumor cells co-expressed CD4, CD43, CD56, CD123, CD45, CD33 and showed partial positivity for CD68 (Figure 2: D, E, F, G, H). One part of nuclei was also positive on p16 and Oct-2. The cells were negative to vimentin, TdT, CD34, CD117, CK5, CK7, p63, p16, SM actin, synaptophysin, PGP 9.5, chromogranin A, PAX-5, CD79a, CD20, CD10, MUM-1, CD138, CD30, CD15, CD2, CD3, CD5, CD7, CD8, granzyme B, perforin, CD13, MPO, CD14, CD163, bcl-2, bcl-6. The final pathohistological diagnosis was BPDCN.

Bone marrow biopsy showed a slightly hypercellular marrow, with CD4-, CD56-, CD123-positive large blast cells accounting for 5–7% of cellularity. Lymphoid, NK, and myeloid lineage-associated antigens were negative.

Karyotype analysis results from the bone marrow aspiration specimen demonstrated tetrasomy of chromosome 2 and monosomy of chromosome 16 in 12 out of 20 analyzed metaphase cells. (47,XX,+2,+2,-16[12]/46,XX[8]).

Based on clinical, radiographic, and predominantly on histological and immunohistochemical findings of cervical and bone marrow biopsy, the patient was diagnosed with BPDCN, but refused further treatments and died two months after the initial diagnosis was established.

Written consent for publication of this article was obtained from the patient's family member.

DISCUSSION

BPDCN is a very rare and aggressive form of lymphoma-like disease derived from precursor of the pDC. Diagnosis is made based on clinical presentation and histological and immunophenotype features of the involved tissue. In the majority of cases it presents with indolent cutaneous lesions, later followed by dissemination and bone marrow and lymph node involvement [10]. A minority of cases present with fulminant leukemia without skin infiltration. Biopsy of involved tissue usually reveals medium-sized blast cells with irregular nuclei, fine chromatin, and at least one small nucleolus. The cytoplasm is scant and agranular. Because of the overlap with other hematopoietic neoplasms such as myeloid sarcoma/AML, T-cell lymphoblastic leukemia/lymphoma, NK-cell lymphoma/leukemia, extensive immunophenotype analysis is necessary [7, 10, 11]. A recent multicentric study suggested that triple positive CD4+CD56+CD123+ phenotype associated with negativity for lineage-specific markers

such as markers for B cells (CD20, CD79a), T cells (CD3), myeloid cells (myeloperoxidase), and monocytes (CD11c, CD163, lysozyme) is a minimum requirement for defining BPDCN [12].

Our patient presented with a quite unique localization of BPDCN in cervix and isthmus uteri. The origin of tumor cells remained unresolved – whether it was bone marrow or cervical mucosa – because BPDCN has an aggressive clinical presentation that probably affects both sites either consecutively or simultaneously. Histopathological features and triple positive (CD4+CD56+CD123+) phenotype in the absence of specific lineage markers clearly point to BPDCN. However, the diagnosis criteria varied from study to study but majority of them included the following five markers: CD4, CD56, CD123, CD303 (also known as BDCA-2), and TCL1 [10]. Heterogeneity of BPDCN tumor cells is more emphasized by occasional CD56 and/or CD123 surface marker expression [7, 11]. An interesting fact is that blasts with immature plasmacytoid dendritic cell phenotype present typically without extramedullary (e.g. skin) disease; on the other hand, mature blast cell phenotype more frequently displays skin/extramedullary involvement [13]. However, a few myeloid-associated antigens have been seen in a significant number of cases [11]. It is highly important to diagnostically differentiate BPDCN from AML or AML-associated leukemia cutis or myeloid sarcoma. BPDCN is characterized by pDC antigens positivity, CD123 and TCL-1, and myeloperoxidase (MPO) negativity, while AML or myeloid sarcoma show MPO positivity and negativity for pDC antigens [14]. In particular, CD68, an antigen typically expressed by granulocytes and histiocytes as well as normal plasmocytoid dendritic cells, is noted in significant number of cases [11]. Another myeloid antigen frequently found in the BPDCN neoplastic cells is CD33, which is the most frequently reported myeloid marker expressed by BPDCN neoplastic cells [11]. Other strong myeloid markers' expression, CD13 and CD117, has also been reported [10]. Neoplastic cells in our case show positivity on both antigens, as well as on CD45 and CD43, which are also often positive on BPDCN cells [10, 11]. Similar triple positive (CD4+CD56+CD123+) cells with blast morphology were found in bone marrow, indicating bone marrow involvement.

Cytogenetic analysis frequently reveals complex aberrations seen in AML or myelodysplastic syndromes [11].An interesting fact is that at the time of diagnosis two-thirds of patients show cytogenetic anomalies [10]. Recent studies showed several structural and numerical chromosomal aberrations, as well as gene mutations associated with BPDCN. Most frequent recurrent published genomic loses are as follows: 5q21 or 5q34, 12p13, 13q13-21, 6q23, monosomy 15, and monosomy 9 [10, 11]. As aforementioned, BPDCN cells can carry multiple genetic abnormalities that overlap with the genetic abnormalities of myeloid and lymphoid neoplasms, but tetrasomy of chromosome 2 and monosomy of chromosome 16 described in this case are not one of them and influence of this numerical chromosomal aberration on the etiology and pathogenesis of BPDCN is unknown.

Because of low incidence, there is no consensus for the optimal therapy for BPDCN. The objective of treatment should be the achievement of complete remission after first-line treatment based on protocols for AML or acute lymphoblastic leukemia and, after that, consolidation with allogeneic hematopoietic stem cell transplantation (allo-HSCT). A recent study confirms that the combination of methotrexate and asparaginase for the frontline treatment could be a good solution with a low toxicity profile, even in elderly patients [10, 12]. Having in mind that the CD123 positivity occurs in virtually all cases, using specific BPDCN CD123-directed cytotoxin (tagraxofusp) consisting of recombinant human interleukin-3 fused to a truncated diphtheria toxin could be a reasonable treatment option. Based on the results of a study carried out by Pemmaraju et al. [15], tagraxofusp was approved as the only treatment specifically indicated for untreated or relapsed BPDCN patients with potential development of adverse events as well as included capillary leak syndrome, hepatic dysfunction, and thrombocytopenia [16].

Conflict of interest: None declared.

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Бластична плазмоцитоидна дендритична неоплазма материце

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САЖЕТАК

Увод Бластична плазмоцитоидна дендритична неоплазма (БПДН) представља редак и врло агресиван хематолошки малигнитет који потиче од прекурсора плазмоцитоидне дендритичне ћелије.

Приказујемо случај захватања грлића материце БПДН, без кожних лезија. Према нашим сазнањима, ово је први забележен случај БПДН локализован у грлићу материце.

Приказ болесника Претходно здрава жена, стара 66 година, јавила нам се проблемом крварења из усмине. Гинеколошким прегледом је уочена крварећа туморска формација грлића материце. Осим блеђе пребојености коже, физикални налаз је био уредан. Анализом крви уочене су анемија и тромбоцитопенија. Компјутеризованом томографијом је радиолошки верификована експанзивна туморска формација грлића материце промера 60 × 42 *mm*. Потом је урађена биопсија наведене промене, а *pH* налаз је показао да се ради о БПДН. Анализом кариотипа из аспирата ћелија коштане сржи утврђена је тетразомија хромозома 2 и монозомија хромозома 16. Болесница је одбила третман и преминула два месеца после постављања дијагнозе БПДН.

Закључак Агресиван клинички ток БПДН, поменута неуобичајена локализација, ретка болест и недостатак слагања о стандардним терапијским опцијама захтевају конструктивно клиничко резоновање и сарадњу медицинских професионалаца из различитих области.

Кључне речи: БПДН; хематолошки малигнитет; агресиван

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Not so innocent bystander – gallbladder varices without portal vein thrombosis

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SUMMARY
Introduction Gallbladder varices (GBV) represent a rare form of ectopic varices that usually occur in patients with portal hypertension and portal vein thrombosis.
Case outline We present a case of a 38-year-old woman with decompensated autoimmune liver cirrhosis who was referred to our institution for evaluation for liver transplantation. She was incidentally discovered to have GBV during a routine B-mode abdominal ultrasonography as part of pre-transplant evaluation. GBV were confirmed by the Color Doppler Sonography, and multi detector computed tomography angiography. Interestingly, portal vein was patent and without thrombus.
Conclusion Despite being asymptomatic in most cases, the presence of GBV is valuable information for a surgeon because they might be a source of potentially catastrophic bleeding, which is particularly poorly tolerated by patients with decompensated liver cirrhosis. Ultrasound has the irreplaceable role not only in discovering GBV, but in prompt diagnosis of rare, but unpredictable and fatal complications as well.
Keywords: gallbladder varices; ectopic varices; portal hypertension

INTRODUCTION

Gallbladder varices (GBV) are rare form of ectopic varices that usually develop in patients with portal hypertension. They represent a form of portosystemic shunting that occurs between the portal vein through the cystic vein branches, and the veins of the anterior abdominal wall [1]. Hence, it is of no surprise that the gallbladder is directly affected by portal hypertension. Portal hypertension may lead to the gallbladder wall thickening secondary to impaired venous drainage. GBV occur with incidence of 12–30% in patients with portal hypertension, are usually associated with portal vein thrombosis (PVT) and are characteristic feature of portal biliopathy [2, 3]. Most of the time they are asymptomatic but their spontaneous bleeding results in hemobilia, recurrent gastrointestinal bleeding or even gallbladder perforation and hemoperitoneum [4].

We present a case of a patient with decompensated liver cirrhosis secondary to autoimmune hepatitis who was diagnosed with GBV during routine abdominal ultrasonography as a part of pre-liver transplant evaluation. The diagnosis was confirmed by the Color Doppler Sonography and abdominal Multidetector computed tomography.

CASE REPORT

A 38-year-old female patient was referred to the Clinic for Gastroenterology and Hepatology of the Clinical Center of Serbia for transplant evaluation due to end stage liver disease secondary to autoimmune hepatitis. She was diagnosed with decompensated liver cirrhosis seven years prior to her current hospitalization and since then she has been admitted several times due to the various complications of end stage liver disease, such as recurrent ascites, jaundice, hepatic encephalopathy, and recurrent gastrointestinal bleeding. During the last admission she had gastrointestinal bleeding and upper esophagogastroduodenoscopy showed grade III varices with "red cherry spots" which were successfully treated by band ligation. Due to worsening Model of End Stage Liver Disease score of 24, she was a transplant candidate. On admission, the patient was hemodynamically stable, without fever or leukocytosis. Her abdomen was distended but non-tender with palpable splenomegaly and positive fluid shift. Cardio-pulmonary exam was unremarkable and skin showed evidence of telangiectasia. Neurological exam was non-focal, and there was no encephalopathy. Pre transplant evaluation included routine abdominal ultrasonography that revealed an enlarged, nonhomogeneous liver with massive splenomegaly of 250 mm in craniocaudal diameter, as well as circular

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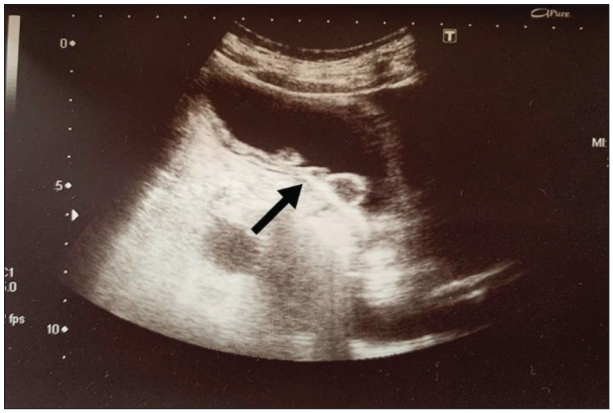

Figure 1. Gallbladder varices on B mode ultrasound

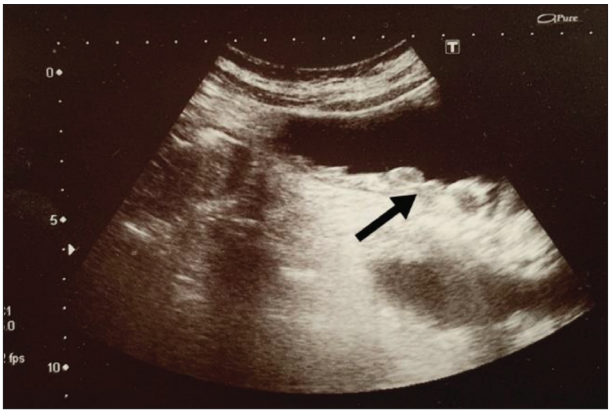

Figure 2. Gallbladder varices on B mode ultrasound

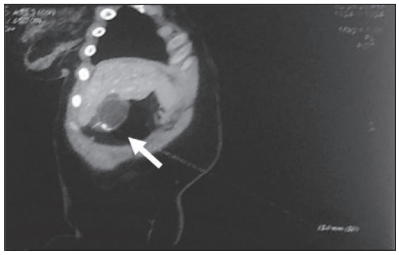

Figure 3. Delaminated gallbladder wall on abdominal multidetector computed tomography

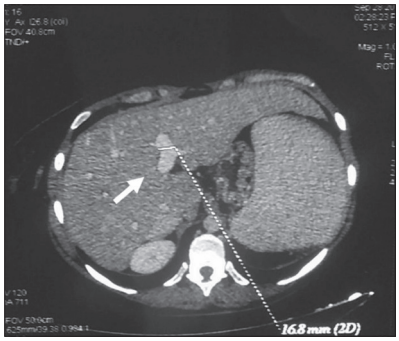

Figure 4. Dilated portal vein branch on abdominal multidetector computed tomography

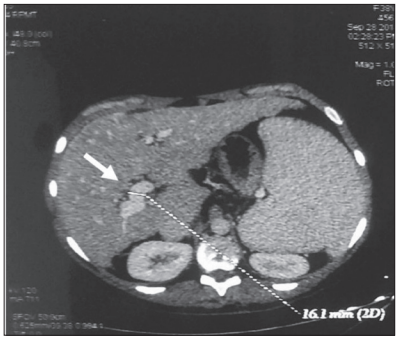

Figure 5. Dilated portal vein branch on abdominal multidetector computed tomography

changes in the gallbladder wall. Doppler sonography of portal system confirmed GBV, however, without a PVT (Figures 1 and 2). Multidetector computed tomography angiography of the abdomen confirmed a thickened and delaminated gallbladder wall with GBV as well as dilated, but patent portal vein without thrombosis (Figures 3, 4, and 5).

DISCUSSION

Ectopic varices represent dilated splanchnic veins, or dilated portosystemic collaterals, which occur along the entire gastrointestinal tract outside the common variceal sites such as gastroesophageal varices and internal hemorrhoids [2]. GBV are a form of ectopic varices seen as a complication of portal hypertension. They consist of enlarged blood vessels in the gallbladder wall or gallbladder fossa, and represent a portosystemic shunt between the cystic branches of the portal vein and the systemic veins of the anterior abdominal wall [1, 2, 3]. The incidence is similar in adult and pediatric population with portal hypertension, estimated to be up to 30% [4]. The majority of patients with GBV also have PVT, however, as our case illustrates, they might develop even in the absence of PVT. The gold standard for diagnosis is the Color Doppler Sonography, which shows the varices as venous flow in the delaminated and thickened parts of the gallbladder wall [3, 5]. If feasible, contrast-enhanced ultrasound can be a valuable further diagnostic tool, while computed tomography scan and magnetic resonance appear to be less sensitive compared to ultrasound.

It is important to consider other etiologies that might mimic GBV and present similarly. These etiologies are more common than GBV and include acute or chronic cholecystitis, gallbladder cancer and porcelain gallbladder to name a few. The absence of mineralization and the presence of vascular enhancement rules out porcelain gallbladder, while the absence of pericholecystic fluid and inflammation make cholecystitis unlikely. A gallbladder cancer can present radiologically in similar fashion, but one would expect to see some degree of local soft tissue invasion or presence of metastatic lesions, which were absent in our case. In spite of their ability to affect the contractility of the gallbladder they are not associated with higher risk for development of cholelithiasis [6]. When present, GBV may cause hemobilia, intra-abdominal hemorrhage, or rupture of the gallbladder as illustrated in several case reports [7]. Ultrasound has the irreplaceable role not only when discovering GBV, but in prompt diagnosis of the rare, but unpredictable and fatal complications as well. [8]. Despite being rare, GBV are the potential cause of detrimental gastrointestinal hemorrhage. The bleeding from GBV is serious because as population with portal hypertension and decompensated cirrhosis tends to be sick and poorly tolerates hemodynamic protuberances. Our case illustrates a rare entity, which should be considered in any patient with planned abdominal surgery, particularly those with

portal hypertension who have increased incidence of GBV. While they usually develop concomitantly with PVT, GBV might be isolated and occur in the absence of PVT, as we have shown in this report.

Considering high availability and low-cost of the color Doppler sonography, which is considered a gold standard for GBV diagnosis, there is no reason for careful evaluation of gallbladder not to be done in every patient with portal hypertension. If GBV are discovered, surgical team should be informed, as it is pertinent information in planning and executing abdominal surgeries. By increasing awareness of this rare portosystemic shunt, we can prevent or decrease the incidence of massive bleeding from GBV, which in turn will decrease perioperative mortality [7–11].

Ethical standards: All procedures performed in studies involving human participants were done 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.

Conflict of interest: None declared.

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Не баш безазлени посматрачи – варикси жучне кесе без тромбозе портне вене

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САЖЕТАК

Увод Варикси жучне кесе (ВЖК) ретка су форма ектопичних варикса код болесника са портном хипертензијом и тромбозом портне вене.

Приказ болесника Приказујемо болесницу стару 38 година, са декомпензованом цирозом јетре на терену аутоимунске болести, која је упућена нашој клиници ради процене за трансплантацију јетре. ВЖК су уочени током извођења рутинског ултразвучног прегледа абдомена (Б-мод) у склопу претрансплантационе припреме. Њихово присуство потврђено је ултразвучним прегледом колор доплером и мултидетекторском компјутеризованом томографском ангиографијом, при чему је портна вена била проходна, без присуства тромбних маса.

Закључак Иако су често асимптоматски, сазнање о присуству ВЖК је од непроцењивог значаја за хирурге, будући да могу бити узрок обилног крварења, које нарочито угрожава болеснике са декомпензованом цирозом јетре. Ултразвучни преглед има незамењиву улогу не само у детекцији ВЖК већ и у правовременој дијагнози претходно поменутих ретких али непредвидивих и фаталних компликација.

Кључне речи: варикси жучне кесе; ектопични варикси; портна хипертензија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Accessory spleen diagnostically hidden, laparoscopically removed – case report and review of the literature

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SUMMARY

Introduction Accessory spleen represents ectopic spleen tissue separated from the body of the spleen, with the percentage share of 10–15% in a population.

Case outline We present a female patient in which immune thrombocytopenic purpura was diagnosed 12 years previously and, after a failed initial treatment, it was decided by a hematologist to perform a laparoscopic splenectomy. The mentioned operation was carried out in a safe and efficient manner wherein the accessory spleen was detected and removed intraoperatively. The operative and postoperative course passed without any complications. The definitive histopathological findings confirmed previously set hematological diagnosis.

Conclusion The laparoscopic approach is a superior modality in terms of diagnostic and therapeutic procedures when it comes to surgical removal of the accessory spleen. Taking into consideration the advantages of this approach presented and proven in literature, even in the case of diagnostically or intraoperatively overlooked accessory spleen or *de novo* discovered after the operation, there should be no dilemma which surgical approach should be applied.

Keywords: spleen; accessory spleen; laparoscopy; splenectomy

INTRODUCTION

The accessory spleen (AS), also known as splenikul or splenul, represents the inherited focal point of the spleen tissue separated from the main body of the spleen. It occurs due to splenic buds not merging during the organogenesis [1]. AS is represented by 10–15% in the general population. In most cases, its dimension is 1–2 cm. The most frequent localization of AS is the posteromedial side of the spleen, spleen hilus, followed by the tail of the pancreas, gastrocolic ligament, large omentum [2].

Diagnostics, or intraoperative detection and surgical removal of the AS is of particular importance in the case of hematological diseases of the spleen. Otherwise, they may grow and lead to a recurrence of the hematological disease for which the patient is subjected to splenectomy [3].

The AS is mainly verified as an incidental finding or are accidentally detected as part of the diagnostic procedures for other diseases. The initial diagnostics are ultrasonography of the abdomen, computerized tomography (CT), and nuclear magnetic resonance (NMR) [4].

Surgical removal of the AS is the only curative treatment modality. As the laparoscopic splenectomy has become the gold standard in the treatment of most diseases of the spleen, it should certainly be given preference over the traditional surgical approach for the treatment of the AS. In addition, laparoscopic splenectomy is a diagnostic and therapeutic option with many benefits [1, 5].

The aim of our work is to present a case in which the laparoscopic splenectomy was a diagnostic tool, in addition to the therapeutic effect, superior comparing to preoperative imaging diagnostics for the detection of the AS in immune thrombocytopenic purpura (ITP).

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.

CASE REPORT

We present a female patient aged 26 years, in which the diagnostics were performed and the primary diagnosis was set by the hematologist. Specifically, the patient was diagnosed with ITP 12 years previously. Since then, she was treated and followed-up by the hematologist. Primary medication (e.g. corticosteroid, immunomodulatory) therapy did not result in the expected therapeutic response. Accordingly, the consultative decision on surgical treatment was

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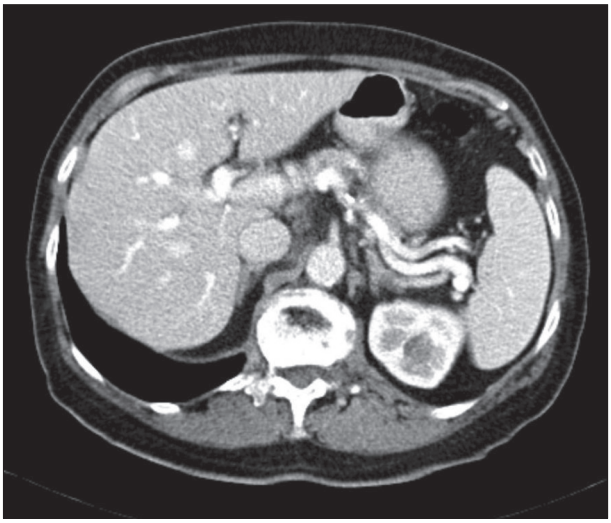

Figure 1. The appearance of the preoperative computed tomography examination

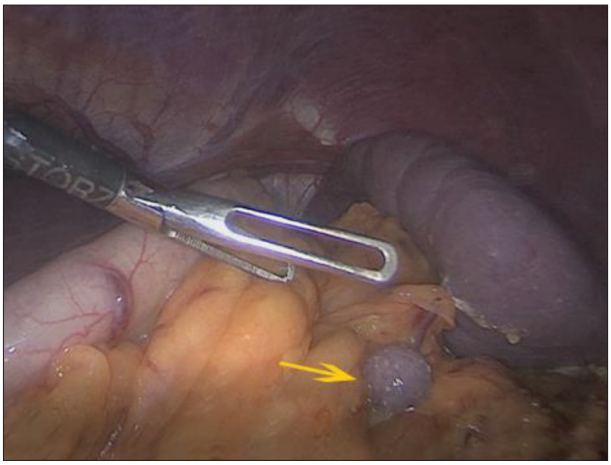

Figure 2. The appearance of the accessory spleen identified intraoperatively (arrow)

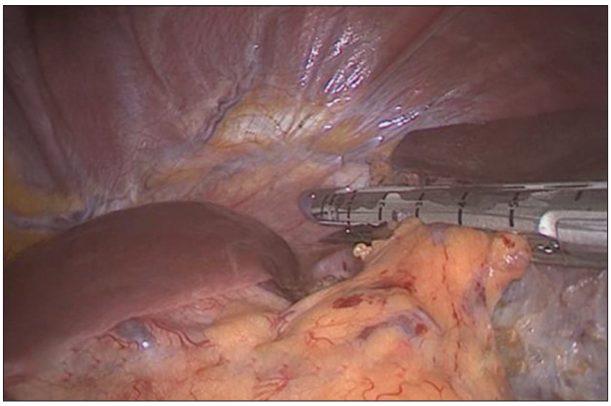

Figure 3. The appearance of the endo-stapler used for hilum of the spleen

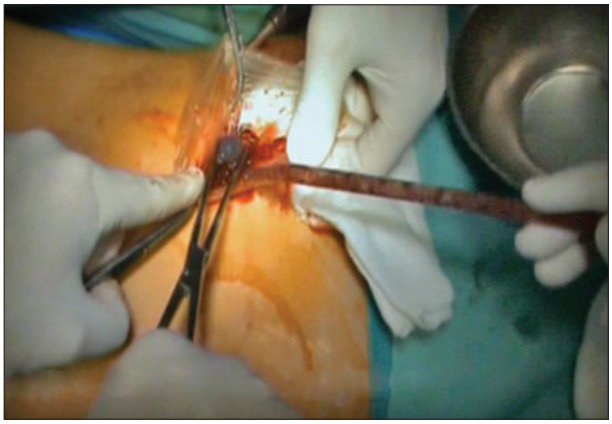

Figure 4. The appearance of the spleen removed from the abdomen in fragments

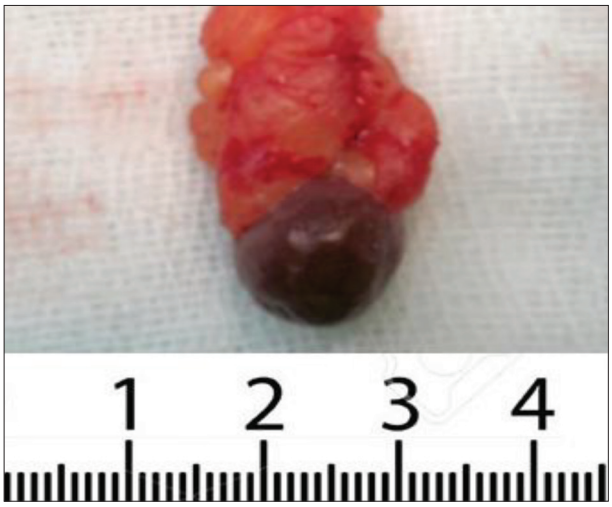

Figure 5. Image of the accessory spleen specimen

made by the hematologist and the surgeon. The laparoscopic splenectomy was to be done.

Upon admission to the clinic, the patient underwent the preoperative CT of the abdomen, where the spleen of normal size was seen, with a diameter of 110 mm in the craniocaudal direction (Figure 1). The patient was set on an operating table in the right lateral position. After adequate preoperative preparation under general endotracheal anesthesia, initially, an artificial pneumoperitoneum was created by using the Veress needle. A port for the laparoscope was placed infraumbilically, and after introducing the camera with a (30°) folded angle, the other working ports were placed in typical locations for the operation. The inspection of the abdomen did not indicate any anomalies. During the mobilization of the spleen, in close proximity to the hilus, an AS of about 1 cm in diameter was identified intraoperatively (Figure 2), which had not been seen at the previous diagnostics. With the use of a bipolar electrosurgical device (LigaSure, Medtronic, Minneapolis, MN, USA) it was entirely removed. Next, we started the liberation and complete mobilization of the spleen by the cutting of splenic ligaments and of short gastric vessels, also with the use of the LigaSure device. Hilus of the spleen was managed by an endovascular stapler with staple feed (Figure 3). After the management of vascular structures of the hilus, the spleen was completely released and placed into a polythene bag for extraction, within which we performed an instrumental destruction of the spleen, which was completely removed from the abdomen in fragments (Figure 4). A silicone abdominal drain was placed in the left subphrenic space, the gas was sucked out and operative incisions were reconstructed by anatomical layers. The prepared AS was

removed entirely from the abdomen (Figure 5) and, with the other fragments of the spleen, was sent for definitive histopathological verification.

The operative and postoperative course was uneventful. The abdominal drain was removed during the second postoperative day, the patient was released from the clinic three days after the surgery with prescribed antibiotic prophylaxis and mandatory postsplenectomy immunization according to the current literature guidelines and according to the guidelines for the prevention of postsplenectomy infections [6, 7].

One month after the operation, a follow-up abdominal ultrasound examination showed normal findings, as did an NMR examination performed six months post-surgery. At the moment of writing the report, the patient is still being followed-up and is monitored by the hematologist.

Definitive histopathological findings of the revised spleen tissue confirmed that there were changes that indicated immune thrombocytopenic purpura.

DISCUSSION

The AS represents ectopic splenic tissue that is separated from the spleen. AS occurs because of that splenic buds placed in dorsal mesogastrium do not merge during the fifth week of embryonic organogenesis [1]. The most common localization of the AS is near the hilum and vascular pedicles of the spleen, the tail of the pancreas, followed by left testicle or ovary due to splenogonadal fusion. It can often be found in the large or small omentum, mesentery of the small intestine, along the greater curvature of the stomach, in the Douglas space, and so on [2, 8, 9].

In the case that we present, AS was positioned near the hilum of the spleen.

Regarding the size and number, AS generally are smaller than 2 cm, rarely can be up to 4 cm in size, and everything bigger than this represents a rare occurrence. Generally, only one AS occurs, two are very rare occurrences, and a larger number is extremely rare [4].

The AS is generally discovered as an incidental finding in the framework of various diagnostic tests that rely on ultrasound, CT, NMR, abdominal scintigraphy, and other tests. Even though previously mentioned modern diagnostic methods are in use, a number of AS remain diagnostically unrecognized [4, 8].

Hematological disorders of the spleen, namely ITP, represent approximately 65% of all indications for splenectomy. These are the patients among which the AS is the most common finding during the diagnostic tests [10]. Detection of AT in hematological patients demands the utmost caution and is of great importance because of the fact that it is very important to detect it and perform a surgical removal; otherwise it can grow and take over the function of the spleen, which leads to disease recurrence [5 ,11].

In our case, despite the diagnostics conducted by hematologists, as well as preoperative imaging diagnostics, the AS was not detected, but we verified it intraoperatively.

Splenectomy represents the only modality of treatment in hematology patients. In cases of trauma or benign diseases of the spleen in which splenectomy is indicated, AS should be preserved and left in the abdomen [1, 8].

Laparoscopic splenectomy is the gold standard in the treatment of hematological diseases of the spleen, undoubtedly with all known benefits that are carried by minimally invasive surgical approach. In one of the recent studies, the superiority of it was confirmed, not only in terms of surgical treatment but also in terms of diagnostics. Namely, Koshenkov et al. [5] have published a study in which the results showed that the intraoperative detection of AS during laparoscopic splenectomy was 100%, while the percentage in pre-operative CT diagnosis was 12.5%.

In cases of a diagnostically and intraoperatively overlooked AS, which is detected during the control diagnostic testing, a repeated surgery with laparoscopic approach should certainly be preferred due to validated greater sensitivity and specificity in the AS detection. One should also take into account possible postoperative complications related to the healing of the incision wound in the classical approach, faster recovery, and, finally, a cosmetic effect, which should not be ignored [12, 13, 14].

The AS, mainly as an incidental finding, is mostly diagnosed in hematological diseases of the spleen, which are most frequently encountered as an indication for splenectomy. Using cameras with 20–30 × optical zoom, a laparoscopic approach represents superior, efficient, and safe modality of detection and of treatment, with extremely rare oversight and low complication rate. In cases where the AS gets overlooked intraoperatively, at reoperation one should not have any dilemma about the approach, in view of the proven benefits of minimally invasive, compared to the classical surgical approach.

Conflict of interest: None declared.

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Акцесорна слезина дијагностички непрепозната, лапароскопски уклоњена – приказ болесника и преглед литературе

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САЖЕТАК

Увод Акцесорна слезина представља ектопично ткиво одвојено од тела слезине, са процентуалном заступљеношћу 10–15% у популацији.

Приказ болесника У нашем раду представљамо болесницу код које је 12 година раније дијагностикована имунолошка тромбоцитопенијска пурпура, те после неуспеле иницијалне терапије од стране хематолога донета одлука да се уради лапароскопска спленектомија. Поменута операција је изведена на сигуран и ефикасан начин, при чему је итраоперативно детектована и уклоњена акцесорна слезина. Оперативни и постоперативни ток су протекли без компликација. Дефинитивни хистопатолошки налаз је потврдио претходно постављену хематолошку дијагнозу.

Закључак Лапароскопски приступ представља супериоран поступак у дијагностичком и у терапијском смислу када је у питању хируршко уклањање акцесорне слезине. Узимајући у обзир до сада литературно представљене и доказане предности овог приступа, чак и у случају дијагностички и/или интраоперативно превиђене акцесорне слезине или *де ново* откривене после операције, не треба да постоји дилема који хируршки приступ треба применити.

Кључне речи: слезина; акцесорна слезина; лапароскопија; спленектомија

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REVIEW ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Prevention of venous thromboembolism with rivaroxaban and apixaban in orthopedic surgery

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SUMMARY

Numerous limitations and side effects of standard anticoagulants require administering new anticoagulant drugs. New peroral anticoagulants of Factor Xa inhibitor group have more advantages, the key ones being: substantial reductions in specific nutrition limitations and drug interaction, no need for routine laboratory monitoring and greatly improved therapy predictability. Rivaroxaban, a selective peroral Factor Xa inhibitor is more effective compared with enoxaparin for venous thromboembolism (VTE) prophylaxis in major orthopedic interventions. Though several single trials demonstrated no difference in hemorrhagic complications, certain meta-analyses with rivaroxaban showed a higher incidence of hemorrhage. Apixaban, a peroral reversible inhibitor of factor Xa approved for the prevention of VTE, compared with European-approved doses of enoxaparin has the efficacy almost equal to the North-American-approved enoxaparin doses without a significant difference in bleeding rates, though ADVANCE I study points towards lower bleeding rates in patients treated with apixaban.

To clarify the contradictory results of the recent meta-analysis related to the comparison between the stated factor X inhibitors and various comparator enoxaparin regimens as well as related to the risk for symptomatic PTE and total bleeding events following major orthopedic surgery, new research will be required. Specificities of rivaroxaban and apixaban, already constituting, according to modern recommendations, an integral part of the VTE prophylaxis protocols after major orthopedic interventions, will enable the establishment of personalized protocols aimed at developing an improved safety profile of each individual patient.

Keywords: prevention; venous thromboembolism; rivaroxaban; apixaban

INTRODUCTION

Prevention of venous thromboembolism (VTE) as a common denominator of deep vein thrombosis (DVT) and/or pulmonary thromboembolism (PTE) as a significant cause of vascular mortality, posttromboflebitis syndrome, chronic thromboembolic pulmonary hypertension, recurrent VTE has great medical, social, and economic significance [1–4]. Like unfractionated heparin (UFH) low molecular weight heparins (LMWH) reduce the risk of VTE at least 60% compared to patients without thromboprophylaxis, but are not convenient for outpatients as they require subcutaneous administration [5, 6]. The incidence of heparin thrombocytopenia, as a serious adverse effect of the applied anticoagulant therapy, is approximately 3–5% in patients treated with UFH in orthopedic surgery and about 0.9% in patients treated with LMWH [7–12].

Oral anticoagulant therapy with vitamin K antagonists (VKA) has many limitations: unpredictability of action, narrow therapeutic window, delayed onset of action, postponed action after discontinuation of therapy, numerous interactions with food and drugs, variable therapeutic effects, and a relatively small percentage of patients in the predicted therapeutic range [13].

The use of heparin anticoagulants is also restricted by the necessity of parenteral administration, the risk of heparin thrombocytopenia with possible potentially endangering thromboses and osteoporosis [14].

In spite of the use of standard, recommended prophylactic anticoagulant regimens with low doses of UFH as well as LMWH, warfarin or recombinant hirudin, the incidence of DVT, confirmed by venography, still ranges 16–30% [15].

Factor X is a well-targeted place for the action of anticoagulant drugs and the primary site of coagulation cascade amplification being positioned at the point of convergence of the external and internal coagulation pathway (Scheme 1) [16, 17]. Direct Xa factor inhibitors such as rivaroxaban and apixaban are not dependent on antithrombin and do not inhibit only the free factor Xa but also factor Xa bound to the complex of prothrombinase on the platelet membrane, and therefore have more advantages even in relation to indirect Factor Xa inhibitors such as fondaparinux and LMWH [16, 18].

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RIVAROXABAN

Pharmacotherapy group and Anatomical Therapeutic Chemical classification

Rivaroxaban belongs to the B01AF subgroup of direct factor Xa inhibitors within the B01A group of antithrombotic drugs [19].

A brief history of the drug

The synthesis of rivaroxaban was achieved under the program "Factor Xa" launched in 1998 by Bayer [20]. In the group of new direct factor Xa inhibitors, rivaroxaban was firstly approved for clinical use in the prevention of VTE in adults undergoing total knee replacement (TKR) and total hip replacement (THR) in 2008 in the EU and Canada [20]. The Food and Drug Administration (FDA) approved rivaroxaban for the prevention of DVT in patients undergoing TKR and THR in 2011 [20].

Pharmacodynamics

Mechanism of action

Rivaroxaban is a highly selective, direct Xa factor inhibitor with oral bioavailability. Rivaroxaban blocks the free factor Xa and the clot connected factor Xa as well as the activity of the prothrombinase complex [18]. The inhibition of factor Xa interrupts the intrinsic and extrinsic cascade pathway of blood coagulation, preventing the formation and development of thrombus. Rivaroxaban does not inhibit thrombin and has no effect on platelets.

Pharmacokinetics

Rivaroxaban causes dose-dependent inhibition of factor Xa and achieves its maximum effect after 1–4 hours following administration [2]. Bioavailability is high, 80–100%.

Due to reduced absorption of rivaroxaban in the distal gastrointestinal tract, the administration of the drug distal to the stomach is avoided [21]. The presence of food delays, the time until the maximum concentration of the drug (T_{max}) is reached and increases the maximum drug concentration (C_{max}) and the area under the curve (AUC) at a dose > 10 mg [22].

Distribution of rivaroxaban is achieved by binding to albumin in 92–95%, and the volume of distribution is 50 liters [21]. The drug cannot be eliminated by hemodialysis due to a high degree of binding to plasma proteins [18].

Biotransformation and elimination: two thirds of the applied drug undergo metabolic degradation, with renal and fecal elimination equally. A third of the administered dose is excreted unchanged in urine following active renal secretion [21].

Metabolism of rivaroxaban is mediated by CYP3A4/5 and CYP2J2 isoenzymes and CYP-independent mechanisms. Rivaroxaban is a substrate for P-glycoprotein (P-gp) [21].

After oral administration the half-life of the drug is 5–9 hours in the young, and 11–13 hours in the elderly [21].

Interactions

The bioavailability of rivaroxaban is increased with the concomitant use of CYP3A4/P-gp inhibitors, such as ketoconazole or HIV protease inhibitors (e.i. ritonavir), which may cause higher concentrations of rivaroxaban and hemorrhage and the concomitant administration of these drugs may be considered contraindicated. Concomitant use of strong CYP 3A4 inducers such as rifampicin, carbamazapine, phenytoin, St. John's Wort can significantly decrease the concentration of rivaroxaban and reduce its anticoagulant effects [21, 22, 23].

Clinical pharmacology

Dosing and indications

Elective TKR and THR require the administration of 10 mg daily rivaroxaban for the primary prevention of VTE. The initial dose is taken 6–10 hours after surgery. Recommended thromboprophylaxis duration is five weeks for THR and two weeks for TKR [21].

Efficacy

In Regulation of **C**oagulation in Orthopedic Surgery to Prevent DeepVenous Thrombosis and Pulmonary Embolism (RECORD) I–III trials, rivaroxaban dose of 10 mg once daily was compared to enoxaparin in a single daily dose of 40 mg, while in the RECORD IV trial enoxaparin at a dose of 30 mg twice daily was administered [24]. The occurrence of DVT, symptomatic or venographically proven asymptomatic, non-fatal PTE and all-cause mortality were registered as the primary efficacy outcome, while the occurrence of major bleeding was monitored as the primary safety outcome.

In the RECORD I, non-inferiority/superiority study examining a group of 4591 patients with THR and treated with the anticoagulants 35 days, it was demonstrated within the primary efficacy outcome that the listed adverse events were registered at significantly lower degree in patients treated with rivaroxaban 10 mg compared to enoxaparin at a dose of 40 mg once daily (1.1% *vs*. 3.7%, $p < 0.001$) [24, 25]. Major episodes of VTE were also lower in the group of patients treated with rivaroxaban compared to enoxaparin (0.2% *vs*. 2.0%, $p < 0.001$). The incidence of major bleeding did not significantly differ between groups (0.3% and 0.1% respectively).

In RECORD II, superiority trial, performed on 2551 patients after THR, rivaroxaban at a dose of 10 mg was administered for 31–39 days, and enoxaparin for 10–14 days. Primary efficacy outcome was revealed in 2% of treated patients with rivaroxaban compared to 9.3% treated with enoxaparin ($p < 0.0001$), without significant difference in bleeding [24, 25].

In the RECORD III, non-inferiority/superiority trial, 2531 patients undergoing TKR were randomized to

thromboprophylaxis with rivaroxaban at a dose of 10 mg once daily and enoxaparin at a dose of 40 mg once daily subcutaneously for 10–14 days in both groups. Within the primary efficacy outcome, significantly lower incidence of adverse events in patients treated with rivaroxaban compared to those treated with enoxaparin (9.6% *vs*. 18.9%, $p < 0.001$) was shown, with comparable rates of significant bleeding [24, 25].

In the RECORD IV randomized non-inferiority/superiority study, which examined 3148 patients after TKR, unlike RECORD III study, the active comparator enoxaparin was administered in the dosage regimen of 30 mg twice daily. The primary efficacy outcome was registered in 6.9% of patients treated with rivaroxaban, as opposed to 10.1% of those treated with enoxaparin ($p = 0.012$), without significant difference in the incidence of major bleeding (0.7% *vs*. 0.3%, respectively) [24, 25].Briefly, single daily oral administration of rivaroxaban was more effective than enoxaparin in North American and European doses in the prevention of VTE after major orthopedic interventions [25].

For orthopedic patients who will not be operated having various forms of immobilizations, the results of the MAGELLAN and MARINER trials are of clinical importance [26, 27]. In the MAGELLAN study with hospitalized medically ill patients, rivaroxaban 10 mg daily was non-inferior to enoxaparin 40 mg daily, while rivaroxaban during 35 days was superior to enoxaparin (administered for 10 days) for the prevention of VTE [26]. In the MARINER study with medically ill patients at increased risk for VTE, after discharge thromboprophylaxis with rivaroxaban 10 mg daily resulted in lower incidence of non-fatal VTE compared to placebo (0.18% *vs*. 0.42%; HR 0.44; 95% CI, 0.22–0.89). Composite of symptomatic VTE or VTE-related death and major bleeding rates were comparable between rivaroxaban and placebo [27]. FDA approved rivaroxaban 10 mg once daily in acutely ill, hospitalized patients at increased risk of VTE, but not at high risk of bleeding [28].

Safety of rivaroxaban administration

Cumulative analysis of all four RECORD studies registered a greater incidence of significant ("major") and clinically relevant ("non-major") bleeding in the rivaroxaban treated group compared to enoxaparin [24]. In RECORD trials calculation of bleeding did not include bleeding from the site of surgery, although it may contribute to major bleeding, wound infection, and the need for reoperation [23, 24].

Rivaroxaban was demonstrated to be more effective than enoxaparin, but with a significantly higher rate of major and clinically relevant "non-major" bleeding [25]. By reviewing 89 publications, rivaroxaban was found to be non-inferior to enoxaparin in thromboprophylaxis after major orthopedic surgery [18]. A meta-analysis of eight randomized clinical studies with 15,596 patients after elective hip and knee surgery showed that patients treated with rivaroxaban had a lower rate of VTE and a lower overall mortality by 44% (RR 0.56; 95% CI 0.39–0.80) compared to enoxaparin. However, prophylaxis with rivaroxaban caused an increased risk of major bleeding by 65% (RR 1.65; 95% CI 0.93–2.93) and clinically relevant bleeding by 21% (RR 1.21; 95% CI 0.98–1.5), which was not statistically significant [29].

Contraindications for the use of rivaroxaban are the following: hypersensitivity to the active substance, clinically significant active bleeding, liver disease associated with coagulopathy and clinically significant bleeding risk, renal insufficiency defined by creatinine clearance < 15 ml/min, concomitant therapy with anticoagulant drugs, pregnancy and lactation [21, 24].

APIXABAN

Pharmacotherapy group and Anatomical Therapeutic Chemical classification

Apixaban belongs to the B01AF subgroup of direct factor Xa inhibitors within the B01A group of antithrombotic drugs [19].

A brief history of the drug

Since 2007, the synthesis of apixaban has been achieved in collaboration with Bristol-Myers Squibb and Pfizer [30]. It has been approved in Europe since 2012 for the prevention of VTE after THR and TKR. The FDA approved apixaban to reduce the risk of thrombosis after THR and TKR in 2014.

Pharmacodynamics

Mechanism of action of the drug

Apixaban is an oral, selective, reversible, direct factor Xa inhibitor that blocks the central protease of the coagulation pathway of factor X, common for the intrinsic and extrinsic pathway of the coagulation cascade. Apixaban inactivates the free factor Xa, the clot-connected factor Xa and prothrombinase complex, responsible for the conversion of prothrombin into thrombin, thereby reducing the formation of thrombin, but not affecting the existing levels of thrombin [31]. Apixaban incompletely inhibits thrombin production by reversibly binding to factor Xa, allowing the formation of a small amount of thrombin needed for the physiological regulation of haemostasis. Consequently, apixaban has a lower risk of bleeding compared with direct thrombin inhibitors [31].

Pharmacokinetics

After oral administration, the maximum concentration of apixaban is achieved after 3–4 hours, and stable concentrations are expected on the third day. Absolute bioavailability is around 50% for doses up to 10 mg [32]. Food intake does not affect AUC or C_{max} of apixaban at a dose of 10 mg, thus the drug can be administred regardless of the meal [32].

Apixaban is 87% bound to plasma proteins, and the volume of distribution is 21 L (Table 1) [32].

Table 1. Pharmacokinetic characteristics of new anticoagulant drugs

Characteristics	RIVAROXABAN	APIXABAN
Absorption	High bioavailability 80–100%	Medium bioavailability 50%
Half-life	5–9 h*/11–13 h**	12 h (8–15 h)
Renal elimination	33%	27%
Hepatic elimination	67%	73%

*generally in young healthy individuals
**generally in the elderly

It is eliminated by multiple pathways (urinary in 27%, non-renal pathways in 73%, mainly fecal and by bile) [32]. The drug metabolism is mediated by CYP3A4/5 and partly via CYP1A2, 2C8, 2C9, 2C19, and 2J2 isoenzymes [32]. The total clearance is 3.3 L/h and the half-life is 12 hours [32].

Intereactions

Multiple elimination pathways suggest that the potential for the interaction of apixaban and other drugs is relatively low [31]. The concomitant use of potent CYP3A4 and P-gp inhibitors, such as azole antimycotics (e.i. ketoconazole, itraconazole, etc.) and HIV protease inhibitors (e.i. ritonavir) are not recommended, while inducers such as phenytoin, carbamazepine, phenobarbital and St. John's wort can be used with increased caution because they decrease the plasma concentration of apixaban and reduce its effect [31].

Clinical pharmacology

Dosage and indications

Apixaban is indicated for primary VTE prophylaxis after THR and TKR at a dose of 2.5 mg twice a day, with a first dose intake 12–24 hours after surgery. The recommended therapy after THR is 32–38 days, and after TKR 10–14 days [32].

Efficacy

ADVANCE-I (Apixaban Dose Orally *vs*. ANtiCoagulation with Enoxaparin), a randomized non-inferiority study performed in 3195 patients after TKR, compared the administration of apixaban at a dose of 2.5 mg twice daily to enoxaparin 30 mg twice a day during 10–14 days. The incidence of total VTE and all-cause mortality (primary efficacy outcome) was comparable in both investigated groups with a similar frequency (9% *vs*. 8.8%) [24, 25].

In the ADVANCE II randomized non-inferiority study involving 3057 patients after TKR apixaban at a dose of 2.5 mg twice daily (initiated 12–24 hours after surgery) was compared to enoxaparin in European doses, 40 mg once daily (introduced 12 hours preoperatively). The primary efficacy outcome (total VTE and all-cause mortality) was observed at a significantly lower rate in patients treated with apixaban compared to enoxaparin (15.1% *vs*. 24.4%, p = 0.001, respectively).

In ADVANCE III study, prophylaxis of VTE in patients undergoing THR, primary outcome was registered in 1.4% of patients treated with apixaban and 3.9% treated with enoxaparin in European doses of 40 mg daily (p < 0.001) [33].

Concerning the previous studies on the efficacy of apixaban in the prevention of VTE, one can conclude that the superiority and comparable safety profile of apixaban versus European doses of enoxaparin (40 mg once daily) was demonstrated, but apixaban was not effective enough compared to the North American doses of enoxaparin (30 mg twice daily) [25]. Efficacy of apixaban at a dose of 2.5 mg twice daily in the prevention od VTE in patients undergoing THR and TKR in ADVANCE II and ADVANCE III were not followed by significant increase in the degree of major and clinically relevant bleeding [34].

The results of the meta-analysis of Gómez-Outes et al. [35], indicated that rivaroxaban had a significantly lower risk of symptomatic VTE compared to enoxaparin (RR 0.48; 95% CI; 0.31–0.75; p = 0.001); compared to insignificant decrease with apixaban (RR 0.82; 95% CI; 0.41–1.64; p = 0.57) and dabigatran (RR 0.71; 95% CI; 0.23–2.12, p = 0.54), with more frequent clinically relevant bleeding with rivaroxaban (RR 1.25; 95% CI 1.05–1.49, p = 0.01) and dabigatran, but less often with apixaban (RR 0.82; 95% CI; 0.69–0.98; p = 0.03). Another meta-analysis with 20 studies and 38,507 patients demonstrated a risk of total VTE significantly lower with apixaban (RR 0.62; 95% CI; 0.47–0.81, p = 0.001) and rivaroxaban (RR 0.7; 95% CI 0.6–0.81, p < 0.001) compared to enoxaparin [36].

Risk of VTE and total mortality is significantly reduced by both rivaroxaban (RR 0.56; 95% CI 0.39–0.80, p = 0.002), and apixaban (RR 0.63; 95% CI 0.42–0.95; p = 0.03) [35]. When data obtained for total symptomatic VTE were analyzed separately for symptomatic DVT and pulmonary embolism, both rivaroxaban (RR 0.4; 95% CI 0.22–0.72; p = 0.002) and apixaban (RR 0.41; 95% CI 0.18–0.95; p = 0.04) significantly reduced the risk of DVT [35].

In the meta-analysis of Gómez-Outes et al. [35], apixaban significantly increased the risk of symptomatic PTE following knee arthroplasty compared to enoxaparin (RR 2.56, 95% CI 1.1–5.98; p = 0.03), unlike rivaroxaban showing an insignificant trend in the reduction of symptomatic PTE (RR 0.89; 95% CI 0.3–2.67, p = 0.84). However, meta-analysis of Ma et al. [37] on six randomized studies with 13,790 patients pointed out that there was no significant difference in the incidence of PTE after knee arthroplasty between apixaban and enoxaparin, (RR 2.0; 95% CI, 0.97–4.12, p = 0.06) as well as between apixaban and enoxaparin in larger North American prophylactic doses of 30mg b.i.d. (RR 1.65; 95% CI, 0.77–3.54, p = 0.2). In addition, the same meta-analysis did not find significant difference between rivaroxaban and enoxaparin in the incidence of pulmonary embolism [37].

Safety profile of apixaban

The rate of all-bleeding and major bleeding of ADVANCE-I, was lower in patients treated with apixaban compared to

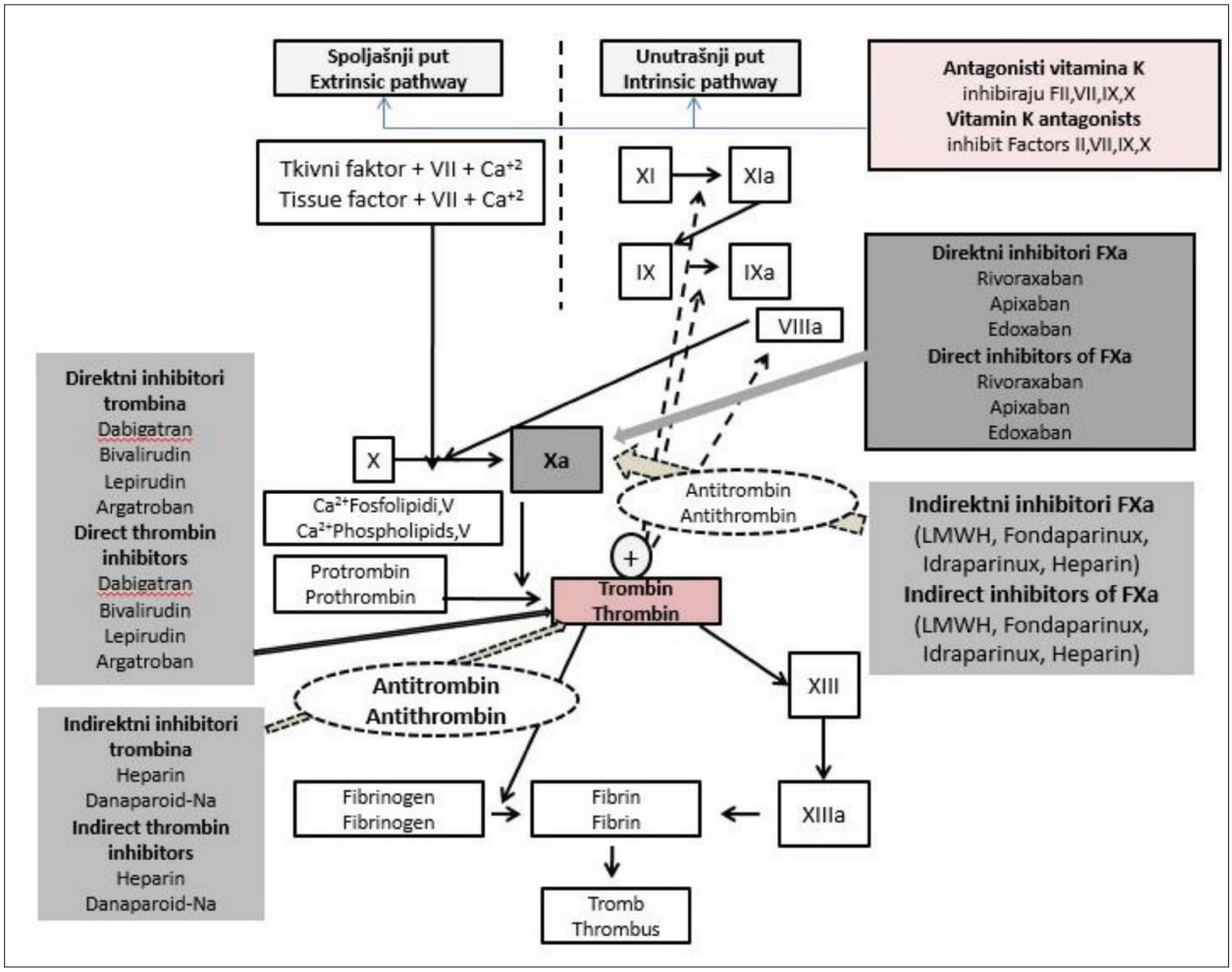

Figure 1. The mechanism of action of anticoagulant drugs

enoxaparin, with the difference even becoming significant for composite of major and clinically relevant non-major bleeding (2.9% *vs.* 4.3%, p = 0.03) [24, 25].

ADVANCE II and ADVANCE III study showed a comparable rate of bleeding in the group treated with apixaban and enoxaparin, also in the case of significant bleeding [23, 33].

The duration of prophylaxis with apixaban was consistent with guidelines, 32–38 days after hip surgery and 10–14 days after knee surgery [33].

When compared to enoxaparin administered at a dose of 30 mg b.i.d. subcutaneously and commenced 12–24 hours after surgery, in patients with elective hip operation in ADVANCE I study, apixaban showed that it did not meet the criteria of non-inferiority compared to enoxaparin but that it had a lower rate of bleeding [32]. ADVANCE trials with apixaban included bleeding from the site of the surgical incision [25].

The results of comparison between apixaban 2.5 mg b.i.d. for 30 days and enoxaparin 40 mg q.d. for 6–14 days in thromboprophylaxis of non-surgical patients (ADOPT study – Apixaban Dosing to Optimize Protection from Thrombosis), indicated that there was no statistically significant difference in the rate of symptomatic DVT but showed significantly higher degree of major bleeding in apixaban group [38].

When major and non-major clinically relevant bleeding in patients treated with apixaban and enoxaparin are analyzed separately, only trends towards fewer bleeding in patients treated with apixaban are observed [38]. In comparison with enoxaparin, the risk of significant major or clinically relevant non-major bleeding is higher with rivaroxaban (RR 1.52; 95% CI; 1.14–2.02, p = 0.004), while comparable bleeding rates with apixaban and enoxaparin were demonstrated after arthroplastic surgery [36, 39].

Apixaban is contraindicated in patients with allergy to apixaban and substances present in the form of a final drug, those with severe hepatic impairment followed by coagulopathy and clinically relevant bleeding risk, and clinically significant active bleeding [32, 33]. Apixaban is not recommended in severe renal insufficiency with creatinine clearance < 15 ml/min. Caution is advised in patients with creatinine clearance of 15–30 ml/min [33].

The advantages of rivaroxaban and apixaban over other drugs from the same Anatomical Therapeutic Chemical group

Oral administration is the advantage of rivaroxaban and apixaban compared with indirect factor Xa inhibitors (fondaparinux, LMWH and UFH) requiring parenteral administration [31]. A lower number of interactions with drugs and foods, the absence of the need for routine laboratory monitoring and a more stable and predictable therapeutic effect have advantages over VKA [13, 32]. Direct inhibitors directly bind to the catalytic site of factor Xa (rivaroxaban and apixaban), while indirect inhibitors (fondaparinux, LMWH, and idraparinux) bind to antithrombin and potentiate antithrombin mediated anti-Xa activity [31].

Section from the guidelines for the prevention of vte with focus on Rivaroxaban and apixaban

In addition to the previously known drugs (i.e. VKA, LMWH), apixaban, dabigatran, rivaroxaban and fondaparinux are recommended for patients undergoing elective knee arthroplasty at least 10–14 days and for elective hip artoplasty 35 days optimally, according to American College of Chest Physicians guidelines, Thrombosis Canada and National Institute for Health and Care Excellence guidelines [23, 40, 41].

The development of anticoagulant drugs of higher quality enables, depending on the characteristics of the patients and the particular pharmacokinetic properties of each drug, better therapy individualization than before the appearance of new anticoagulants. The most important pharmacokinetic characteristics of these new anticoagulant drugs are provided in Table 1 [21, 32, 42, 43].

In the event of moderate bleeding caused by the use of apixaban and rivaroxaban, local hemostasis measures and standard general hemorrhage measures with supstitution of certain blood products are taken. In case of a life-threatening bleeding for patients treated with rivaroxaban and apixaban, andexanat alfa, a first-in-class recombinant modified factor Xa protein, has been approved in Europe and the USA for reversal of anticoagulant activity [44, 45]. Currently, the use of andexanet alfa is limited due to the availability and cost. Prothrombin complex concentrate, activated prothrombin complex concentrate, and recombinant activated factor VIIa may be also administered [45].

CONCLUSION

In conclusion, rivaroxaban, a selective oral factor Xa inhibitor, is more effective than enoxaparin administered in both of the mentioned regimens in VTE prophylaxis in major orthopedic interventions. Although some studies state that there is no difference in the incidence of hemorrhagic complications, certain meta-analyses have shown a greater incidence of haemorrhage with rivaroxaban. Apixaban, an oral reversible factor Xa inhibitor, has proved more effective in the prevention of VTE compared to European doses of enoxaparin, and almost as effective as North American doses. No significant differences in bleeding rates have been identified, although one study (ADVANCE I) indicates a lower incidence of bleeding in those treated with apixaban [24].

New research is required in order to clarify the contradictory results of certain statistical analyzes in recent meta-analyses comparing the mentioned factor X inhibitors and the different regimens of comparator enoxaparin, related to the risk of symptomatic PTE and overall bleeding after major orthopedic surgeries [35, 36, 37].

It remains a challenge for future studies and clinical practice to determine the place of factor Xa inhibitors, rivaroxaban and apixaban in the prophylaxis of VTE, to define undoubted benefits and to remedy their deficiencies, bearing in mind the pharmaco-economic aspect and the unavoidable social dimension of VTE as one of the leading causes of mortality and morbidity in the world.

NOTE

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Превенција венског тромбоемболизма ривароксабаном и апиксабаном у ортопедској хирургији

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САЖЕТАК

Бројна ограничења и нежељена дејства стандардних антикоагулантних лекова намећу потребу за применом нових антикоагулантних лекова. Перорални антикоагулантни лекови из групе инхибитора фактора *Xa* имају више предности, од којих су најважније непостојање посебних ограничења у исхрани, значајно мањи број интерреакција са лековима, рутинска примена без посебног лабораторијског мониторинга, стабилнији и предиктабилнији терапијски ефекат. Ривароксабан, селективни перорални инхибитор фактора *Xa*, ефикаснији је од еноксапарина у профилакси венског тромбоемболизма при великим ортопедским интервенцијама. И поред тога што поједине студије наводе да нема разлике у настанку хеморагијских компликација, одређене метаанализе са ривароксабаном показале су већу учесталост хеморагија. Апиксабан, перорални реверзибилни инхибитор фактора *Xa*, показао се ефикаснијим у превенцији венског тромбоемболизма у односу на европске дозе еноксапарина, а готово је подједнако ефикасан као северноамеричке дозе. Нису утврђене значајније разлике у степену крварења, мада студија *ADVANCE-I* указује на мању учесталост крварења код оних лечених апиксабаном. Ради разјашњења контрадикторних резултата у новијим метаанализама у односу на поређење наведених инхибитора фактора *X* и различитих режима компаратора еноксапарина, као и у односу на ризик за симптоматски плућни тромбоемболизам и укупна крварења после великих ортопедских операција, биће неопходна нова истраживања. Специфичности ривароксабана и апиксабана, који према савременим препорукама већ чине саставни део протокола профилаксе венског тромбоемболизма у великим ортопедским интервенцијама, омогућиће успостављање персонализованих протокола како би се успоставио бољи сигурносни профил код сваког болесника.

Кључне речи: превенција; венски тромбоемболизам; ривароксабан; апиксабан

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REVIEW ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Genetic basis of otosclerosis

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SUMMARY
Introduction Otosclerosis is a disorder of the bone labyrinth and stapes resulting in conductive hearing loss. The genetic basis of otosclerosis still remains unknown.
We aimed at reporting a comprehensive review of up-to-date knowledge on genetic basis of otosclerosis.
Methods Narrative literature review was undertaken to summarize the data about genetics of otosclerosis.
Results Genetics of otosclerosis has not been studied extensively and the literature on this topic is scarce. However, knowledge of genetic basis of otosclerosis is recently increasing. We have presented an overview of the knowledge of association of genetic markers with otosclerosis, gained from linkage analyses, candidate-gene studies, and modern high-throughput genomic studies.
Conclusion Due to its complex pathophysiology, otosclerosis is not a disease whose genetic base will be easily understood. Multiple omics analysis and bioinformatics will lead to elucidation of genetic basis of otosclerosis.
Keywords: otosclerosis; genetics; linkage analyses; candidate-gene studies; high-throughput genomic studies

INTRODUCTION

Otosclerosis is a disorder of the bone labyrinth and stapes known to affect only humans. Unlike all other bones in the body, the human otic capsula undergoes very little remodeling following development. Otosclerosis is a process of pathologic remodeling within a bone that is normally refractory to remodeling. The foci of otosclerotic bone are silent clinically, until the movement of stapes is impaired by invasion of the stapediovestibular articulation. Otosclerosis is a disease of inflammatory character where there is abnormal bone production in the otic capsule and around the base of the stapes. It is one of the most common causes of conductive hearing loss [1]. Its etiology is not fully understood. Some of the proposed causes are genetics, viral diseases and autoimmune reactions [2]. Among viral agents, measles are prominent.

The primary symptom produced by the otosclerotic lesions is conductive hearing loss, usually bilateral, with the onset between the age 20 and 30 years. The magnitude of the hearing loss is directly related to the degree of fixation of the stapes' footplate [3]. The patients with otosclerosis may also exhibit vestibular disturbance.

Some studies have demonstrated the effect of vitamin D deficiency and of some heavy metals, especially calcium, on the progression of otosclerosis [4]. Therefore, it is speculated that dietary regime for patients with otosclerosis could be based on fruits and vegetables containing specific nutrients [5].

Surgical treatment, such as stapedectomy, is used in the treatment of otosclerosis, but the disease can also be managed through hearing amplification with hearing aids [6].

The genetic cause underlying otosclerosis development still remains unknown. The fact that otosclerosis affects multiple members in large families indicates its strong genetic foundation. Also, sporadic forms of otosclerosis have been identified. Linkage analysis have been used to identify genetic loci/genes responsible for otosclerosis in families segregating an autosomal dominant form of the disease [7]. So far, eight genetic loci have been mapped, designated *OTSC1-5, OTSC7-8* and *OTSC10* loci [8, 9]. Although a genetic cause is evident in otosclerosis, none of the genes involved in otosclerosis development have been proven in these loci. Several genetic association studies have been conducted to determine genetic background of the sporadic forms of otosclerosis. Quite a few genes and molecular pathways have been implicated in otosclerosis development, playing roles in bone metabolism, immune system, inflammation, and endocrine system [8, 10, 11, 12]. Candidate gene studies were directed by considering physiologic processes that could be important for otosclerosis development. For that purpose, genes and proteins involved in bone remodeling were among the first to be analyzed [12–15]. Also, the "building

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Table 1. Candidate genes associated with otosclerosis

Gene	Full name of the gene	Protein function	Type of the study	Reference
COL1A1	Type I collagen A1	strengthens and supports many tissues in the body, including cartilage, bone, tendon, skin, and the white part of the eye (the sclera)	association study	[16]
			case control studies	[17, 18, 31, 32, 33]
COL1A2	Type I collagen A2	encodes the pro-alpha2 chain of type I collagen whose triple helix comprises two alpha1 chains and one alpha2 chain	case control study	[32]
COL2A1	Type II collagen A1	encodes the alpha-1 chain of type II collagen, a fibrillar collagen found in cartilage and the vitreous humor of the eye.	animal studies, case reports	[34, 35]
ACE	Angiotensin converting enzyme	central component of the renin–angiotensin system (RAS), which controls blood pressure by regulating the volume of fluids in the body. It converts the hormone angiotensin I to the active vasoconstrictor angiotensin II	case control studies	[36, 37]
AGT	Angiotensinogen	component of the renin-angiotensin system (RAS), a hormone system that regulates blood pressure and fluid balance	case control studies	[30, 36]
ATII	Angiotensin-II	hormone that may act on the central nervous system to regulate renal sympathetic nerve activity, renal function and blood pressure	case control study	[38]
ATIIR	Angiotensin-II receptor	receptor for angiotensin-II	case control study	[38]
RELN	Reelin	extracellular matrix protein	genome-wide association studies	[41]
			case control study	[13, 15, 29, 42, 43]
TGFB1	Transforming growing factor beta 1	multifunctional cytokine family	case control studies	[12–15, 29]
BMP2	Bone morphogenetic protein 2	multi-functional growth factors that belong to the transforming growth factor beta	case control studies	[12–15]
BMP4	Bone morphogenetic protein 4	multi-functional growth factors that belong to the transforming growth factor beta	case control study	[12–15]
SERPINF1	Serpin peptidase inhibitor, clade F	pigment epithelium-derived factor – potent inhibitor of angiogenesis	family study - whole exome sequencing	[44]
CD46	Cluster of differentiation cell surface antigen 34	measles virus receptor, transmembrane phosphoglycoprotein	case control study	[40]
HLA-B40	Human leukocyte antigen	human leukocyte antigen (HLA) proteins complex	case control study	[39]

blocks" of the skeletal system have been legitimate candidates for investigation [16, 17, 18].

We aimed at reporting a comprehensive review of up-to-date knowledge on the genetic basis of otosclerosis.

SEARCH STRATEGY

We used an approach characteristic for a narrative review, consisting of critical analysis of the literature published in electronic or paper-based journal articles. We searched the PubMed database from 1980 to December 2018 using the terms "otosclerosis" AND "gene" OR "genetic" OR "familial" [19]. Studies were identified from the titles and abstracts by the primary reviewers (BZ, JG) and the secondary reviewer (SP). The ethical norms of the Declaration of Helsinki were followed.

GENETIC BASIS OF OTOSCLEROSIS: AN OVERVIEW

Here we present an overview of the knowledge of association of genetic markers with otosclerosis, gained from the association studies of genetic markers and otosclerosis using diverse methodology, starting from linkage analyses, through candidate-gene studies, to modern high-throughput genomic studies, such as genome-wide association studies (GWAS), microarray, and next generation sequencing (NGS).

LINKAGE ANALYSES

Linkage analyses have contributed to the identification of genetic loci associated with otosclerosis. Eight genetic loci have been mapped, designated *OTSC1-5*, *OTSC7-8*, and *OTSC10* loci. *OTSC1* locus was mapped on chromosome 15q25-q26 and contains 33 genes. It was identified in large Indian and Tunisian families [20, 21]. *OTSC2* locus with 152 genes was located on chromosome 7q34-36 and found in Belgian and British families [22]. *OTSC3* locus was mapped to 6p21.3-22.3 and identified in Cypriot and Tunisian families [23]. This locus contains 488 genes and maps to the major histocompatibility complex (MHC) locus. One study revealed that the MHC locus have been associated with otosclerosis in Greek patients with otosclerosis [24].

OTSC4 locus comprising 74 genes was located on chromosome 16q21-23.2 and described in an Israeli family [25]. *OTSC5* locus with 59 genes was defined in a Dutch family and located on chromosome 3q22-24 [26]. *OTSC7* locus containing 66 genes was mapped to chromosome 6q13-16.1 and identified in a Greek family [27]. *OTSC8* locus with 24 known and 121 predicted genes was mapped in a Tunisian family to chromosome 9p13.1-9q21.11 [28]. *OTSC10* locus was identified in a Dutch family on 1q41–44 chromosome and it comprised 306 genes/predicted genes [9]. Loci *OTSC6* and *OTSC9* have been reserved by the Human Genome Organization Gene Nomenclature Committee but are still not published. Although a genetic cause is evident in otosclerosis, none of the genes involved in otosclerosis development have been proven in these loci.

CANDIDATE-GENE STUDIES

Additionally, several genes have been shown to be disease-causing/disease-related for otosclerosis. Genes influencing dysregulation of bone remodeling within the otic capsule were investigated as otosclerosis has been identified as the primary disorder of bone remodeling. Bone morphogenetic proteins (BMPs) and TGF-β1, members of the TGF-β superfamily, are critical regulators of bone turnover. These proteins work as specific growth factors and also as inflammatory cytokines. Out of 20 different BMPs identified so far, only BMPs 2–7 have a central role in the embryonic endochondral bone development and later in new bone formation and repair [13]. Elevated expression levels of BMPs contribute to the increased bone turnover in active phases of otosclerosis. A significant association between clinical otosclerosis and variants in *BMP2* and *BMP4* genes was demonstrated. However, it is concluded that rare variants in *BMP2* and *BMP4* are not a major genetic component in the otosclerosis, at least in the German otosclerosis population [14]. Nonfunctional variants influence reduced downstream *BMP* signaling, namely phosphorylation of Smad1/5/8 [14]. As for the other gene involved in the regulation of bone turnover, *TGF-β1*, a significant association between variants in *TGF-β1* and clinically and histologically confirmed otosclerosis in Hungarian and in British populations was found [15, 29].

Type I collagen *COL1A1* gene was already shown to be involved in the development of osteogenesis imperfecta, a disease similar to otosclerosis by its major characteristics, namely conductive hearing loss [30]. An American study demonstrated a significant association of otosclerosis and *COL1A1* [16]. The same group further revealed a significant association between clinical otosclerosis and the Sp1 binding site variant in the first intron of *COL1A1* [17]. A replication study on German patients with otosclerosis identified haplotypes including the Sp1 binding site variants associated with otosclerosis [18]. A significant association between otosclerosis and *COL1A1* were additionally confirmed in Egyptian, Turkish, and Tunisian studies [31]. Despite finding that in Spanish samples of 100 cases and 100 matched controls the previous findings were not replicated [32], results of a comprehensive meta-analysis are supporting the fact that *COL1A1* and otosclerosis have been strongly associated [33].

Variations in *COL1A2* gene could indirectly influence *COL1A1,* thus possibly impacting otosclerosis as well. A German study showed that some of the *COL1A1* otosclerosis-associated variants alter binding of transcription factors that regulate transcription of *COL1A2* [18]. They have confirmed that targeted deletion of *COL1A2* in the mouse model leads to a mild conductive hearing loss. However, a Spanish group found no evidence in favor of *COL1A2* genes association with otosclerosis [32]. Type II collagen, the main collagen of cartilage, is encoded by the *COL2A1* gene. Autoreactivity to COL2A1 was proposed as a cause of otosclerosis [34]; nevertheless, this finding has been revisited [35].

Association with genes in the renin-angiotensin-aldosterone system and otosclerosis was also demonstrated [36]. Variants in angiotensinogen gene (*AGT*) and angiotensinogen converting enzyme gene (*ACE*) in a French-Caucasian cohort were related to higher plasma concentrations of ACE and also associated with a higher risk of otosclerosis development. A replication study done in a larger Belgian-Dutch population was unable to confirm these findings [37]. Also, four members of the activation pathway of AGT cascade were not expressed at protein level in otosclerotic stapes footplates [38]. However, it is possible that other variants in renin-angiotensin-aldosterone system could be associated with otosclerosis.

Class I MHC has been described in otosclerosis when *OTSC3* was mapped and the frequency of HLA-B40 was significantly lower in patients with otosclerosis than in healthy blood donors [28, 39].

Additionally, an environmental factor, namely persistent measles infection, was implicated in the development of otosclerosis [2]. A novel splice variant in the *CD46* receptor gene was detected and a potential association between measles virus infection and otosclerosis was demonstrated [40].

HIGH-THROUGHPUT GENOMIC STUDIES

Genomic studies performed using high-throughput methodology are not numerous, but their contribution to the elucidation of the genetic basis of otosclerosis is significant. One of these studies represents the first successful genome-wide association study for a hearing impairment [41].

A genome-wide association study recognized a significant association of the *RELN* gene, coding for an extracellular matrix protein important in brain development and synaptic plasticity, and the pathogenesis of otosclerosis [41]. This association was observed in various European and non-European populations [29, 42, 43]. Nevertheless, variants in the *RELN* gene might be associated with a specific otosclerosis-like phenotype, clinically not distinguishable from otosclerosis [38]. The *RELN* gene is not actively expressed in adult stapes footplates and further studies are needed to determine the role of *RELN* in the pathophysiology of otosclerosis [13].

A whole-exome sequencing study in four families with inherited otosclerosis identified several rare heterozygous *SERPINF1* (serpin peptidase inhibitor, clade F) variants suggesting that it may be a common pathogenic pathway in the otosclerosis development [44]. *SERPINF1* gene encodes PEDF (pigment epithelium-derived factor), which is shown to be involved in the regulation of bone density and inhibition of angiogenesis.

The review of the literature that covers candidate genes associated with otosclerosis is presented in Table 1.

CONCLUSION

Otosclerosis is a complex genetic disorder, without a clearly defined genetic basis. Genetics of otosclerosis has not been studied extensively and the literature on this topic is scarce. High-throughput methodology, such as NGS, has transformed genetic and biomedical research, enabling genetic profiling of each patient. Nowadays, it is widely used for diagnostics, monitoring, and administering appropriate molecular-targeted and gene therapy for many diseases. As for research, NGS has become a powerful tool in GWAS. It contributes to the discovery of new disease-causing and disease-related genetic markers of complex rare diseases, which could be implemented in clinical practice [45]. Using NGS in families affected by otosclerosis, analyzing disease-affected and disease-nonaffected family members, sharing the same genetic background, can add to understanding the molecular basis of the disease [46].

However, due to its complex pathophysiology, otosclerosis is not a disease whose genetic base will be easily understood. High-throughput genotyping will provide a vast amount of data, but more effective data analysis tools are missing [47, 48]. Databases that contain genomic variant data, collected using the microattribution approach principle, could assist in elucidating the genetic basis of complex rare diseases [49, 50]. Unquestionably, bioinformatics is a bottleneck of research in finding reliable genetic associations with otosclerosis. Also, gene–gene and gene–environment interactions should be considered. Multiple omics analysis, including epigenomics, transcriptomics, proteomics and radiomics, together with powerful bioinformatics will eventually lead to implementation of the knowledge on genetic basis of otosclerosis in clinical practice.

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Генетичка основа отосклерозе

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САЖЕТАК

Увод Отосклероза је поремећај коштане капсуле лавиринта и слушних кошчица ува, који доводи до губитка слуха због немогућности провођења звука. Генетички узрок отосклерозе је непознат.

Циљ овог рада је био да се сачини свеобухватни преглед савремених сазнања о генетичкој основи отосклерозе.

Методе За приказ података о генетици отосклерозе коришћен је наративни преглед литературе.

Резултати Генетика отосклерозе није много изучавана и литературни подаци о генетичкој основи отосклерозе су оскудни. Међутим, у новије време, проширују се знања о генетичкој основи отосклерозе. Овде је приказан преглед знања о асоцијацији генетичких маркера и отосклерозе, која су резултат анализа генетичке повезаности, као и асоцијативних студија гена кандидата и свеобухватних анализа генома.

Закључак Отосклероза због своје комплексности није болест чија ће генетичка основа бити лако расветљена. Анализе омика и биоинформатика ће допринети разумевању генетичке основе отосклерозе.

Кључне речи: отосклероза; генетика; анализа генетичке повезаности; студија гена кандидата; свеобухватна анализа генома

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REVIEW ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Genotoxicity test methods – a tool for DNA and chromosome damage biomonitoring

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SUMMARY

Nowadays, life is highly influenced by intense growth of various industries, high levels of pollution, and other environmental factors with harmful effects on human health. Therefore, cytogenetic monitoring is essential for detection of changes in the structure of chromosomes, which occur because of the effects of various genotoxic agents. In this review, we shall apprize the theoretical and experimental aspects of several tests for assessment of genotoxicity in humans such as Micronucleus assay, Comet assay, Chromosomal aberrations assessment and Sister chromatid exchanges analysis. These methods are accepted by the World Health Organization as standard tests for genotoxicological screening in humans. The methods are sensitive and confirm the cellular genotoxic effects of various genotoxicants.

Keywords: genotoxicology; cytogenetic screening; biomonitoring; Micronucleus assay; Comet assay

INTRODUCTION

On a daily basis, humans are directly exposed to various genotoxic agents of physical or chemical nature, both professionally and incidentally, or they occur due to their different lifestyle, habits, or addictions. Genotoxic agents, which are present in our immediate surroundings, represent a potential health risk for each exposed individual, and increase the risk of various non-communicable diseases, especially cancers. The effect on each exposed individual depends on the degree of exposure to a given factor, the way of the genotoxicant is eliminated and the genetically determined differences among individuals [1, 2, 3]. Some of the human genotoxicants come as pollutants from technological processes or from uncontrolled manufacturing of certain chemical substances and their products [4–8]. Genotoxic substances that are present in various manufacturing processes mainly represent a direct hazard to the workers involved in the process, as well as to local population in these industrial areas. Agents that are produced during manufacturing processes and that eventually enter the composition of those products, represent a potential health risk for a larger population, which include the consumers of these products. This group of potentially genotoxic agents includes acrylamide, organic solvents, organic compounds of metals, and heavy metals. The main features of genotoxic agents are essential for understanding their toxic effects, as well as the comprehension of the transport dynamics, distribution, and excretion from the body [2, 8]. Genotoxic agents may be broadly classified into factors of chemical nature (acrylamide, polychlorinated organic compounds, pesticides, organic solvents, and heavy metals) or factors of physical nature [short-wavelength electromagnetic energy such as ultraviolet radiation and ionizing radiation (IR)]. IR is one of the main sources of genotoxicity [2, 6]. Some genotoxic agents are capable of damaging DNA due to their chemical and physical features. The harmful effects of genotoxic agents for mutations induction do not necessarily manifest in the organism that is exposed to them, sometimes not even in the first generation. Instead, these harmful effects can be manifested in the following generations. There is an evidence that long-term exposure to low doses of certain genotoxicants induce changes in the structure of chromosomes that would not be phenotypically visible, but can be passed on to the future generations. However, while high doses of these agents are lethal or toxic, small doses are cumulative, and mutagenic effects are activated or manifested in the next generations [7, 8].

Genetic toxicology (or genotoxicology) studies the impact of genotoxic agents on the process of transmission of inherited traits, with particular emphasis on the possible health effects. It also studies the mechanisms of genetic damage, the substances that cause it, and improves the methods and experimental models that can determine such changes [7, 8, 9]. Potential changes in the chromosomes include chromosome breakage and rearrangement of the fragments as a result of destruction of chromosomal structure [9, 10, 11]. Genotoxic effects of various agents can be detected with several tests for genotoxicity assessment. These tests have the ability to reveal damage in the DNA molecule, as well as the changes in the structure and the number of chromosomes, which are extremely important for the transmission of hereditary traits and are involved in induction of carcinogenesis [9–17]. In genotoxicology there are several available tests applicable on human cells.

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In this paper we apprize the theoretical and experimental aspects of commonly used tests for assessment of genotoxicity in humans such as Micronucleus Assay (MA), Comet assay (CA), Chromosomal aberrations (CAs) assessment and Sister chromatid exchanges (SCEs) analysis. Our aim is to give a brief review of these genotoxicity testing methods, as a tool for DNA and chromosome damage biomonitoring.

Micronucleus Assay (procedure and principles)

MA is widely used assay for measurement of DNA damage and genotoxicity in the cells. Upon exposure to genotoxic agents, the cell may be damaged and divided, and after that it will form a small micronucleus in addition to the main nucleus. To perform MA, venous blood sample is being collected in heparinized blood collection tubes. In our laboratory, we follow the blood culture protocol according to Fenech [13]. After 44-hour-long incubation of the cells, Cytochalasin B is added to each culture to block cell cytokinesis, and cultures are re-incubated at 37°C for further 28 hours. Cytochalasin B blocks the cytokinesis because it inhibits actin assembly and thus prevents separation of daughter cells after mitosis, leading to formation of binucleated cells. Treatment with Cytochalasin B ensures that only the cells, which were divided, are scored. After 72 hours of cultivation, cells are harvested, fixated, and fixed lymphocytes are stained by Giemsa, and finally examined with light microscopy (×40 and ×100). Micronuclei are independent chromatin structures, completely separated from the core. They are created as a result of condensation of acentric chromosome fragments or whole chromosomes that failed to incorporate into the core of the newly formed nuclei. The average size of micronuclei may vary from 1/3 to 1/16 of the nucleus size. The appearance, as well as the number of micronuclei is an important quantitative biomarker for DNA damage, resulting from various genotoxic agents *in vitro* or *in vivo* [13, 16–21]. During the examination of the microscopic slides it must be taken into account, not to score binucleated cells with irregular shapes or with two nuclei different greatly in size, neither should binucleated cells be confused with poorly spread multi-nucleated cells [22].

The micronuclei are scored as positive if they are distinguishable from the two main nuclei, in case if they are less than one-third the size of the main nuclei, and if they have similar staining intensities to the main nuclei. Cells with irregularly shaped nuclei, more than two nuclei, and those with nuclei of different sizes in a single cell, should not be scored [13, 18].

The number of micronuclei per binucleated cells provides a measure of chromosome breakage. Micronuclei are generated due to exposure to genotoxic agents, especially IR [6, 23]. Increased formation of small and large micronuclei, as an indicator of chromosomal instability, has been found in medical workers who are professionally exposed to IR [24].

Comet Assay (procedure and principles)

The Comet Assay (CA) is rapid and sensitive procedure for quantification of damage and repair of DNA molecules at the level of individual cells [12, 25, 26, 27]. This method is sensitive in detecting low levels of DNA damage (measuring DNA strand breaks) in the cells with absence of mitotic activity. The method does not require a large number of cells per sample, it is inexpensive, relatively easy to apply, and is widely used as a genotoxicity test. However, it is considered optimal for genotoxic effects detection on various agents. The cells embedded in agarose gel on a microscope slide are lysed with detergent and high salt to form nucleoids containing supercoiled loops of DNA linked to the nuclear matrix [5, 12]. Nucleoids are subjected to electrophoresis, resulting in formation of structures, which resemble comets. The former cell nucleus, which does not migrate, and the broken fragments stretched in the electric field, forms the "head" and the "tail" of the comet, respectively. The method itself was named after the characteristic appearance of the nucleoids. The intensity of the comet tail relative to the head reflects the number of DNA breaks. This is due to loops, which contain a break and become free to extend towards the positive charged electrode (anode). Shorter DNA fragments travel faster through the gel, because of the difference in molecular weight. For better understanding of the results from the CA it should be taken into consideration that the tail of the comet is formed by DNA loops attached to the nuclear matrix while cut-off fragments leave the nucleus and could not be observed [12, 25, 26]. In addition, the results by Georgieva et al. [28] are the principle proof that Comet assay may be used for studying the higher-order chromatin structures at a single-cell level. Most often, the gel electrophoresis is followed by fluorescence microscopy in order to visualize the migration of the damaged DNA. Fluorescent microscope connected to a computer, and a special computer software, are used to measure several important parameters of the comets, including their tail length and the percentage of DNA in the tail (tail intensity). The comet tail length reflects the size of the DNA fragments and the level of damage. The tail intensity indicates the portion of the genome that is affected by the damage [25, 26]. In literature, the most commonly mentioned embodiment of CA in alkaline conditions is the method by Singh et al. [29].

In the genotoxicological research, two versions of CA are widely used: the neutral method for detection of DNA double-strand breaks, and the alkaline method, which detects DNA single-strand breaks and alkali-labile lesions [25]. In addition to the staining with fluorescent dyes (propidium iodide, ethidium bromide, SYBR Green and others), the comets can be also stained with the silver staining method [30, 31, 32]. Therefore, the comets can be visible on a conventional microscope, which is an important advantage for some laboratories. However, after the fluorescence staining, the agarose gels could be dried and re-stained with silver, for their documentation and future analyses. Nadin et al. [32] also described modifications of the silver staining method, which significantly increases the sensitivity/reproducibility of CA and preserves the comets for long periods. In 2014, Osipov et al. [33] reported that DNA comets can also be visualized and analyzed using Giemsa staining. They explain the high sensitivity of Giemsa staining with the Romanowsky-Giemsa effect. They propose this staining as a result of the stain photo-stability

and the higher resolution of bright-field imaging compared to fluorescence imaging.

Chromosomal aberrations

Human peripheral lymphocytes, particularly T-lymphocytes are commonly used cells in the studies of genotoxicity [34, 35, 36], since approximately 80% of lymphocytes recirculate throughout the body, which means that lymphocytes are able to leave the peripheral blood, pass through the spleen, lymph nodes and other tissues and re-enter the circulation. Therefore, lymphocytes with damaged DNA arising in any part of the body shall appear in the peripheral blood. CAs are considered biomarkers for assessment of exposure to occupational genotoxicants in human biomonitoring studies.

Scoring the specific unstable CAs, such as acentric fragments, dicentric chromosomes, and ring chromosomes in peripheral blood lymphocytes in persons exposed to various genotoxic agents, is reliable method to detect and possibly measure the exposure [37, 38, 39]. The high rates of CAs are observed in subjects occupationally exposed to low levels of IR [37]. In meta-analysis of cytogenetic studies performed in four Italian laboratories in the period 1965–1993, Bonassi et al. [38] reported significantly higher frequencies of CAs in medical workers exposed to low doses of IR. Garaj-Vrhovac et al. [37] also reported higher rates of CAs in workers exposed to IR, but the differences among those were not significant. Multiple studies have shown a significant correlation between induction of CAs and the risk of cancer [14, 15].

Sister chromatid exchanges

SCEs are currently being considered to be an exposure biomarker on genotoxic agents such as carcinogens. Monitoring their exposure, as DNA damaging agents, their frequency increases substantially so they have been commonly used as an indicator of genotoxic effects in cells. This is an excellent tool for the quantitative and qualitative evaluation of DNA damage, and it detects the genotoxic agents' ability to enhance the exchange of DNA between two sister chromatids. SCEs are defined as a symmetrical exchange at one locus between sister chromatids, and appears to involve DNA breakage and repair mechanism during the S phase of the cell cycle, which does not alter the overall chromosomal morphology. Significant increases of SCEs frequencies have been reported in studies of the human population occupationally exposed to various biohazardous agents, including radiation, dust, fibers, fumes, organic, and inorganic chemicals [39–42].

DISCUSSION

Advantages and disadvantages of genotoxicity test methods

Due to their numerous advantages, the genotoxicity test methods as tools are widely used for DNA and chromosome damage biomonitoring especially in human biomonitoring studies. However, they also have some limitations. MA is a relatively inexpensive method whose micronuclei scoring can be performed on various cell types relevant for human biomonitoring such as lymphocytes, fibroblasts, exfoliated epithelial cells, and other cell types with mitotic activity. However, the main disadvantage of this assay is that it does not detect all structural CAs, but only the acentric fragments. Another disadvantage is the cytotoxicity of Cytochalasin B, which varies among cell types and sometimes even among the subtypes of the same cell type. Next, in order to perform the MA, a high number of cells should be taken (approximately 1000 binucleated lymphocytes per sample). With these improvements the MA will become more sensitive and specific, which will increase its applicability in large-scale screening studies.

One of the most important advantages of CAs is that the DNA damage can be measured in any (nucleated) cell type, whereas the MA is limited to the cells having mitotic activity [14]. The CA is rapid, inexpensive, relatively easy to perform, and detects a broad variety of primary DNA lesions which cannot be identified by other tests. It is sensitive to very low levels of DNA damage, and requires a small amount of cells per sample. Common feature of MA and CA is that both micronuclei and comets appear because of the damage of nuclear DNA. However, Fluorescent in situ hybridization (FISH) analysis promotes better understanding of the mechanisms of their formation, and often complements the MA and CA [7].

Various cytogenetic end-points (including CAs, SCEs, and micronucleus), have already been utilized as biomarkers of cancer susceptibility in non-carriers [43]. Epidemiological evidence supports the predictive value of elevated frequency of CAs in peripheral blood lymphocytes [44]. Indeed, in Nordic [44], Italian [45] and Czech cohort studies [46], the authors evaluated the association between the frequency of CAs, SCEs, or micronucleus in peripheral blood lymphocytes and the subsequent risk of cancer.

Luzhna et al. [47] confirm that hypomethylation of heterochromatin in the pericentromeric regions is related to chromatin decondensation, which leads to improper chromosome segregation and exclusion into micronucleus. Global methylation has been related to more relaxed chromatin, increased gene expression, elevated DNA damage, and chromosomal breaks, which form micronucleus with acentric chromosome fragments. According to this, overall loss of DNA methylation has been proposed as a valid biomarker for cancer. Due to the alteration of DNA methylation patterns has been related to many diseases, including cancer, this alteration has potential for clinical application as a prognostic biomarker. Recently, van Leeuwen et al. [48] developed a transcriptomic network analysis of micronucleus-related genes based on the literature and a case study on children and adults who were differentially exposed to air pollution. Using a pathway tool MetaCore (Clarivate, Philadelphia, PA, USA) the authors retrieved 27 genes and gene complexes involved in micronucleus formation. Such genes were mainly associated with cell cycle checkpoints, spindle assembly, and aneuploidy. In a biochemical approach, repair enzymes in the extract induce

breaks at damage sites; and the breaks are measured with CA. The nature of the substrate lesions defines the repair pathway to be studied [49].

The extent of DNA migration depends directly on the DNA damage present in the cells. It should be noted that DNA lesions consisting of strand breaks after treatment with alkali either alone or in combination with certain enzymes (e.g. endonucleases) increase DNA migration [14]. Spivak et al. [50] suggested the use of comet-FISH assay for examination of the initial DNA damage and subsequent repair in some gene region. Findings by Horvathova et al. [51] suggest that the patterns of migration of domain-specific signals may depend on the localisation of breaks within or around the probed region. In addition, Zeller et al. [52] in their study of human exposure to formaldehyde comparatively investigated it in order to be able to identify a possible effect of the exposure schedule on changes in gene expression.

CONCLUSION

Considering the fact that today's living is highly influenced by intense growth of many industries and environmental pollution, genotoxicity test methods are extremely important for monitoring of the changes in the structure of chromosomes and DNA damage, which occur as a result of the influence of various genotoxic compounds. In this paper we focused on the effects of genotoxic agents on human cells, which can be analyzed with previously noted tests for assessment of genotoxicity. We defined several basic genotoxicity test methods, which can be applied as tools for DNA and chromosome damage biomonitoring in human population.

Conflict of interest: None declared.

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Тестови генотоксичности – алатке за биомониторинг оштећења ДНК и хромозома

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САЖЕТАК

Данашњи живот је под великим утицајем интензивног ефекта различитих индустрија, високог нивоа загађења и других фактора животне средине са штетним утицајем на здравље људи. Због тога је цитогенетски надзор од суштинског значаја за детекцију или потврду различитих промена структуре хромозома, које настају због дејства различитих генотоксичних агенаса.

У овом прегледу анализирамо теоретске и експерименталне аспекте, као и позитивне и негативне стране, неколико тестова за процену генотоксичности код људи, као што су микронуклеусни тест, комет тест, процена хромозомских аберација и анализа размена сестринских хроматида. Ове методе су прихваћене и одобрене од стране Светске здравствене организације као стандардни тестови за скрининг генотоксичности хумане популације. Све те методе или тестови су осетљиви и потврђују ћелијске генотоксичне ефекте настале под утицајем различитих генотоксичних средстава.

Кључне речи: гентоксичност; цитогенетски скрининг; биомониторинг; микронуклеусни тест; комет тест

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REVIEW ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Podocytopathies

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SUMMARY

Podocytopathies include a wide spectrum of primary or secondary glomerular diseases that are the consequence of the podocyte injuries. The damage of podocytes can occur due to congenital or acquired disorders of podocyte transcriptional regulators, altered components of the slit diaphragm complex, abnormal assembly, or function of the actin-based cytoskeleton, dysfunction of membranes or cytoplasmic proteins, and mitochondrial injury. Podocytes reactions to injurious stimulus include FP effacement, apoptosis, and loss of podocyte, developmental arrest associated by mild proliferative activity, and dedifferentiation with moderated proliferation. Based on histopathological findings, podocytopathy may be diagnosed such as minimal change nephropathy; focal segmental glomerulosclerosis, diffuse mesangial sclerosis, or collapsing glomerulopathy while in relation to their etiology can be categorized as idiopathic, genetic, and reactive. Podocytopathies may be diagnosed due to podocyte morphological changes, immunohistochemistry, circulating and urine biomarkers, and genetic analysis. The primary clinical focus in prevention should be to reduce the factors that can damage the podocytes and cause hyperperfusion/hypertrophy of the glomerulus. Nowadays, control of systemic and intra glomerular hypertension by pharmacological blockade of angiotensin II is a central in the prevention strategy, while regeneration of podocytes by stem cells is therapeutic strategy of the future.

Keywords: steroid resistent nephrotyc syndrome; glomerulosclerosis; foot process effacement; mesenhimal-epithelilal transition

INTRODUCTION

Glomerular dysfunctions that result from podocyte damage or loss are referred with one name as podocytopathies. Podocyte, a key cell involved in podocytopathy, is a highly specialized, terminally differentiated, atypical visceral glomerular epithelial cell which has an essential role in at least five functions: glomerular permselectivity, dynamic structural support for the glomerular structure, remodeling the glomerular basement membrane (GBM), endocytosis of filtered proteins, and production of vascular endothelial growth factor and platelet-derived growth factor required for proper functioning of glomerular cells [1].

Better knowledge of the podocytes biology and etiopathogenesis of their damage during the last two decades has opened up new possibilities for diagnosis, treatment, and prevention of podocytopathies why they rank very attractive topic both in basic research and in clinical studies [2]. This review article aims to provide an analysis of the current literature about a mechanism of podocyte injury, classification of podocytopathies and their phenotypic variations, as well as the diagnostic and therapeutic possibilities for podocytopathies available to date.

MECHANISM OF PODOCYTE INJURY

Podocytes functions depend on their highly specialized and unique architecture that includes:

a) the slit diaphragm complex (SD) that is an unique intercellular connection that integrates the structural components of different types of cellular contacts, including tight, adhesion, slit and neural connections [2, 3];
b) the actin-based cytoskeleton which is the main strength and weakness of the podocytes including associated proteins and adhesion proteins [4];
c) the membrane structures that are on one hand exposed to the urinary area (in the Bowman's capsule) and on the other hand indirectly communicate with the vascular space via the GBM [1];
d) the current internal and external biochemical signals that contribute to maintaining normal glomerular function [1, 5, 6].

The damage of podocytes can occur due to congenital or acquired disorders of

a) transcriptional regulators [Wilms tumor 1(WT-1) zinc finger protein, PAX2, LIM homeobox transcription factor 1β (Lmx1b), the Notch signaling and Wnt pathway];
b) altered components of the SD complex (nephrin, podocin, CD2AP, Neph1 and others);
c)abnormal assembly or function of the actin-based cytoskeleton;
d) expression and localization of the membrane (apical and basal side) proteins [α3β1-integrin, dystroglycan complex, transient receptor potential cation channel 6 (TRPC6), podocalyxin];

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e) dysfunction of cytoplasmic proteins;
f) mitochondrial injury, and extracellular matrix protein alteration (laminin β2 encoded by LAMB2 gene) [7].

There are at least100 ways to damage the podocytes [8]. Since podocytes are postmitotic cells they typically are unable to regenerate by proliferation in response to injury [4]. Therefore, they react to injurious stimuli in limited manner, which may include:

1) changes in phenotype without alteration in podocytes number such as foot process (FP) effacement;
2) apoptosis and loss of podocyte;
3) developmental arrest associated by mild proliferative activity;
4) dedifferentiation and re-entrance into the cell cycle with mitotic catastrophe [4, 9].

FOOT PROCESS EFFACEMENT WITHOUT PODOCYTE LOSS

FP effacement is a non-specific podocyte reaction to injury or damage characterized by retraction, widening, and shortening of the FP due to:

a) actin cytoskeleton condensation into a narrow band within cytoplasm adjacent to the GBM;
b) loss of the normal three-dimensional interdigitating architecture;
c) a redistribution of the components of slit diaphragm to the cytoplasm and the apical plasma membrane. With the electronic microscope, the podocytes look flat giving the appearance of a continuous cytoplasmic sheet covering the GBM.

Podocyte FP effacement is found in proteinuric renal diseases including focal segmental glomerulosclerosis (FSGS), minimal change disease (MCD), immunoglobulin A nephropathy, and diabetic nephropathy [9, 10]. It can be reversible, as is the case in MCD under the corticosteroid therapy, while in FSGS that is refractory to existing therapies, FP effacement is usually irreversible. However, the main difference determining the outcome of the podocytopathy is the number of the podocytes, which is preserved only in non-progressive glomerulopathies.

PODOCYTE INJURY AND DEPLETION

Podocyte depletion is the main step in progressive nephropathy. It may be absolute, or relative. Absolute podocyte depletion is either a consequence of sublethal injury that leads to podocyte detachment from underlying GBM or from the lethal injury due to apoptosis or necrosis. Relative podocyte depletion happens in cases where the normal number of podocytes is insufficient to cover the GBM due to enlargement of the glomerulus because of its hypertrophy.

Wiggins et al. [11] proposed a five-step model of podocyte response to glomerular enlargement in the aging rat. In stage 1, the glomerulus is normal, as is the number and function of podocytes; in stages 2 and 3 the podocyte is subjected to "compensated" hypertrophy, while in stage 4 "decompensated" podocyte hypertrophy is associated with altered podocyte biology [11]. Podocyte size is decreased in relation to glomerular volume with consequently increased width of FP and decreased perm selectivity of glomerular filtration barrier, which leads to an increase in proteinuria. Finally, in stage 5, the number of hypertrophied podocyte is decreased with changed podocyte biology and reduced specialized podocyte machinery. Therefore, relative or absolute podocyte loss leaves uncovered GBM with consequent development of FSGS [9, 11]. According to this model, initial response, during the adaptive phase, is podocyte hypertrophy to cover an expanded GBM surface but the resulting mechanical stretch induces a shift in podocyte phenotype, favoring structural instability and decreased SD function [11]. "Adapted and decompensated" stages of podocyte hypertrophy have been reported in many congenital and acquired chronic kidney diseases (CKD) [9]. Using rat models of regulated podocyte depletion it was documented that 20% of podocytes loss causes mesangial expansion alone, more than 20% of podocytes leads to the appearance of denuded areas of GBM resulting in synechia formation, 20–40% podocyte loss results in segmental sclerosis, while more than 60% podocyte loss ends in glomerular global sclerosis [12].

PODOCYTE INJURY AND PROLIFERATION

Podocyte injury may be the consequence of mitotic catastrophe leading to delayed cellular maturation, dedifferentiation, with either low, manifesting as diffuse mesangial sclerosis (DMS) or high rates of proliferation manifesting as collapsing glomerulopathy (CG).

CLASSIFICATION OF PODOCYTOPATHIES

Podocytopathies may be classified according to histopathology and etiology as suggested by Barisoni et al. [9]. The four histological patterns of podocytopathies including MCD with unchanged the number of podocytes per glomerulus, FSGS with podocytopenia, DMS with podocytopenia and low proliferative index and CG with podocytopenia and marked proliferation are grouped in three etiological categories: idiopathic, genetic and reactive [9]. As an alternative to this classification based on histological description [9], Ahn and Bomback [13] have recently shown how podocytopathies can be classified according to pathogenesis and response to treatment (Table 1). According to them, podocytopathies are classified into those in which circulating hyper permeability factor causes podocyte injury, toxic podocytopathy caused by direct action of toxins or cytokine mediated ± APOL1 overexpression, hereditary podocytopathy as consequence of mutations causing structural or functional abnormalities of podocytes, and hyperfiltration mediated podocytopathies caused by adaptive changes due to excessive nephron workload (Table 1).

MCD is most often (80–90%) presented in childhood as an idiopathic steroid-sensitive nephrotic syndrome (SSNS)

Table 1. Pathogenesis-based classification of podocytopathies*

Type of podocytopathy	Causes and Pathogenesis	Pathology	Clinical Manifestation	Treatment	Recurrence after transplantation
Permeability factor–mediated	Circulating factor causing podocyte injury	MCD, FSGS with extensive FPE	Sudden-onset nephrotic syndrome	Immunosuppression, plasma exchange	Common; sometimes immediate
Toxic	Direct toxicity or cytokine mediated ± APOL1 overexpression	MCD, FSGS (frequently collapsing) ± endothelial tubuloreticular inclusions	Variable clinical course; slowly progressing CKD or nephrotic syndrome	Removal of toxic injury	Possible; usually several months later
Genetic	Mutation causing structural or functional abnormalities of podocytes	MCD, MesGN, FSGS	Steroid-resistant nephrotic syndrome	RAAS inhibitors	Rare
Hyperfiltration mediated	Adaptive changes due to excessive nephron workload	FSGS (frequently perihilar) with glomerulomegaly and segmental FPE	Slowly progressive proteinuria without edema and hypoalbuminemia	RAAS inhibitors	Rare

*Modified from [13];
CKD – chronic kidney disease; FPE – foot-process effacement; FSGS – focal segmental glomerulosclerosis;
MCD – minimal change disease; MesGN – mesangioproliferative glomerulonephritis; RAAS – renin-angiotensin system

that is considered an autoimmune disease with a poorly understood its genetic background. Hildebrandt's group identified EMP2 (epithelial membrane protein 2), as a rare cause of the nonsyndromic autosomal-recessive form of SSNS [14] and Izzedine et al. [15] discovered a loss of podocyte dysferlin expression as a cause of syndromic MCN associated with limb-girdle muscular dystrophy type 2B (LGMD2B). Recently, large genome-wide association studies has identified three loci that explain about 14% of the genetic risk for SSNS [16]. The strongest association was found for the *CALHM6* gene, which is important for regulating the immune response to infection. These findings suggest that a genetically determined risk of immune deregulation may be a key component in the pathogenesis of SSNS [16].

FSGS is usually manifested in childhood as idiopathic steroid resistant nephrotic syndrome (SRNS). For FSGS two main pathogenic mechanisms are assumed: either (1) an alteration of the immune system resulting in the production of a putative circulating glomerular permeability factor; or (2) mutations in more than 50 structural genes of the glomerular filtration barrier, mainly in the podocytes for which they are named hereditary podocytopathies [17]. Genetic mutations that are responsible for non-syndromic FSGS include those that encode SD associated and adaptor proteins such as NPHS1, NPHS1+ NPHS2, NPHS2, CD2AP, TRPC6, PTRO, CRB2, PLCe1, actin-based cytoskeleton complex and signaling including at least ACTN4, MYH9, INF2, MYO1E, ARHGAP24, ARHGDIA and ANLN or nuclear transcriptional factor (WT1) [9, 17–24]. Syndromic FSGS may be a consequence of genetic mutations that are responsible for mutations in GBM proteins such as the mutated COL4 genes in Alport syndrome, WT-1 in Denys-Drash, and Frasier syndrome, LMX1B in nail-patella syndrome, metabolic disorders such as GLA, encoding galactosidase A in Fabry disease, and mitochondriopathies (mitochondrial tRNA mutations in MELAS syndrome, and COQ2 mutations [9, 17, 18, 25]. The best-studied susceptibility gene (a genetic variant that represents important risk factors in the presence of a "second hit") APOL1 (G1 and G2 alleles encoding apolipoprotein L1) is a major cause of podocytopathy among African Americans, formerly called hypertensive CKD [18].

Reactive FSGS occur in CKD with reduced renal mass (*e.g.*, renal dysplasia, surgical renal mass reduction, reflux nephropathy, chronic interstitial nephritis) or in the presence of initially normal renal mass (obesity, sickle cell anemia, or cyanotic congenital heart disease) [9, 18].

DMS is characterized by the presence of different podocyte phenotype (increased expression of cytokeratin) and increased expression of proliferative markers, such as Ki67. DMS be idiopathic or due to genetic mutations, such as WT-1 mutations (Denys-Drash syndrome) [25] and mutations of LAMB2, encoding laminin 2 chain (Pierson syndrome) [26]. Congenital nephrotic syndrome of the Finnish type is caused by homozygous or compound heterozygous mutations in NPHS1, encoding nephrin [27]. Podocyte, which does not express nephrin, pass through detachment and cause progression to end-stage kidney disease, usually in the early childhood [27].

CG is defined by the presence of segmental capillary tuft collapse (wrinkling and folding) in at least one glomerulus, in association with podocyte hypertrophy and/or hyperplasia. It shows podocyte proliferation rather than podocyte depletion, and the actin cytoskeleton may disappear [11]. In fact, podocytes returned in the primordial embryonic stages through the process of epithelial-mesenchymal transition. During epithelial-mesenchymal transition, podocytes regain a cuboidal shape and loss of primary and FP. The markers of maturity such as synaptopodin, podocalyxin, GLEPP1, and CALLA are replaced by PAX2 and cytokeratin, and E- and P- cadherins are replaced by the N- cadherin. Further, transcriptional marker WT-1 is lost while expression of Ki67 is increased, and immature podocytes re-enter the cell cycle and proliferate. Increased vimentin and intermediate filaments contribute to the high podocytes migration capacity, which with vivacious podocyte hyperplasia seems

to generate the apparent pseudo crescents within Bowman's space [11]. According to the etiology, CG may be idiopathic, genetic, and reactive [9].

DIAGNOSIS OF PODOCYTOPATHIES

Podocytopathies may be diagnosed based on the data from the history of the disease, podocyte morphological changes, immunohistochemistry, circulating and urine biomarkers, and genetic analysis [28].

Podocytopathy should be considered when patients have increased proteinuria with albuminuria, or nephrotic syndrome with or without hyperfiltration, or hypofiltration. Hereditary podocytopathy is very likely in child with SRNS especially if they are from a consanguinity marriage, and/or have syndromic features [19].

The visualization of structural changes on glomerular filtration barrier has been carried out in the clinical practice by scanning or transmission electronic microscope since the end of the thirties of the last century. During the last few years, different super-resolution microscopic techniques were developed to enable new insights into podocyte morphology [29]. These super-resolution microscopy approaches include three dimensional structured illumination microscopy, stimulated emission depletion microscopy and localization microscopy (stochastic optical reconstruction microscopy and photo activated localization microscopy). Their resolutions reached down to 80–20 nm and could be used to image and further quantify podocyte FP morphology [29]. Furthermore, high-magnification helium ion microscopy produce high-quality subnanometer-resolution images of glomerular structures [7]. For imaging of podocytes *in situ* multiphoton laser microscopy allows imaging structures up to several hundred micrometer in depth within the tissue while multiphoton microscopy, light sheet microscopy is currently used to visualize larger tissue volumes and therefore image complete glomeruli in their native tissue context [29]. Furthermore, atomic force microscopy has been used to study the change of mechanical properties of podocytes [29].

An immunohistochemical examination of podocytes cytoskeleton-specific proteins expression, including ezrin, podocalyxin, synaptopodin, and nephrin may be useful in predicting a clinical course of podocytopathy [28].

A circulating biomarker, such as permeability factor, soluble urokinase-type plasminogen activator receptor, failed to meet expectations as the diagnostic tool and a therapeutic target for FSGS [30].

Urine markers, such as the ratios of the number of podocytes or podocytes mRNA with creatinine, and the podocin nephrin mRNA ratio showed correlation with histological outcome as well as or better than clinical biomarkers, with highly sensitivity and specificity [28, 31].

Finally, genetic diagnosis is now possible for more than 50 monogenetic podocytopathies. Recent progress in high flow sequencing and continuous reduction of whole exome sequencing costs, made genetic testing more accessible and less time-consuming [18, 19, 32]. Mutation analysis should be offered to all individuals who manifest with SRNS before the age of 25 years, especially to those with congenital (less then three months), infantile (3–12 months), familial and syndromic SRNS, as well as in resistance to calcineurin inhibitors, and before kidney transplantation [19, 20, 32, 33].

PODOCYTE-TARGETED THERAPIES

Podocyte- targeted therapy can be carried out by inhibition of rennin angiotensin aldosterone system (RAAS), administration of immunosuppressive drugs and through the methods that achieve regeneration of the podocytes [7, 32, 34, 35, 36].

RAAS inhibition has been demonstrated to lower proteinuria by 40–50% in patients with SRNS [32]. In the PodoNet cohort, RAAS inhibition alone was associated with partial proteinuria remission in 21% and even maintained complete remission in 27% of patients [32]. RAAS blockade by either angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers achieves a renoprotective effect primarily by inhibiting vasoconstriction of the efferent arterioles and thus reduces intraglomerular pressure and hyperfiltration. However, the beneficial effect of these drugs can be realized also by direct action on the glomerular cells including the podocytes [37, 38]. Studies have shown that ACE type 2 (ACE2) which is found in the podocyte body and in the slit diaphragms has the potential to antagonize the action of Ang II [37]. In addition, Ang-(1–7) has been demonstrated to attenuate podocyte injury by down regulation of MAPK (p38, ERK1/2 and JNK) phosphorylation [38].

Advances in podocyte biology and pathogenesis of proteinuric disease unveiled unexpected mechanisms of action of widely used immunosuppressive drugs, which are independent of their traditional immunomodulatory function [39]. Glucocorticoids and levamisole have non-immune mediated, reno-protective effects on podocytes; they stabilize actin (increase of nephrin and activity of Rho-A), attenuate podocyte apoptosis (restoration of Bcl-2 and reduction of p53) and thereby prolong the survival of the podocytes [38]. Non-immunologic effect of calcineurin inhibitors, (CsA and FK506) is realized by preventing synaptopodin degradation by cathepsin L and thus they increase the stability of podocyte cytoskeleton [39]. Rituximab also improves stability of actin cytoskeleton. The mechanism of this action is achieved by rituximab binding to acid sphingomyelinase-like phosphodiesterase 3b protein (SMPDL-3b), a putative acid-sphingomyelinase (ASMase) [39]. The restoration of SMPDL-3b expression in podocytes by rituximab prevents the disruption of stress fiber (synaptopondin) and podocyte apoptosis. Abatacept acts to stabilize actin cytoskeleton by blocks B7-1 signaling and restoring β1 integrin activation [39]. However, Rapamycin, which inhibits mTOR activity, may have dual effects on podocytes, positive effect by suppressing autophagy as documented in diabetic nephropathy) and negative one, by development of podocyte damage and increase proteinuria. In general, the beneficial effects of immunosuppressive drugs in the treatment of hereditary podocytopathy are small in relation to adverse effects of

this therapy. The findings in the PodoNet cohort argue against a relevant nephroprotective effect of calcineurin inhibition – or other immunosuppressive therapies – in children with genetic forms of SRNS and support the notion that such patients should be spared immunosuppressant side effects [32, 36]. A promising example of an innovative gene specific treatment option is successful use of CoQ10 in children with SRNS due to genetic defects leading to CoQ10 deficiency [32].

Finally, having in mind that podocytes are postmitotic cells, the identification of effective ways to promote podocyte regeneration has become a major focus for therapeutic research. The two progenitor pools have recently been identified in multiple studies: parietal epithelial cells, and cells of renin lineage [40]. A reasonable podocyte replacement goal should be to simply increase podocyte number to that above the critical scarring threshold (20% podocyte loss), which limits/prevents segmental sclerosis progressing to global.

CONCLUSION

Effective podocyte depletion is the common driving force of the progressive podocytopathies. The classification of podocyte diseases needs to be improved by new markers of podocyte phenotype that will override traditional morphologic analysis and will serve as new bases for therapeutic intervention. The primary clinical focus in prevention should be to reduce the factors that can damage the podocytes and cause hyperperfusion/hypertrophy of the glomerulus. Nowadays, a control of systemic and intraglomerular hypertension (pharmacological blockade of angiotensin II) has a central role in the prevention strategy while a regeneration of podocytes by stem cells is therapeutic strategy of the future.

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Подоцитопатије

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САЖЕТАК

Подоцитопатије укључују широк спектар примарних или секундарних гломерулопатија које су последица оштећења подоцита. Могу се јавити услед конгениталних или стечених поремећаја транскрипционих регулатора, измењених компонената дијафрагме прореза, абнормалног састава или функције актинског цитоскелета, дисфункције мембранских или цитоплазматских протеина и оштећења митохондрија. На штетне утицаје подоцити реагују губитком ножних наставака, апоптозом и некрозом, застојем у развоју повезаним са пролиферативном активношћу и мезенхимно-епителном транзицијом. На основу хистопатолошких налаза, подоцитопатије се деле на нефропатију са минималним променама, фокалну сегментарну гломерулосклерозу, дифузну мезангијалну склерозу и колапсну гломерулопатију, а у односу на етиологију могу бити идиопатске, генетске и реактивне.

Дијагноза подоцитопатија се може поставити на основу морфолошких и имунохистохемијских промена, плазматских и уринарних биомаркера и налаза генетских мутација. Примарни клинички фокус у превенцији подоцитопатије треба да буде смањење фактора који могу да оштете подоците и изазову хиперперфузију/хипертрофију гломерула. Савремена контрола системске и интрагломерулске хипертензије фармаколошком блокадом ангиотензина II је централна у стратегији превенције, а регенерација подоцита матичним ћелијама је терапија будућности.

Кључне речи: нефротски синдром отпоран на стероиде; гломерулосклероза; губитак ножних наставака подоцита; мезенхимално-епителна транзиција

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

COVID-19 impact on women on both sides of the frontline – the American College of Cardiology Women in Cardiology Section's International Working Group perspective

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SUMMARY
At the beginning of the SARS Co V2 (COVID-19) pandemic, women worldwide represented the majority of health care workers.
As part of the fight against the pandemic, women health care workers became a part of the significant frontline response.
This led to unique challenges that affected women physicians as well as the women patients they were taking care of. The American College of Cardiology Women in Cardiology International Working Group set up a webinar to discuss the challenges being faced by women physicians and women patients in various parts of the world and look towards finding possible solutions for these issues in a webinar themed "WIC Global Perspectives: COVID-19."
Keywords: COVID-19; pandemic; sex differences; pregnancy; women in cardiology; discrimination

INTRODUCTION

The American College of Cardiology's Women in Cardiology (WIC) section was established in 1996 aiming to support female members advance their careers. Irrelevant of parity achieved at medical schools' graduation level, the percentage of women opting for cardiology remained low, prompting the WIC Section and its Leadership Council to strive to recruit more women both nationally and internationally [1, 2]. In 2018, the ACC WIC International Working Group (WG) was launched. Its first meeting gathered over 50 WIC from 30 countries during the European Society of Cardiology annual meeting, after a year of diligent fieldwork aimed to define its agenda. The WG's scope was set to globally promote #EquityBasedMeritocracy – hashtag coined by its founding Chair, Dr. Biljana Parapid – for WIC trained in the United States who either returned to their home institutions outside the USA or opted practicing elsewhere, and also to give an opportunity and facilitate early career cardiologists worldwide to find a mentor and build a collaboration (Figure 1). First speed mentorship table was held only two months later, during the ACC Middle East annual meeting in Jeddah hosted by Dr. Mirvat Alasnag. Medical students, trainees, adult and pediatric cardiologists equally, as well as cardiac surgeons, who were wholeheartedly supported by their male mentors and colleagues, participated in fruitful discussion with all ACC WIC faculty who joined (Figure 2) [3].

The ACC WIC International WG endeavored throughout 2019 to promote education and grew its global membership particularly through social media, which became the silver lining of the 2019/2020 SARS-CoV2 pandemic. As women worldwide turned into key frontline workers in part due to initial mis-perception that they are less prone to SARS-CoV2 infection, ACC worked diligently across its sections to maintain its core values present in times of adversity. Simultaneously, the ACC WIC Leadership Council worked closely with the ACC WIC International WG and in response to the concerns raised by women physicians, opted to address issues linked both to women's health and women's battling adversity both as doctors and members of the academic community.

At the same time, the UN Women's report stated disturbing statistics hallmarking a setback in achieved gender equality so far due to

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Figure 1. The ACC WIC International WG Launch (Aug 26, 2018);
top row, ACC WIC International WG Launch, meeting wrap up (left to right): Drs. Martha Gulati (USA), Sharon Mulvaugh (Canada), Biljana Parapid (Serbia), Denisa Muraru (Italy), Hariette van Spall (Canada), Ami Bhatt (USA), Fina Mauri Fere (Spain), Mirvat Alasnag (KSA), Ing Han Lin (Singapoor), Angela Maas (the Netherlands), Alexandra Frogoudaki (Greece), Janneke Wittekoek (the Netherlands), Bharati Shivalkar (Belgium), Toniya Singh (USA), Cara Hendry (UK), Hannah Sinclair (UK), Khalida Soomro (Pakistan);
lower row left, ACC WIC Leadership presence at ESC 2018 (left to right): Drs. Ami Bhatt (ACC MA Chapter WIC delegate), Biljana Parapid (ACC WIC Leadership Council member, ACC WIC International Working Group founding Chair), Toniya Singh (ACC WIC Leadership Council Chair elect), Mary Norine – Minnow Walsh (ACC President), Mirvat Alasnag (ACC Interventional Cardiology Council member);
lower row right ACC WIC International WG Launch, beginning of the meeting: Drs. Jelena Nedeljković (Serbia), Mirvat Alasnag (KSA), Angela Maas (the Netherlands), Janneke Wittekoek (the Netherlands), Bharati Shivalkar (Belgium), Toniya Singh (USA), Alexandra Frogoudaki (Greece), Martha Gulati (USA), Lia Crotti (Italy), Ing Han Lin (Singapore), Biljana Parapid (Serbia), and Silvia Castelleti (Italy)

Figure 2. The ACC WIC International WG Speed Mentoring Tables (Oct 26, 2018) with Drs. Mirvat Alasnag (KSA), Alison Bailey (USA), Biykem Bozkurt (USA), Dipti Itchhaporia (USA), Roxana Mehran (USA), Biljana Parapid (Serbia), and Nireen Okasha (Egypt)

Figure 3. The ACC WIC International WG webinar (Jun 08, 2020) entitled "Women in Cardiology Global Perspectives: COVID-19" organized under the auspices of the WIC Leadership Council Chaired by Drs. Mirvat Alasnag (KSA) and Biljana Parapid (Serbia) with keynote speakers Drs. Shrilla Banerjee (UK), Manal Alasnag (KSA), Sondos Samargandy (KSA), and Sharonne N. Hayes (USA)

pandemic [4]. The "WIC Global Perspectives: COVID-19" webinar held on June 8, 2020 gathered experts in the field (Figure 3) who drafted this brief report aiming to distribute better the messages shared with the audience [5].

SEX DISPARITY IN MORTALITY FROM COVID-19 INFECTION

Historically, male sex has been associated with worse clinical outcomes in previous pandemics, infections, and famine. The epidemics due to coronaviruses (severe acute respiratory syndrome or SARS virus and the Middle Eastern Respiratory Syndrome (MERS) resulted in case fatality rates of 21.9% in males and 13.2% in females [6, 7].

In the majority of countries that have submitted disaggregated data to the World Health Organization – data broken down by sex difference and not just total infection and mortality figures – the infection rate in men is 50% of total, but male mortality varies from between 50% and up to 75% of total mortality [4].

Preliminary Wuhan data showed rates of infection in males ranging 51–66.7% and mortality rates of 2.8% in men *vs*. 1.7% in women, equating to just under a 2:1 mortality ratio for male:female [8, 9]. Italians report 58% of the infected population to be male, who also carried 70% of the COVID-19 related deaths [10].

Yet, global healthcare workforce is dominated by women where up to 85% of nurses in Europe and the Americas are female, as are 46–53% of physicians, which explains why 75% of COVID-19 confirmed infections among healthcare workers were women [4].

Still, the issue of mortality remained, which is explained by critical immune-modulating genes location on the X-chromosome, and in particular the gene that codes for the TLR-7 protein, which is of paramount importance in the detection of single-stranded RNA viruses, such as the coronaviruses [11]. Additionally, while one X-chromosome is usually inactivated in each female cell, the gene coding the TLR-7 protein somehow escapes this inactivation, meaning that women produce more of this protective protein and hence amplify the immune response to COVID-19 [12].

Estrogen provides a protective role, which regulates and makes the response to COVID-19 infection more appropriate. The ACE-2 receptor (with its gene also on the X-chromosome) is used as the portal of entry by the virus. After entry into the cell, the virus causes a downregulation of the ACE-2 receptor. Estrogen opposes this action and also directly suppresses viral replication to provide a two-pronged defense against COVID-19 [13, 14].

When we look into sex as a risk factor, men are known to adhere to hygiene principles less and ask for help later for classical risk factors whose burden is more prominent [13, 15]. Our Chinese colleagues have shown that men with SARS-CoV2 infection carry additional viral and bacterial infections [8].

Therefore, in summary, infection rates in the general population are similar between males and females; however, in healthcare, due to the fact that a significant part of the work force is female, there is a higher incidence of infections in females. Mortality rates, though, remained much higher in males, as a result of sex- and gender-based factors. Sex-disaggregated data are essential to understand variations in risk, infection, and disease.

JOURNEY WITH HIGH-RISK PREGNANCY DURING COVID-19 PANDEMIC

Some of the most vulnerable patients in an overwhelmed healthcare system during a pandemic are the patients with high-risk pregnancies and their babies. Even in tertiary care facilities and during non-pandemic times, these cases are challenging. For each case, the interplay between maternal and fetal factors requires understanding, risk assessment, and meticulous planning for the delivery of comprehensive multidisciplinary healthcare [16, 17].

Pregnant women seem to have the same risk of becoming infected with COVID-19 as women who are not pregnant [16, 17, 18]. However, from historic data of other viral illnesses and recent pandemics, once infected, pregnant women have a high risk of severe infection. There are reports of increased rates of preterm deliveries and stillbirths in addition to maternal respiratory complications and maternal mortality [18, 19]. The WHO-China Joint Mission on Coronavirus Disease 2019 reported on a cohort of 147 COVID-19 PCR-positive pregnant patients. One percent developed critical illness requiring mechanical ventilation for respiratory failure with or without organ support in the intensive care [20].

Delayed recognition and obstacles to access healthcare are well-recognized causes for an increase in both maternal and fetal mortality rates [21]. Moreover, the physiological changes that occur during pregnancy mimic early presentations of both cardiac and respiratory disease. It is well established that during pregnancy, oxygen consumption increased by up to 30%. To meet demands, cardiac output increases. Hence, it is not uncommon to see tachycardia and shortness of breath at rest during pregnancy. As the pregnancy progresses, there may be basal lung atelectasis [17]. This makes visual triaging very tricky, especially if it is done virtually as is often the case in the current pandemic.

The American College of Obstetricians and Gynecologists developed a risk assessment pathway for pregnant outpatients with suspected or confirmed COVID-19. Such efforts ensure that appropriate channeling of patients into needed healthcare services is done in a timely manner for each case [22]. Peripartum management checklists have also been developed by many centers to outline the preplanned multidisciplinary care needed for women with COVID-19. These checklists identify where the patient will be admitted and the teams that need to be activated upon admission of the patient. Details of the intrapartum management and postpartum management for the mother are charted. Similarly, for the newborn, the care plan includes the clinical samples to be taken as well as the timing of theses samples [23].

Early testing may lead to false positive results due to contamination with maternal fluids. For this reason, the Centers for Disease Control and Prevention recommends testing all neonates born to women confirmed or suspected at the age of 24 hours. If initial results are negative, testing is repeated at 48 hours of age using nasopharyngeal, oropharyngeal, or nasal swabs for RT-PCR [18]. For research purposes, there are centers that take samples from amniotic fluid, umbilical cord, placenta, and rectal swabs for the neonate.

A systematic review of COVID-19 in newborns reported a very small number of COVID PCR-positive cases. There were no adverse perinatal outcomes found and most had no or mild symptoms. Studies that tested breast milk reported negative COVID results [24]. The virion has been seen on electron microscopy of placental tissue. However, there is much uncertainty regarding vertical transmission. Therefore, based on current literature, there is no evidence to support the absolute contraindication of breastfeeding nor temporary separation of mothers from their newborn. Caution must be advised due to the risk of direct postnatal droplet transmission. Most centers are using shared decision-making between the mother and the clinical team on a case-by-case basis with the option of expressed breast milk [18].

Another challenge during this pandemic is the newborn with congenital heart disease. The British Congenital Cardiac Association has listed the high-risk groups and included single ventricle patients and all infants less than 12 months of age. For those already on medications, such as ace-inhibitors and aspirin, they recommend continuing mediations. For those who need interventions, most hospitals are restructuring their pathways to accommodate the most urgent cases. Minimal interventions are favored and case-by-case plans are made as institutions face these uncharted waters. The learning curve has been steep. But the silver lining of this pandemic has been the support of colleagues across the world in sharing experiences and our agility to reshape and restructure our services.

COVID-19 AND FAMILY CHALLENGES FOR WORKING WOMEN

The World Health Organization has expressed concerns about the COVID-19 pandemic' mental health and psychosocial ramifications generally [25]. A total of 68.7% of frontline medical staff who were women reported a feeling of anxiety regarding their safety and the safety of their families among the participants [26]. Women are less likely to have access to personal protective equipment or correctly sized equipment. Therefore, the effects of this crisis on working women are substantial, and its long-term consequences from depression, suicide, possible self-harm and mood-related issues are genuine and concerning.

During the pandemic, the closedown of schools and daycare centers have shifted the burden of care and schooling of children to working mothers [27].

In response to these challenging times, healthcare establishments are modifying work arrangements to be more flexible with opportunities for both men and women to work from home. Strict infection control guidelines and specialized fitting protected equipment have started to be implemented. Appreciation and acknowledgement of health staff drive and effort by hospital managements and governments, in addition to providing onsite and online

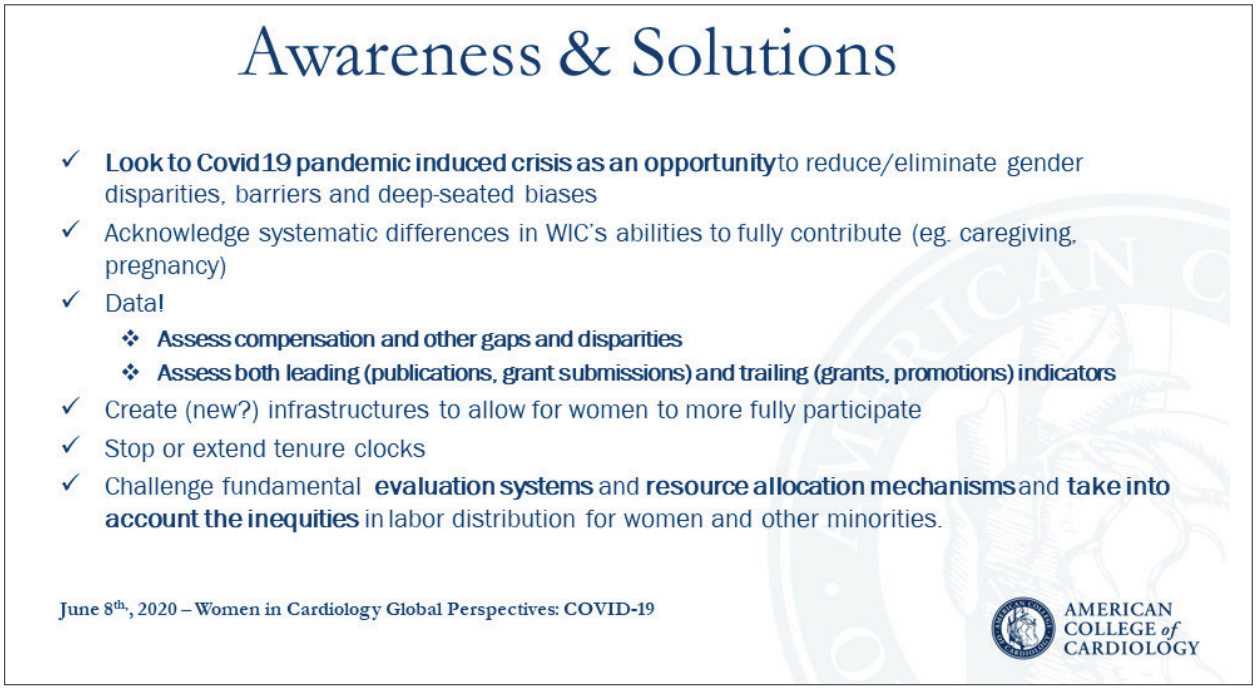

Figure 4. Awareness and solutions for academic advancement during COVID-19 pandemic

psychological support, will help decrease the psychological toll of this pandemic on working women. There is a slow shift in what used to be the social norms, with more men participating in the domestic chores and child care in an equal partnership in these difficult times.

On the bright side, communities have come together to help and support working mothers offering help in childcare and house chores. Additionally, this has led to an open dialogue that has shed light on the unequal distribution of domestic chores, which has led to a discussion on social norms related to this. Thus, it became more acceptable to share domestic responsibilities among men and women, especially in dual-career houses brought to by the social, health and economic demand of COVID-19 crises. Furthermore, many working establishments came to adjust their regulation and schemes allowing remote working and outsourcing, which will improve the balance between work and home that many women are striving to achieve.

COVID-19 AND LOST OPPORTUNITIES FOR WOMEN

Women in medicine, particularly in academic medicine, have disproportionately been adversely affected by the COVID-19 pandemic, all but reversing recent gains. Even before the pandemic, women *vs*. men cardiologists in the United States faced barriers – importantly, more responsibility for housework, childcare, and supervising family activities [1]. At work, women cardiologists experience more discrimination and higher burnout rates [1, 28], are less likely to participate in research and receive less encouragement to do so, while at the same time perform more service work ("office housework"), diverting energy and time from activities that drive career advancement. These factors contribute to women cardiologists holding fewer leadership roles and academic promotions and working for substantially lower pay for similar work [29].

The pandemic has further exacerbated domestic workloads, particularly among those with school-age children, threatening to widen the gender academic productivity divide. Women have experienced challenges in academic productivity as COVID-19 has resulted in less direct/on-site work and more remote work. In contrast, academic productivity for men, who are on average less responsible for childcare, may have benefitted. Supporting this hypothesis is evidence that pre-prints and manuscript submissions by women have declined, while increasing/stable among men, with the greatest declines in medicine and among first-author submissions by women (Figure 4) [30]. Lack of face-to-face work means that women and minorities, who previously had less access to informal mentoring and coaching, are now truly "on their own." Younger female cardiologists, already disadvantaged, are also at risk for being disproportionately affected by the many cancelled meetings and lost speaking opportunities – important in and of themselves, but also for networking, since women tend to be less well known.

Finally, in the COVID-19 era, the work is fundamentally different. In-person inequities are heightened with remote work. If it was difficult to be recognized when physically present at a meeting, video meetings render women even less "visible" – and "Zoom fatigue" is real. Women working directly on COVID-19-related science are less likely to be authors [31], cited, or featured in media stories [32, 33], and when featured, often are not afforded their professional title of "Dr." when men are afforded theirs [34]. Taken together, if nothing is done to support women cardiologists at home and at work, these factors are likely to delay academic

promotions and leadership opportunities, and to lead to more women leaving the cardiology workforce altogether.

The pandemic will subside, but without action these inequities will not, and progress made in advancing women in cardiology will be reversed. We must use this crisis as an opportunity to reduce and/or eliminate gender disparities, barriers, and deep-seated biases. We must challenge the fundamental evaluation systems and resource allocation mechanisms and fully take into account the inequities in labor distribution for women and other minorities. We must acknowledge and address the systematic differences in women cardiologists' ability to fully contribute (e.g. caregiving, pregnancy). Data are needed on compensation, leading (publications, grant submissions) and trailing (grants, promotions) indicators in order to create infrastructures that will allow women to more fully participate and succeed.

CONCLUSION

From the beginning of the SARS-CoV2 pandemic, the world has seen an unprecedented healthcare crisis and also its finest moment in social solidarity and bridging gaps of care and need for all the sick and disadvantaged. Women physicians already suffering biases in both clinical and academic settings are at a higher risk of losing the ground they have gained so far. While looking for solutions, their unique circumstance should be taken into account. Flexibility in work and academic production will go a long way in mitigating these issues. Continuing to work on maintaining a strong network of mentors and sponsors through events such as these by the ACC WIC International Work Group helps us stay connected and work toward actionable solutions.

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Утицај инфекције *COVID*-19 на жене са обе стране прве линије фронта – становиште Интернационалне радне групе Секције за жене кардиологе Америчког колеџа кардиолога

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САЖЕТАК

На почетку пандемије *SARS CoV*2 (*COVID*-19) жене широм света представљале су већину здравствених радника.

У склопу борбе са пандемијом, жене здравствени радници постале су значајан део снага прве линије одбране. Ово је довело до јединствених изазова који погађају жене као лекаре и жене које оне збрињавају. Интернационална радна група Секције жена кардиолога Америчког колеџа кардиолога организовала је вебинар како би размотрила изазове са којима се суочавају жене лекари и жене пацијенти широм света и покушала да пронађе могућа решења за ове проблеме кроз вебинар насловљен „Глобалне перспективе жена кардиолога: *COVID*-19".

Кључне речи: *COVID*-19; пандемија; сексуалне разлике; трудноћа; жене у кардиологији; дискриминација

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Radiotherapy and COVID-19 pandemic – a review of the current recommendations

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SUMMARY
Cancer patients are at high risk for developing severe symptoms with a high mortality rate due to infection of COVID-19. Radiation therapy is one of the main treatment modalities of central nervous system tumors and lung cancer. Radiotherapy is often delivered in a number of fractions, which implies many visits to the radiotherapy center and thus possibly more exposure to the COVID-19. The convenient compromise between the exposure of the patients to the SARS-CoV-2 virus and the optimal treatment is questionable. The most used measures in radiotherapy centers are classification of patients into priority groups and frequent use of hypofractionation. From the beginning of the COVID-19 outbreak, only a few expert group consensuses of radiotherapy treatment are published. In this paper we briefly review available practical recommendations of the expert groups for radiation therapy and oncology as well as the expert opinions for radiotherapy of the central nervous system tumors and lung cancer during the COVID-19 pandemic.
Keywords: COVID-19; radiotherapy; brain tumors; lung cancer

INTRODUCTION

A novel RNA virus named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was first described in Wuhan City, Hubei Province, China in December 2019 [1]. Coronavirus disease 2019 (COVID-19) pandemic has affected all the aspects of the public health, but also treatment processes, as well as the treatment of cancer patients [2].

Radiation therapy (RT) firmly stands as one of the most used modality of cancer treatment since the discovery of the X-rays and radium. External beam radiation therapy (EBRT) is used in 52.3% cancer patient [3].

The important question in patients with aggressive cancers and short overall survival is how and whether to make a compromise between the treatment and the reduced exposure to COVID-19.

The most used measures in RT centers are classification of patients into urgency groups and treatment with hypofractionation schedules [2]. Hypofractionation schedules provide a reduced number of visits to the RT centers and thus reduce exposure to the virus [4].

To date, there are a few published guidelines and RT schemes for cancer patients in the era of the COVID-19 pandemic. In this paper we are primarily focused on brain tumors and lung cancer RT treatment, with a critical review of the guidelines.

RADIATION THERAPY OF BRAIN TUMORS IN THE ERA OF COVID-19

Brain tumors and nervous system tumors make up 2.5% of cancer deaths [5]. The last revised classification of brain tumors was introduced in 2016 by the World Health Organization (WHO) [6]. RT is often one of the key modalities of the treatment of brain tumors (Figure 1).

European Society for Medical Oncology (ESMO) divided priorities for RT during COVID-19 pandemic into high, medium, and low priority [7]. ESMO high priority group for RT

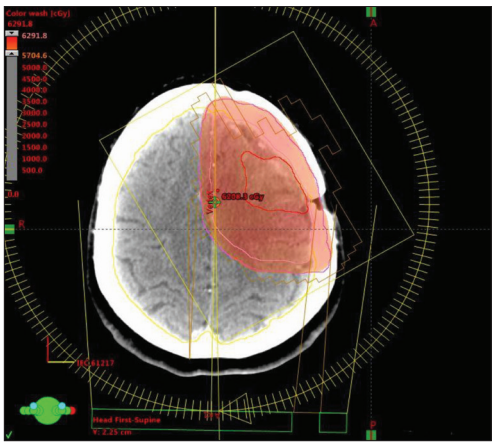

Figure 1. Example of postoperative radiotherapy plan in a patient with glioblastoma planned with volumetric modulated arc therapy technique and standard fractionation scheme treated at the Institute for Oncology and Radiology of Serbia during the pandemic of COVID-19; the dose prescribed to the planning target volume is 60 Gy; planning target volume (purple contour and color wash) is encompassed by the 95% isodose; the volumetric modulated arc therapy field arrangements are represented with yellow arcs

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includes newly diagnosed glioblastoma, isocitrate dehydrogenase (IDH) wild-type, the lower WHO grade gliomas, IDH-mutant with relevant clinical manifestations, as well as the adult medulloblastoma [7].

Standard radiation scheme for younger or fit patients with glioblastoma is 60 Gy in 2 Gy daily fractions with concomitant temozolomide (TMZ) [8]. Others with poor performance status (PS) and older than 70 years are suitable for hypofractionation with 40 Gy in 15 daily fractions as well as 34 Gy in 10 fractions [8].

In the literature, the data of the overall survival (OS) among elderly patients who were treated with standard and hypofractionated RT are different. Mak et al. [9] found that patients treated with hypofractionation RT had poorer OS than others with standard course RT. In contrast, Roa et al. [10] found no differences in OS among the groups. The addition of the oral TMZ to hypofractionation RT of glioblastoma may improve survival more than RT alone [11].

Recommendations of the hypofractionated schemes for older patients and patients with poor PS with glioblastoma is reasonable with or without pandemic of COVID-19. However, there is a lack of data about safety of hypofractionated regimens in younger patients with good PS. A Meta-analysis by Liao et al. [12] showed that hypofractionated RT is efficacious for patients older than 70 years, while in younger patients and others with good prognostic factors it is yet to be determined. Balakrishnan et al. [13] proposed treatment options for brain tumors during the COVID-19 pandemic. Among other authors' recommendations, for younger fit patients they recommended hypofractionated RT with 60 Gy in 20 fractions, with simultaneous integrated boost (SIB) technique and with concomitant TMZ. From a radiation oncologist's point of view, radiation with the SIB technique may produce toxicity different than with standard fractionation, which is important in young patients. However, Zhong et al. [14] reported mild acute and late toxicities in patients with glioblastoma treated with SIB intensity-modulated RT (IMRT) and TMZ.

According to ESMO, the high priority group for RT includes lower WHO grade gliomas, IDH-mutant with relevant clinical manifestations [7]. Medium priority for RT of gliomas is lower WHO grade gliomas, IDH-mutant [7]. For low grade gliomas, Balakrishnan et al. [13] suggested delaying RT or offering RT at progression. Mohile et al. [15] proposed to delay diagnostic surgeries and adjuvant therapy during the COVID-19 pandemic in stable patients and if the adjournment will not compromise further complete resection. For low-grade astrocytomas and 1p/19q co-deleted tumors, delay of all therapies in asymptomatic patients should be considered [15]. Yang et al. [16] found that patients treated with chemotherapy four weeks before the onset of COVID-19 symptoms were related to an increased risk of mortality. In general, hematological toxicities as well as the opportunistic infections are observed in patients with oral TMZ [17]. Patients with O^6-methylguanine DNA methyltransferase unmethylated promotor may have little or no benefits from oral TMZ, while there is the risk of hematological and other toxicities. Along with toxicities and the immunosuppressive condition, patients with cancer are at a greater risk for severe COVID-19 manifestations [17].

For medulloblastoma, Balakrishnan et al. [13] suggested the beginning of the treatment within 4-6 weeks after surgery with a possible start of the posterior fossa boost, followed by craniospinal RT with IMRT or volumetric modulated arc therapy (VMAT) [13]. Also, they proposed treatment for other brain tumors, mostly regarding treatment postponement or hypofractionation regimens.

Pediatric brain tumors are often different from adult brain tumors [18, 19]. In accordance with that, pediatric brain tumors will not be discussed here.

RADIATION THERAPY OF LUNG CANCER IN THE ERA OF COVID-19

Lung cancer is the main cause of cancer death in men, while in women it is breast cancer and colorectal cancer [5].

Expert groups for lung cancer RT as well as single institutions gave their opinions on susceptible changes in RT during the COVID-19 outbreak. The European Society for Radiotherapy and Oncology (ESTRO) and American Society for Radiation Oncology (ASTRO) made a consensus statement with recommendations for lung cancer radiation considering risk reduction and reduced RT administration [20].

An ESTRO-ASTRO statement presented by Guckenberger et al. [20] revealed as a strong consensus that in terms of risk reduction the curative treatment for stage III non-small cell lung cancer (NSCLC), as well as for limited stage small cell lung cancer (SCLC) and palliative NSCLC, should not be delayed. In the phase of the risk reduction, they were in consensus on the need not to change standard RT regimens in favor of more hypofractionated schemes. However, hypofractionated RT may be changed to more hypofractionated schemes in palliative NSCLC [20]. When concurrent radiochemotherapy is planned for stage III NSCLC, hypofractionated RT should not be applied [20]. Some of the expert participants of the ASTRO-ESTRO consensus who support hypofractionation in concurrent radiochemotherapy strategy for stage III NSCLC suggested RT regimens of 60–66 Gy in 22–30 fractions and 50 Gy in 20 fractions [20].

ESMO consider three groups of priority for lung cancer RT to be the high, medium, and low priority group [21]. The high priority group for RT comprises inoperable stage II-III NSCLC and limited stage SCLC in concurrent or sequential approach with chemotherapy as well as conditions suitable for palliative radiation such as spinal cord compression or superior vena cava obstruction [21].

An example of definitive RT planned during the COVID-19 pandemic for stage III NSCLC is presented in Figure 2.

Faivre-Finn et al. [22] proposed stereotactic ablative RT (SABR) for early stage NSCLC but with other specific limitations regarding the tumor size and the distance from the chest wall. Fractionation and dose schedules vary from 30–34 Gy in one fraction to 48–54 Gy in three fractions

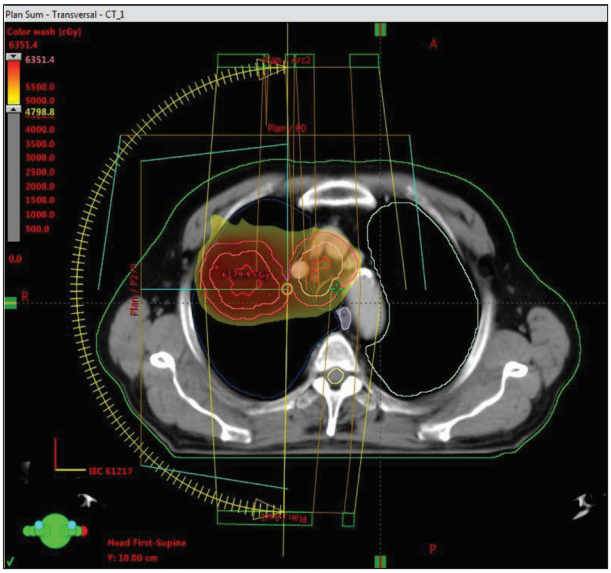

Figure 2. Example of dose distribution in volumetric modulated arc therapy plan for definitive radiotherapy in a patient with stage III non-small cell lung cancer treated at the Institute for Oncology and Radiology of Serbia during the pandemic of COVID-19; the dose prescribed to the primary planning target volume (purple contour) is 50 Gy with a sequential boost of 10 Gy to the secondary planning target volume (light pink contour) conventionally fractionated; the volumetric modulated arc therapy field arrangements are represented with yellow arcs

[22]. For central tumors, hypofractionated regimen is considered with a dose of 50–60 Gy in 15 fractions [22].

Not only for inoperable early stage NSCLC but also for operable NSCLC, stereotactic body RT (SBRT) may be a solution for the treatment in the era of COVID-19 [4]. Aside from having a better outcome with surgical resection, Moore et al. [23] concluded that definitive RT may be a feasible curative approach for stage II NSCLC.

Radiation pneumonitis (RP) is one of the toxicities that is observed in patients with lung cancer treated with RT. The mechanism of RP is correlated with treatment factors as radiation dosimetry, irradiated lung volume, radiation treatment technique, as well as with patient characteristics [24]. Considering α/β ratio of normal lung parenchyma, a question about safety of hypofractionation and therefore possible toxicity is posed. Barriger et al. [25] reported 9.4% of RP in patients treated with SBRT. Lung volume that received 20 Gy (V20) was a predictor of toxicity rather than gross tumor volume or planning tumor volume. Moreover, Jin et al. [26] reported that hypofractionation may be a better option for smaller tumor volumes. It should be kept in mind that many patients have concurrent or sequential chemotherapy or immunotherapy and their synergistic effect with radiation may increase the risk of pneumonitis. Palma et al. [27] showed that elderly patients treated with concurrent chemoradiation with carboplatin-paclitaxel chemotherapy are at the highest risk for symptomatic pneumonitis. Similarity between symptoms of RP and SARS-CoV-2 may be fatal if it remains unrecognized. Shaverdian et al. [28] suggested that patients with RP symptoms should be tested for COVID-19 infection, regarding different treatments for these conditions.

CONCLUSION

RT remains one of the key treatment options for lung cancer and central nervous system tumors in the era of the COVID-19 pandemic. To date, in June 2020, for central nervous system tumors there is no published RT expert group consensus as there is for lung cancer, with the exception of individual expert opinions. Since the postponement of RT may have detrimental impact on tumor control and OS, we suggest compliance with the recommendations of the expert consensus where available. Individual expert opinions are encouraged as guidelines where expert consensuses are not available. Moreover, in the absence of evidence-based safety about modified regimens, we can only recommend an individual approach to every patient taking into account all relevant factors. Whenever possible, standard treatment with all precaution measures for the prevention of COVID-19 should be applied.

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Радиотерапија и пандемија *COVID*-19 – осврт на тренутне препоруке

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САЖЕТАК

Болесници оболели од рака су под великим ризиком од развијања тешке клиничке слике и високог морталитета услед инфекције *COVID*-19. Зрачна терапија је један од кључних начина лечења тумора централног нервног система и рака плућа. Радиотерапија се најчешће примењује у већем броју фракција, што захтева много долазака у радиотерапијски центар и самим тим већи ризик од изложености инфекцији *COVID*-19. Компромис између оптималног третмана рака уз смањену изложеност инфекцији *COVID*-19 је упитан. За сада, најчешће мере које се примењују у радиотерапијским центрима су класификација болесника у приоритетне групе и чешћа примена хипофракционих режима зрачења. Од почетка пандемије *COVID*-19 објављено је само неколико консензуса експертских група за радиотерапију. У овом раду смо укратко прегледали доступне практичне препоруке експертских група за зрачну терапију и онкологију за лечење тумора плућа и централног нервног система, али и појединачна експертска мишљења током трајања пандемије *COVID*-19.

Кључне речи: *COVID*-19; радиотерапија; тумори мозга; рак плућа

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HISTORY OF MEDICINE / ИСТОРИЈА МЕДИЦИНЕ

Лекарке и супруге лекара – чланице Женског друштва (1875–1915)

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САЖЕТАК

Уочи Херцеговачког устанка (1875) и Првог српско-турског рата (1876–1877) у Србији су основана два удружења са хуманим циљевима рада која ће у свим ратним сукобима у којима је учествовао српски народ пружати значајну помоћ здравственој служби. Прво од њих било је Женско друштво, основано маја 1875, а друго је било Српско друштво Црвеног крста, основано фебруара 1876. године. Уочи ратова заједнички су организовала курсеве за обуку добровољних болничара и болничарки, током ратова су установљавала резервне болнице, прикупљала новчане прилоге, санитетски материјал и одећу за рањенике и избеглице. У мирнодопском времену, поред осталих својих активности, радила су на подизању свести становништва о важности хигијене и правилне исхране. У Женском друштву су посебно биле активне лекарке и супруге лекара, чији пример су следиле жене из њиховог окружења. Рад чланица Женског друштва био је драгоцен нарочито у подружницама, јер су у културно заосталим сеоским срединама заједно са својим мужевима радиле на здравственом просвећивању.
Кључне речи: лекарке; супруге лекара; Женско друштво; добровољне болничарке

УВОД

Прва грађанска удружења у Кнежевини Србији настала су убрзо после доношења Турског устава (1838) и конституисања државне управе (1839). Већ 1841. основано је Друштво српске словесности, претеча данашње Српске академије наука и уметности. Закон о еснафима (1847) омогућио је настанак првих удружења занатлија и трговаца. У другој половини 19. века настала су три нова удружења од којих су два – Техничарска дружина (1868) и Српско лекарско друштво (1872) – била професионална, док је Друштво за пољску привреду (1869) окупљало делатнике различитих професија. Удруживање ради заједничког деловања постало је не само израз потребе грађанства већ и начин друштвеног ангажовања, те је до краја века у Београду настало преко четрдесет различитих удружења [1]. Као једно од првих међу њима, 1875. године основано је Женско друштво. Институционално организовање дало је ширу основу активностима које су жене и до тада спорадично предузимале. Тако су за време сукоба са Турцима 1862. београдске госпође припремале завојни материјал и радиле као болничарке, куварице и праље у болницама, ангажовале су се у акцијама прикупљања новчаних прилога за изградњу Народног позоришта (1864, 1873) и Варошке болнице (1864), као и у организовању прославе педесетогодишњице Другог српског устанка [2].

Женско друштво је по свом карактеру било хуманитарно удружење, засновано на идејама просветитељства и доброчинства. Настало је по угледу на удружења жена првенствено у Немачкој и Русији, али и другим европским земљама, у времену када се сматрало да је брига о другима специфична женска мисија. Иако се по данашњим мерилима чланице овог Друштва не би сврставале у феминисткиње, оне су од самог почетка радиле на побољшању положаја женске деце, њиховог образовања и оспособљавања за самосталан живот. Све остале активности Друштва такође су биле усмерене ка помоћи и заштити најугроженијих категорија друштва – у првом реду старих и болесних жена и самохраних мајки.

ОСНИВАЊЕ И РАД ЖЕНСКОГ ДРУШТВА У МИРНОДОПСКИМ ПЕРИОДИМА

Женско друштво је основано 17/29. маја 1875, иницијативом Катарине Миловук (1844–1913), управнице Више женске школе. У Оснивачком одбору, који је чинило још 13 угледних жена, била је и Варвара Машин, супруга др Јована Машина, једног од оснивача Српског лекарског друштва. Прва председница Друштва била је Катарина Миловук, потпредседница Јелена Грујић, а Варвара Машин једна од чланица прве Управе. Циљеви друштва, дефинисани Правилима, били су „усавршавање женског пола у правцу саморадње“, помагање „сиротих и невољних“ и обука сиромашних жена „за добре и ваљане служитељке и раденице“. У чланство је примана свака женска особа

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старија од 17 година, без обзира на њен брачни статус, вероисповест и народност [3].

При оснивању Друштва, један од важних циљева Катарине Миловук био је да у чланство привуче жене из друштвене и политичке елите јер је у патријархалној Србији подршка утицајних људи била неопходна за његов опстанак и омасовљење. Угледу Друштва значајно су доприносила покровитељства српских владарки и жена из Краљевског дома. Међу њима посебно место припада првој покровитељки кнегињи и краљици Наталији Обреновић, која је била највећи добротвор овог Друштва. Од самог почетка Друштво је имало највећу подршку у круговима интелигенције, људи школованих у иностранству, међу којима су лекари заузимали истакнуто место, посебно у унутрашњости земље. Својим погледима на живот и животним навикама стеченим у европском културном миљеу из којег су потицали или су се у њему школовали, лекари су у свом друштвеном окружењу били носиоци просветитељства. Они су први указали на чињеницу да је за здравље породице, поготово на селу, образовање жене – домаћице од кључне важности. Зато су многи од њих не само подржавали ангажовање својих супруга у женским друштвима већ су и сами учествовали у њиховом раду. Супруге лекара су углавном потицале из средњих и виших друштвених слојева и имале су солидно опште образовање. У Друштву су такође биле активне лекарке, чији је број у Србији постепено растао од 1879. године, када се са студија вратила др Драга Љочић, прва Српкиња која је стекла звање доктора медицине.

У мирнодопским периодима Друштво је радило на образовању сиромашних девојчица и на заштити најугроженијих категорија друштва – сирочади, сиромашних ученика из унутрашњости, самохраних мајки и старица без породице. Ради остварења својих циљева основало је неколико установа – Раденичку школу 1879, комисиону продавницу – Пазар 1882, Ђачку трпезу 1899, Дом за убоге и изнемогле старице 1900. и Ћилимарску школу са радионицом у Пироту 1907 [4]. Друштву припада заслуга за оснивање првог женског часописа *Домаћица*, који је излазио од 1879. до 1941. Часопис је од 1888. године уређивао Литерарни одбор. Значајно постигнуће Друштва било је и оснивање Српског женског савеза (1906), који је окупио сва женска удружења Краљевине Србије. Савез се исте године прикључио Међународном женском савезу, који је у том тренутку имао преко осам милиона чланова [5].

ДЕЛАТНОСТИ ЖЕНСКОГ ДРУШТВА У ТОКУ РАТОВА

Иако се Друштво није припремало за деловање у току рата, његове прве активности биле су последица Херцеговачког устанка (1875). Из ратом захваћених подручја народ је избегао и трпео велику оскудицу, па су се чланице Друштва ангажовале у прикупљању и слању новчаних прилога и одеће за избеглице. Све до Првог светског рата чланице Друштва су у сваком ратном сукобу у коме је Србија учествовала међу првима притицале у помоћ, сакупљале новчана средства, израђивале санитетски материјал и радиле као добровољне болничарке. Због тога је у овом раду период до 1915. године посматран као посебна целина.

По избијању Првог српско-турског рата, јуна 1876. године, Друштво је основало своју болницу у Београду. Од септембра 1876, када је др Марија Зиболд заменила бечког лекара Штајнера на месту управника, Болница је највећим делом – од организационих послова, администрације, економије и лечења рањеника, била у рукама жена. Чланице Друштва и добровољне болничарке обављале су комплетну негу рањеника, помагале при превијањима, учествовале у расподели терапије, дежурале уз постеље оперисаних. Помоћ коју су пружале рањеницима при одржавању преписке са породицом била је важан вид емотивне подршке [6, 7]. Прерано преминули докторанд Милан М. Радовановић (1849–1878), у то време лекарски помоћник у Болници, описујући њен рад одао је велико признање Друштву и његовим чланицама [8]. Значај Болнице огледа се у чињеници да је то била прва или једна од првих женских болница у Европи, не само по саставу особља већ и по томе што је била финансирана средствима једног женског друштва (Слика 1).

Чланице Друштва биле су ангажоване и у резервним болницама у унутрашњости. У свом рапорту кнезу од 26. фебруара 1878, начелник Санитета Врховне команде др Владан Ђорђевић посебно је похвалио рад „Параћинског одбора госпођа“ у време док је на његовом челу била Јадвига Гонсиоровска, супруга др Казимира Гонсиоровског [9].

Током Српско-бугарског рата 1885. године чланице Друштва које су биле обучене за болничарке радиле су у

Слика 1. Особље прве женске болнице Београдског женског друштва 1876/77; у првом реду слева: Милка Котуровић, шеф болнице др Марија Фјодоровна Зиболд, Божена Снећивна и Даринка Вујић; стоје: др Фећушин и др Раиса Свјатловска; (Музеј науке и технике, Збирка Музеја Српског лекарског друштва, *MNT.T*:11.7.1130)
Figure 1. The staff of the First Women's Hospital of the Belgrade Women's Society 1876/77; from the left: Milka Koturović, head of the hospital Dr. Marija Fyodorovna Zibold, Božena Snećivna and Darinka Vujić; standing: Dr. Feđušin and Dr. Raisa Svjatlovska; (Museum of Science and Technology, Collection of the Museum of the Serbian Medical Society, MNT.T: 7/11/11)

резервним болницама, којих је највише било у Београду. Остале чланице су шиле рубље и завојни материјал у радионици у двору. За заслуге током тог рата краљ Милан Обреновић је одликовао преко 220 чланица Друштва златном и сребрном Медаљом краљице Наталије [10].

Непосредно по објави мобилизације уочи Првог балканског рата, чланице Друштва су покренуле широку делатност прикупљања прилога за породице војних обвезника. Раденичка школа је прекинула рад и била претворена у радионицу за шивење, а управа је решила да отвори и своју болницу. С одобрењем управе Војног санитета, Болница Женског друштва је радила под називом ХВ резервна болница у Београду и била је смештена у згради Основне школе „Свети Сава" у Мекензијевој улици. Први управник је био др Миладин Свињарев, а касније др Милан Васић. Друштво је финансирало хонораре иностраних лекара који су радили у Болници, док су главне надзорнице биле председница друштва Даница Соларовић и потпредседнице Мила Николић и Боса Стефановић [11]. Дужност нудиља у болесничким собама вршиле су све чланице Управе, поједине редовне чланице, али и жене које нису биле чланице Друштва. Оне су се бринуле о набавци потребног болничког материјала, исхрани болесника и одржавању хигијене. Надлежни су често истицали ову болницу као узор у погледу чистоће, реда и правовремене лекарске помоћи рањеницима. Болница Женског друштва је међу последњима завршила свој рад 11. септембра 1913. године. Лечено је укупно 1750 рањеника и болесника, а Управа је о њима водила бригу и при отпусту, снабдевајући их одећом, обућом и новцем. Све чланице Друштва које су радиле у болници Женског друштва одликоване су Крстом милосрђа и медаљама Црвеног крста [12, 13]. Чланице подружница Друштва широм Србије такође су радиле у резервним болницама у својим местима или у близини.

САРАДЊА ЖЕНСКОГ ДРУШТВА СА ЛЕКАРИМА И ДРУГИМ УДРУЖЕЊИМА

Врло успешна сарадња Друштва са лекарима како у Београду, тако и у унутрашњости, у подружницама, остварена је непосредно по оснивању Женског друштва. Од 1881. године, када је Друштво преузело бригу о ратној сирочади, о њиховом здрављу су се старали београдски лекари др Јосиф Холец, др Младен Јанковић, др Лаза К. Лазаревић и други. Лечиле су их, такође, ученице Раденичке школе и штићенице Дома старица у Београду, често им лично обезбеђујући потребне лекове. Лекари који своје услуге нису наплаћивали, попут др Богосава Завађила и др Милана Васића, почетком 20. века проглашавани су за добротворе Друштва [14, 15]. Лекари у унутрашњости земље, обично они чије су супруге биле чланице подружница, водили су бригу о здрављу ученица подружинских школа. Осим бесплатних систематских прегледа ученица, прегледали су просторије школа, па су често због неадекватних услова у њима ограничавали број полазница. По савету лекара, чланице подружнице у Лозници су почетком 1896. године прикупиле новац за куповину Беринговог серума против дифтерије, болести која је у то време имала високе стопе леталитета. Од прикупљених прилога купљен је довољан број доза за серопрофилаксу ученица Раденичке школе и сиротиње [16]. Подружнице су у сарадњи са лекарима такође организовале здравствено-просветна предавања за грађанство.

Посебно блиску сарадњу Друштво је развило са Српским друштвом Црвеног крста непосредно по његовом оснивању, 1876. године. Уочи Првог српско-турског рата, на позив Друштва Црвеног крста Женско друштво је финансирало набавку пакетића првог завоја за војнике [17]. Чланице Друштва су те године похађале прве курсеве за добровољне болничаре и болничарке које је организовао Лекарски одсек Друштва Црвеног крста. Први курс је одржан у априлу, у Варошкој болници у Београду (за мушке полазнике), а предавач је био др Казимир Гонсиоровски, управник Болнице. Полазнице су курс похађале у Великој школи, где је предавач био др Павле Стејић, градски физикус [18]. Други курс, организован у јуну, мушкарци су похађали у Војној болници, а предавач је био санитетски капетан др Илија Милић. Жене су похађале предавања др Јована Валенте у Великој школи [19]. Међу првим добровољним болничаркама које су завршиле курс биле су чланице Женског друштва Милка Котуровић, наставница Више женске школе, и Љубица Луковић.

Током 1892. године Женско друштво и Друштво Црвеног крста су покренули иницијативу заједничког организовања курса за обуку болничарки за рад у ратним и мирнодопским условима. Значајно је истаћи да у то време, осим Школе за бабице, у Србији није постојала школа за образовање средњег медицинског кадра. Наредне, 1893. године друштва су дефинисала питања своје сарадње потписивањем Статута на основу ког је расписан конкурс, а кандидаткиње је изабрало Женско друштво. Наставни план и Правила за хонорарне и добровољне болничарке израдио је Санитетски одбор Црвеног крста, а позив за упис на курс објављен је у листу *Домаћица*. Настава је започела 1/13. децембра и трајала је три месеца. Курс је похађало десет приправница за хонорарне нудиље и шест за болничарке [20].

ЛЕКАРКЕ И СУПРУГЕ ЛЕКАРА КАО ЧЛАНИЦЕ ЖЕНСКОГ ДРУШТВА

Будући да архива Женског друштва није сачувана, главни извори података током истраживања идентитета чланица били су нам годишњи извештаји о раду Друштва објављивани у *Домаћици*, уз које су публиковани и спискови имена чланица. Од око 2000 чланица, колико је Друштво имало само у Београду у периоду од 1875. до 1915. године, утврдили смо идентитет 682 чланице (име и презиме, породично порекло и/или име и професија супруга, понекад и професија чланице). Међу њима је 10 лекарки и преко 80 супруга лекара,

Слика 2. Др Јован и Даница Валента (*Atelier von Franz Aberle, Ungarische Weisskirchen*, 1872); власништво породице
Figure 2. Dr. Jovan and Danica Valenta (Atelier von Franz Aberle, Ungarische Weisskirchen, 1872); property of the family

што износи око 14% од укупног броја идентификованих чланица. У групи идентификованих чланица најзаступљеније су биле супруге официра (око 30%), те се може се закључити да је скоро половина чланица Друштва у престоници потицала из два веома важна и утицајна друштвена круга – војног и медицинског. Из непотпуних података о чланицама подружница идентификовали смо преко 40 лекарки и супруга лекара, што значи да је готово у свакој подружници радила и често била међу оснивачицама бар једна супруга лекара који је радио у том месту.

Међу првим чланицама Женског друштва примљене су као почасне чланице две руске лекарке Раиза Свјатловска и Марија Зиболд (1877). Поред њих, чланице су биле и др Даринка Банковић, рођена Клајн-Маленић, др Марија Вучетић Прита, др Љубица М. Гођевац, рођена Ђурић, др Марија Ђурић, др Драга Љочић Милошевић, др Јадвига Олшевска, др Наталија Николајевић и др Василија Стојиљевић. У мирнодопском периоду оне су најчешће бринуле о ученицама Раденичке школе или су радиле у Ђачкој трпези, док су током ратова биле ангажоване у болницама Женског друштва.

Супруге лекара су имале веома значајну улогу, не само у раду Друштва и пропагирању његове делатности већ и на другим пољима. Њихов здравствено-просветни рад био је посебно важан у културно слабије развијеним срединама, у подружницама. У односу на супруге официра, у Управи Друштва су биле малобројније, али су зато биле ангажованије у двема најзначајнијим хуманитарним установама Друштва – у Управи и надзору Ђачке трпезе и у Управи Дома госпођа. Највеће пожртвовање испољавале су током ратова, када су, често уз своје супруге, радиле као болничарке у резервним болницама. Поједине супруге лекара биле су чланице Друштва по десет и више година, док је врло мали број оних које су напуштале Друштво после периода краћег од пет година.

Међу супругама лекара, по високим функцијама које су обављале у Друштву, издвајају се Варвара Машин, Даница Валента и Марија Холец.

Варвара Машин, рођена Вшетечка (Нимбурк, Чешка око 1828–?, пре 1910) у свом родном граду упознала је Јована Машина (1820–1884), младог лекара на почетку каријере. Након венчања 1846, Машини су четири године живели у Ваљеву, где је Јован службовао као окружни физикус, а потом у Београду. У породици је рођено шесторо деце, међу којима је био Светозар, први супруг Драге Луњевице, потоње српске краљице. Јован Машин је у Београду службовао у цивилном, потом у војном санитету, а тринаест година (1860–1873) био је дворски лекар. Сматран је једним од најстручнијих лекара и био је члан Друштва српске словесности и Српског ученог друштва. Као супруга тако угледног човека, Варвара Машин је била међу највиђенијим женама свог доба. Као што је речено, била је једна од оснивачица Друштва и чланица прве Управе. За почасну чланицу изабрана је 1905. године.

Даница Ј. Валента, рођена Стефановић (?–1904) била је друга супруга др Јована Валенте (1826–1887). Дужност председнице Друштва вршила је само неколико месеци, од априла 1878. до јануара 1879. Даничин супруг Јован Валента такође је био угледан лекар са богатом радном каријером. Био је управник Болнице округа и вароши Београда, хонорарни професор хигијене у Великој школи и у Вишој женској школи, члан Српског ученог друштва и један од оснивача Српског лекарског друштва (1872). Даница је била врло ангажована у раду Женског друштва, била је благајница Друштва, чланица Литерарног одбора, проценитељка у Пазару и надзорница Ђачке трпезе. Породица Валента је због Јованове службе на месту физикуса Округа пиротског од 1882. до 1886. живела у Пироту, а Даница је била иницијатор оснивања подружнице Друштва у том граду. У време Српско-бугарског рата, уз супруга Јована је радила у тамошњој резервној болници. Одликована је златном Медаљом кнегиње Наталије 1878. и Медаљом Црвеног крста (Слика 2).

Значајан печат у раду Друштва оставила је породица Холец. Марија Холец је била супруга санитетског пуковника и управника Војне болнице у Београду др Јосифа Холеца (1835–1898), који је такође био један од оснивача Српског лекарског друштва. Марија Холец је обављала важне дужности у Друштву – била је потпредседница, главна надзорница резервне болнице 1886, чланица одбора и надзорница Ђачке трпезе. Кћи Јосифа и Марије Холец, Катарина, била је чланица Друштва чак 55 година, од 1881. до своје смрти, 1936. године. Као секретар и главни администартор, 28 година је водила и усмеравала рад Друштва.

Осим Данице Валенте, у подружницама су биле посебно активне Милева Кужељ и Персида Краков. Милева Кужељ, супруга окружног лекара др Јарослава Кужеља (1846–1928), у Чачку је веома успешно реактивирала подружницу која се била готово угасила. Била је председница подружнице и чланица њене управе до 1905. године. Међу оснивачицама подружнице у Књажевцу 1901. године била је Персида Краков, супруга др Сигисмунда Кракова, санитетског поручника и трупног лекара ХВИ пешадијског пука у Књажевцу.

ЗАКЉУЧАК

У оснивању Женског друштва и његовом раду учествовале су жене које су по свом рођењу или својим брачним и породичним везама припадале политичкој, војној, културној и научној елити српског друштва. Од преко 2000 чланица Друштва у Београду, скоро четвртину су чиниле лекарке, супруге и кћерке лекара, што илуструје значај и углед Женског друштва, као и став српске елите према друштвеном ангажовању жена. У афирмацији и остваривању постављених циљева Друштву су помогла покровитељства спрских владарки и жена из Краљевског дома, у првом реду краљице Наталије Обреновић. Чланство лекарки такође је доприносило угледу и омасовљењу Друштва. Истрајне у настојању да упркос бројним препрекама стекну високо образовање, одлучне у борби за своја радна права и статус у друштву, оне су биле узор многим женама. Супруге лекара су се посебно ангажовале током ратова радећи као болничарке, чиме су дале значајан допринос раду српског санитета. У мирнодопским периодима, својим здравственопросветним радом утицале су на развијање хигијенских и здравствених навика сеоског становништва (Табела 1).

ЗАХВАЛНОСТ

За уступљену фотографију др Јована и Данице Валенте захваљујемо се господину Милошу Лазаревићу из Београда.

СУКОБ ИНТЕРЕСА

Аутори изјављују да нема сукоба интереса у вези са овим радом.

Табела 1. Лекарке и супруге лекара чланице Женског друштва и његових подружница 1876–1915; у табели су називи места наведени поред имена чланица у подружницама, а нису навођени уз имена чланица Београдског одбора; следе затим година приступања, функције у управи друштва и име супруга (код којих је то било могуће утврдити); у табели је „с." скраћеница за реч супруга.
Table 1. Female physicians and physicians' wives – members of the Women's Society and its affiliates 1876–1915; in addition to their names and places of their membership, listed are also the years of accession and positions they held within the Society, as well as the spouses' names (when known); the *c.* in the table stands for *spouse of.*

ЛЕКАРКЕ
1. Вучетић-Прита др Марија, (1905), с. др Николе Вучетића.
2. Гођевац др Љубица, рођ. Ђурић, (1900), чланица уређивачког одбора *Домаћице*, с. др Милорада Гођевца.
3. Љочић Милошевић др Драга, (1895), лекарка питомица Женског друштва и ученица Раденичке школе, с. Аранђела Раше Милошевића, политичара.
4. Матић др Милица, Ражањ (1912).
5. Маленић-Банковић др Даринка, (1900), чланица Литерарног одбора, аутор текстова о хигијени и здрављу у *Домаћици*.
6. Николајевић Давидовић др Наталија, (1914).
7. Олшевска др Јадвига, Лозница (1895), Пожаревац (1898).
8. Стојиљевић др Василија, (1914).

СУПРУГЕ ЛЕКАРА
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2. Атанасијевић Софија Соја С., (1900), с. др Ставре Атанасијевића.
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Female physicians and physicians' wives – members of the Women's Society (1875–1915)

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SUMMARY

Prior to the Herzegovina Uprising (1875) and the First Serbian–Turkish War (1876–1877), two associations were established in Serbia with humane work goals that provided great assistance to the health service throughout the war conflicts in which the Serbian people participated. The first of these was the Women's Society, established in May 1875, and the second one was the Serbian Red Cross Society, established in February 1876. Shortly before the wars, they organized training courses for voluntary paramedics and nurses, during the wars they established reserve hospitals, collected money, medical supplies, and clothing for the wounded and the refugees. In peacetime, among other activities, they worked to raise public awareness of the importance of hygiene and proper nutrition. Female physicians and physicians' wives were particularly active in the Women's Society, and were followed by women around them. The work of the female members of the Women's Society was especially invaluable in the subcommittees, as they worked together with their husbands to promote health education in culturally primitive rural areas.

Keywords: physicians; physicians' wives; Women's Society; voluntary nurses

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LETTER TO THE EDITOR / ПИСМО УРЕДНИКУ

Challenges arising from the residency program for traditional Chinese medicine postgraduate students in China

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Dear Editor,

The Chinese residency program ("5 + 3" system) became formalized and institutionalized nationwide in 2015, covering all the primary medical specialties, which included traditional Chinese medicine as well [1]. Traditional Chinese medicine is an experience-based specialty that has been inherited and developed through the handing-down teaching strategy for thousands of years, and this strategy has been proven effective for Chinese medical education [2]. Interestingly, traditional Chinese medicine postgraduate students nowadays are subjected to the US-style residency program, which is tailored for Western medicine education.

Unfortunately, the feedback of the residency program seems to be lower than expected due to some notable limitations. There are two key aspects of the current restrictions, which include curriculum set and clinical training [3]. The curriculum set issues are that the courses have a much shorter duration, weak pertinence, and absence of timeliness. Traditional Chinese medicine postgraduates used to take at least a one-year course on campus prior to participating in a two-year program for clinical training or medical research training. However, over 80% of postgraduate students only take a three-year residency program at a designated hospital during their entire postgraduate career as requested. Consequently, this inhibits students from possessing a sufficient professional knowledge base prior to starting clinical training. Additionally, residents with inadequate profession can be a real threat to patient safety.

The purpose of clinical training is to lay a foundation for residents to be independently engaged in health care via targeted and systematic teaching and practice [4]. Nevertheless, three leading limitations arise as follows:

1) The training duration of each student's specialty (sub-subjects of traditional Chinese medicine) accounts for less than 1/3 of the entire program duration;
2) Students may not obtain competent support and guidance from their teaching peers at the hospital;
3) Negative activating emotions can be bred among students as a trend of neglect during their daily work or even becoming free labor at hospitals.

Although the residency program has been occurring in China for almost five years, this trend seems to continue. More importantly, most postgraduate students are subjected to the repetitive writing of medical records and may not be trained in typical traditional Chinese medicine since Chinese medical doctors are likely to undertake an unimaginable workload every day due to China's large population.

Notably, the aforementioned limitations are threatening the quality of the traditional Chinese medicine education. This, in turn, contributes to the graduation of unqualified physicians of traditional Chinese medicine and low-quality health care services. Therefore, the National Health Commission of China has to emphasize those problems and elaborate a localized and comprehensive residency program for the traditional Chinese medicine postgraduates, which will ultimately offer all Chinese citizens high-quality care.

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

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

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

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

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

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

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу

оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant* – Cross Check (iThenticate). Радови код којих се докаже плагијаризам/аутоплагијаризам биће одбијени, а аутори санкционисани.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (http://www.nlm.nih.gov/mesh)*.

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом. Уколико је рад у целости на српском језику, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад и претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе и актуелну тему чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°*C*), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада који чине – насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, рад из историје медицине и преглед литературе до 5000 речи, а за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3000 речи; радови за остале рубрике могу имати највише 1500 речи.
Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике“ у CA се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).
Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видеоприказа у *e*-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публи-

кације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примере навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *http://www.nlm.nih.gov/bsd/uniform_requirements.html*. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) и измирити накнаду за обраду чланака (*Article Processing Charge*) у износу од 3000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*Article Processing Charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату / накнаду за обраду чланка, као доказ о уплатама, уколико издавач нема евиденцију о томе. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за обраду чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

НАПОМЕНА. Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доле наведене адресе и број телефона.

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

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