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Two American scientists, left, David Julius (born November 4, 1955) and right, Ardem Patapoutian (born October 2, 1967), won this year's Nobel Prize in Medicine for understanding key mechanisms by which people feel heat, cold, touch and their own bodily movements. Today, their discoveries are used to develop therapeutic modalities for a wide range of diseases, including chronic pain.

Dva američka naučnika, levo, Dejvid Džulijus (rođen 4. novembra 1955) i desno, Ardem Patapoutijan (rođen 2. oktobra 1967), dobitnici su ovogodišnje Nobelove nagrade za medicinu za razumevanje ključnih mehanizama pomoću kojih ljudi osećaju toplotu, hladnoću, dodir i sopstvene pokrete tela. Danas se njihova otkrića koriste za razvoj terapijskih modaliteta za širok spektar bolesti uključujući hronični bol.

ORIGINAL ARTICLES

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Clinical significance of matrix metalloproteinase (MMP)-2 and MMP-9 expression in laryngeal squamous cell carcinoma

Klinički značaj ekspresije matriks metaloproteinaza (MMP)-2 i MMP-9 kod skvamocelularnog karcinoma larinksa

Dejan Rašić*, Aleksandar Perić*†, Biserka Vukomanović Djurdjević†‡, Jelena Sotirović*†, Nenad Baletić*†, Milanko Milojević*†, Ljubomir Pavićević*, Aleksandar Dimić*

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Abstract

Background/Aim. The previous researches suggest that matrix metalloproteinases (MMPs) have the ability to degrade extracellular matrix components and play an important role in malignant tumour progression and metastasis. The aim of this study was to investigate MMP-2 and MMP-9 expression in the tissue of laryngeal squamous cell carcinoma (LSCC) and to evaluate their clinical significance. **Methods.** In this prospective study the samples of tumour tissue of seventy patients with LSCC (45 glottic and 25 supraglottic), and samples of laryngeal mucosa of 70 patients with chronic laryngitis were immunohistochemically stained for MMP-2 and MMP-9. We studied the relationships between MMPs tissue expression and clinical and histological characteristics of the patients with LSCC in comparison to the patients with chronic laryngitis. **Results.** MMP-2 and MMP-9 expression was significantly higher in the tissue of both glottic and supraglottic LSCC than in chronically inflamed laryngeal mucosa ($p < 0.001$ and $p < 0.001$, respectively). There were positive correlations between epithelial MMP-2 expression and the presence of perineural invasion ($r = 0.515$, $p < 0.008$), lymphovascular invasion ($r = 0.559$, $p < 0.004$) and disease recurrence ($r = 0.415$, $p < 0.039$) in supraglottic LSCC, as well as between MMP-2 expression and the presence of the exophytic type of tumour growth ($r = 0.347$, $p < 0.020$) in glottic LSCC. Epithelial MMP-9 expression was associated with lymphovascular invasion ($r = 0.331$, $p < 0.026$) and the presence of the exophytic tumour growth ($r = 0.474$, $p < 0.001$) in glottic LSCC. **Conclusion.** MMP-2 and MMP-9 can be used as potential biomarkers for the assessment of LSCC progression.

Key words:
biomarkers; glottis; immunohistochemistry; larynx, neoplasms; laryngitis; matrix metalloproteinases.

Apstrakt

Uvod/Cilj. Prethodna istraživanja sugerišu da matriks-metaloproteinaze (MMP) imaju sposobnost da razgrađuju komponente vanćelijskog matriksa i imaju veliku ulogu u progresiji malignih tumora i nastanku metastaza. Cilj ove studije je bio da se ispita ekspresija MMP-2 i MMP-9 u tkivu skvamocelularnog karcinoma larinksa (SCKL) i da se proceni njihov klinički značaj. **Metode.** U prospektivnoj studiji, uzorci tumorskog tkiva 70 bolesnika sa SCKL (45 sa karcinomom glotisa i 25 sa karcinomom supraglotisa) i uzoraka tkiva sluznice larinksa 70 bolesnika sa hroničnim laringitisom bojeni su imunohistohemijski na MMP-2 i MMP-9. Ispitivana je povezanost između nivoa imunohistohemijske ekspresije MMP i kliničkih i histoloških karakteristika bolesnika sa SCKL u odnosu na bolesnike sa hroničnim laringitisom. **Rezultati.** Ekspresija MMP-2 i MMP-9 bila je značajno viša u tkivu glotičnog i supraglotičnog SCKL u odnosu na ekspresiju ispitivanih MMP u tkivu hronično inflamirane sluznice larinksa ($p < 0,001$ i $p < 0,001$, redom). Nađena je pozitivna korelacija između epitelne ekspresije MMP-2 i perineuralne invazije ($r = 0,515$; $p < 0,008$), limfovaskularne invazije ($r = 0,559$; $p < 0,004$) i pojave recidiva bolesti ($r = 0,415$; $p = 0,039$) kod supraglotičnog SCKL, kao i između ekspresije MMP-2 i pojave egzofitičnog tipa rasta tumora ($r = 0,347$; $p = 0,020$) kod glotičnog SCKL. Ekspresija MMP-9 u epitelu bila je povezana sa limfovaskularnom invazijom ($r = 0,331$; $p = 0,026$) i pojavom egzofitičnog tumorskog rasta ($r = 0,474$; $p < 0,001$) kod glotičnog SCKL. **Zaključak.** Endopeptidaze MMP-2 i MMP-9 mogu biti korišćene kao potencijalni biomarkeri za procenu progresije SCKL.

Ključne reči:
biomarkeri; glotis; imunohistohemija; larinks, neoplazme; laringitis; matriks metaloproteinaze.

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Introduction

Laryngeal malignant tumours make about 1%–2% of all malignant tumours in the human population [1]. Laryngeal squamous cell carcinoma (LSCC) is more common in male than in female population, occurring usually between the fifth and seventh decade of life. The most important etiological factors for LSCC occurrence are tobacco smoking, consumption of alcoholic drinks, gastroesophageal reflux, human papillomavirus (HPV) infections, air pollution, a diet low in fruits and vegetables. Alcohol in combination with tobacco significantly increases the risk of laryngeal cancer [1].

The prognosis of LSCC depends on the region involved in malignant disease formation, actual stage of disease, which can be determined by Tumour Node Metastasis (TNM) classification. Survival is significantly reduced due to the cancer development and spread of the disease (local or regional recurrence and distant metastasis occurrence) [2]. In recent years, molecular investigations have discovered many mechanisms of oncogenesis in malignant tumours. The expression of the specific tumour markers is associated with development and biological behaviour of laryngeal carcinoma. Prognostic value identification of some tumour markers would be of great help in selecting treatment modalities, which is of crucial significance for the prognosis of the disease. Matrix metalloproteinases (MMPs) are the family of 23 endopeptidases, or gelatinases, structurally very similar proteolytic enzymes which have the ability to degrade extracellular matrix components [3]. Thus, MMPs play important role in tumour progression and metastasis. The elevated levels of MMPs can be detected in the tumour tissue and serum of patients with advanced cancer. MMPs may be activated by various agents including other protease. Depending on the type of substrate which degrades or structural domains, MMPs can be divided into 6 subgroups. MMP-2 and MMP-9 have the ability to destroy the type IV collagen, which is a very important component of basement membrane [4]. This is a key moment, which is necessary for tumour growth and distant metastasis onset. Some published papers have shown that MMP-2 and MMP-9 may play important roles in the progression of the head and neck squamous cell carcinoma [4, 5]. However, the results regarding the correlation of MMP-2 and MMP-9 expression levels with clinical and pathological characteristics, as well as with prognosis of LSCC, are not consistent. The identification of patients with a high risk of tumour development, the presence of subsequent locoregional tumour recurrence, as well as the existence of regional metastases, are very important for the prognosis of the disease, as well as for the choice of the best treatment modality.

The aim of the study was to evaluate the clinical significance of MMP-2 and MMP-9 expression in the tissue samples of patients with LSCC, comparing their expression in glottic and supraglottic region of the larynx. To our knowledge, this is the first study completely investigating the relationships between the MMPs expression, clinical characteristics of LSCC, 5-year survival rates, and the presence of lymphovascular and perineural invasion of the malignant cells in patients with LSCC.

Methods

Study population

This prospective, observational, cross-sectional study included a total of 140 patients, 70 with histologically verified LSCC and 70 with chronic laryngitis. The patients with the malignant tumour were divided into groups with respect to: the localization of the tumour in the larynx (supraglottic, glottic); TNM stages of the disease; a degree of tumour differentiation (good, moderate, poor); the disease stages (I, II, II, IV); the types of tumour growth (infiltrative, exophytic, mixed); the development of locoregional recurrence (with or without disease recurrence); disease-free interval (with or without that), and 5-year survival (with or without 5-year survival). The patients diagnosed, treated and followed-up for LSCC for a period of minimlly 5 years (between 2010 and 2017) in our Clinic for Otorhinolaryngology were included in this prospective, observational study. The control group consisted of patients diagnosed and treated for chronic laryngitis in the same period. Pathological and immunohistochemical analyses of tissue specimens were performed at the Institute of Pathology by the same experienced pathologist. The research was approved by the Ethics Committee of our Institution. A written informed consent was obtained from all participants to use their medical data.

The criteria for inclusion in the study were histologically verified LSCC and histologically verified chronic inflammatory changes in laryngeal mucosa, including the patients with keratosis (hyperkeratosis, parakeratosis, etc). All patients enrolled in the study underwent a primary surgical treatment. They were interviewed about their profession, history of malignant diseases in the family, their habits and health status. The criteria for exclusion from the study were other malignant diseases, previous radiotherapy or chemotherapy, previously performed surgical treatment of laryngeal tumour, systemic diseases affecting the larynx, laryngeal benign pseudotumours (vocal fold oedema, polyps, granulomas, etc).

Histopathological examination

Tissue specimens, that were obtained during the laryngomicroscopy or laryngectomy, were fixed for 24 h in 4% buffered formaldehyde solution. Then, they were washed by water and dehydrated by concentrated ethanol (70% up to absolute), then lipofilled in xylene and embedded in paraffin. Paraffin blocks were sectioned at the thickness of 3–5 μm. The sections were stained with hematoxylin-eosin (HE). The type and dimensions of tumour, histological and nuclear grade, perineural and lymphovascular invasion, keratinisation of tumour, spread beyond the larynx or in the regional lymph nodes were determined by the preparations. Determination of TNM status was done according to the TNM classification of American Joint Committee on Cancer (AJCC) [6].

Immunohistochemical staining

Immunohistochemical staining includes a series of technological procedures: deparaffining after cutting the sections of 3–4 μm form paraffin mold and drying phase following the sinking in xylene, alcohol and distilled water then proteolytic digestion. Deparaffined sections were cooked twice in a microwave oven in a cuvette with 250 mL of citrate buffer solution (10 mmol/L) at a maximum temperature, for five min. After that, sections were cooled in a citrate buffer at the room temperature for 30 min and washed with distilled water two times for thirty sec. The next phase involved the blocking of endogenous peroxidase: tissue sections were placed in 3% hydrogen peroxide for five min; then washed with distilled water, overlaid with a phosphate-buffered saline three times for two min. Immunohistochemical staining was performed with human anti-MMP-2 and anti-MMP-9 antibodies (R&D Systems, Inc, Minneapolis, USA). The analysis of MMP-2 and MMP-9 expression levels was performed semi-quantitatively on the basis the intensity of staining cytoplasm of epithelial and stromal cells by light microscopy. The staining was negative when no cells stained for MMP-2 or MMP-9 were found. Immunoreactivity was graded from 1 to 3 : 1 = weak (0%–10% stained cells), 2 = moderate (10%–50% stained cells) and 3 = strong (more than 50% stained cells).

Study power and the number of participants

According to the results of the previous study performed by Peschos et al. [7], we expected the difference of 20% in the expression of MMP-9 between LSCC tissue and chronic laryngitis tissue. The type I error (α-level) was set to 0.05. Using z-test (differences in proportions between independent groups), we calculated the minimum of 70 participants would be required in each group to raise the study power of 80%. For the calculation of the number of participants in each group, the G*Power 3.1.9 programme (Heinrich Heine Universität, Düsseldorf, Germany) was used.

Statistical analysis

Statistical analysis of the data was done with the statistical software package, SPSS Statistics 18 (SPSS INC, Chicago, Illinois, USA). As the majority of variables were expressed as categorical data (frequencies), we used the χ^2 test to compare the values of different frequencies. In case of continuous data, the variables were presented as mean value ± standard deviation (SD), median, min and max values. Kolmogorov-Smirnov test was used for the evaluation of the normality of the data distribution. The levels of statistical significances between the two groups were assessed by the *t*-test and Mann-Whitney test. A one-way analysis of variance (ANOVA) was used to calculate the differences among three groups of participants. To assess the levels of the statistical differences, we performed a *post-hoc* Dunn-Bonferroni correction. For the comparison of the disease free interval and 5-year survival between glottic and supraglottic LSCC, we used Kaplan-Meier analysis. The Pearson's and Spearman's correlation analyses were used to establish the value of relation between parameters. All the differences were estimated at $p < 0.05$ to be statistically significant.

Results

The mean age of patients with LSCC was 59.4 (± 7.1) years, range from 40–79 years. There were 60 (85.7%) male and 10 (14.3%) female patients. Among them, 45 (64.3%) of patients had glottic LSCC and 25 (35.7%) of patients had supraglottic LSCC, mean age of 59.7 (± 6.8) years and 58.8 (± 7.7), respectively. The majority of patients with glottic LSCC were in T1/T2 stage, whereas the majority of supraglottic LSCC patients were in T3/T4 stage. Table 1 shows the distribution of patients according to T and N stages. The most common stage of glottic LSCC was I (53.3%), and the most common stage in supraglottic LSCC was IV (40.0%). It was found that more frequent lymph node metastases (N+ status) were present in supraglottic than in glottic LSCC (40% *vs* 4.4%) (Table 1). The 5-year survival rates for glottic LSCC and supraglottic LSCC were 77.8% and 76.0%, respectively. The disease-free interval was present in 71.1% of the glottic LSCC patients and 68.0% of the supraglottic LSCC. We found no significant difference regarding the disease-free interval and 5-year survival between glottic and supraglottic LSCC ($p = 0.835$ and $p = 0.776$, respectively).

The positive MMP-2 expression in epithelial cells was detected in 65 (92.9%) of patients with LSCC (Figure 1a). In stromal cells, positive immunostaining was found in 66 (94.3%) of cases. In patients with the diagnosis of chronic laryngitis, the MMP-2 expression was negative in 72.9% of specimens (Figure 1b).

The positive MMP-9 epithelial immunostaining was detected in all LSCC specimens (Figure 2a). Positive stromal expression of MMP-9 was detected in 69 (98.6%) of LSCC

Table 1

Distribution of patients according to T and N stages

Region of larynx	T	n (%)	N	n (%)
Glottis	T1/T2	36 (80)	N0	43 (95.6)
	T3/T4	9 (20)	N+	2 (4.4)
Glottis total		45 (100)		45 (100)
Supraglottis	T1/T2	7 (28)	N0	15 (60)
	T3/T4	18 (72)	N+	10 (40)
Supraglottis total		25 (100)		70 (100)

T1-T4 – tumour stage; N – lymph node; N0 – without lymph node metastasis; N+ – with lymph node metastasis.

specimens. In patients with chronic laryngitis, moderate MMP-9 expression was found in 47.1% of tissue specimens (Figure 2b). Our results revealed significantly higher MMP-2 and MMP-9 expression in both epithelium and stromal tissue specimens of patients with glottic and supraglottic LSCC in comparison to patients with chronic laryngitis ($p < 0.001$ and $p < 0.001$, respectively). These results are presented in Figure 3.

In patients with glottic LSCC, we found a positive correlation between the epithelial and stromal MMP-2 expression ($r = 0.315$, $p < 0.05$). We also found a positive relationship between the epithelial MMP-2 and epithelial MMP-9

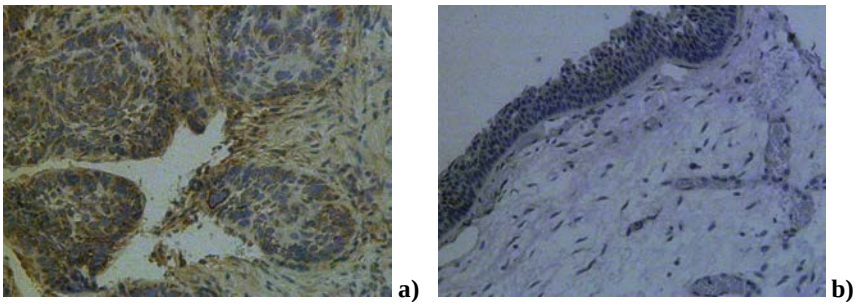

Fig. 1 – Immunoreactivity of matrix metalloproteinase (MMP)-2 in (hematoxylin-eosin, x40): a) laryngeal squamous cell carcinoma (LSCC); b) chronic laryngitis.

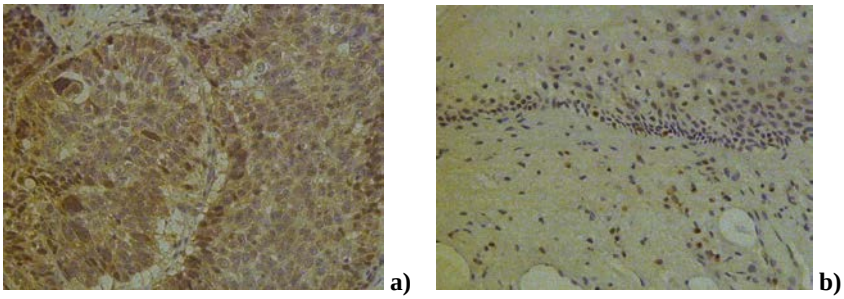

Fig. 2 – Immunoreactivity of matrix metalloproteinase (MMP)-9 in (hematoxylin-eosin, x40): a) laryngeal squamous cell carcinoma (LSCC); b) chronic laryngitis.

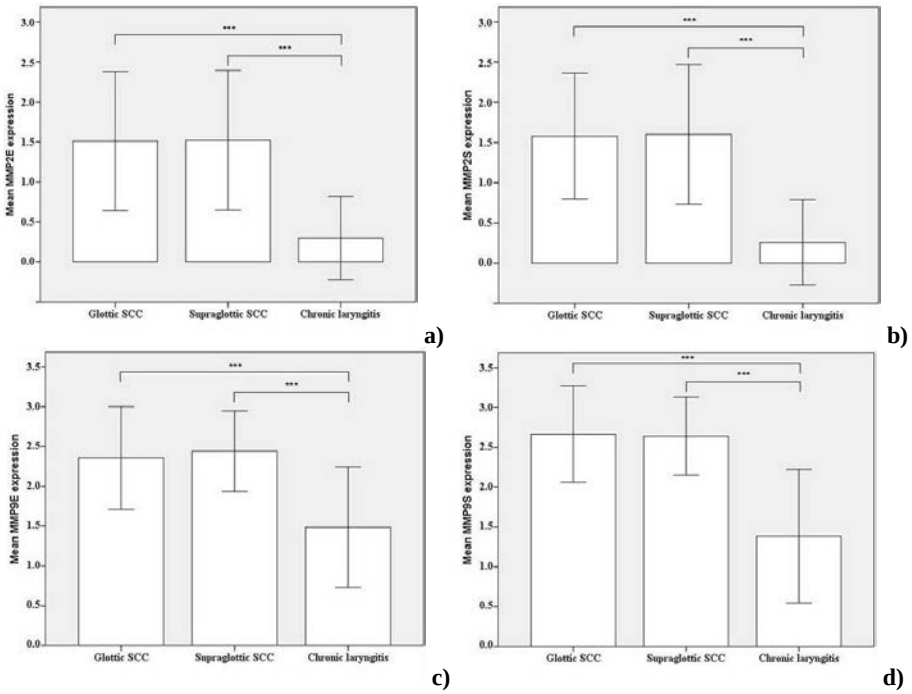

Fig. 3 – Comparison of matrix metalloproteinase (MMP) expression in tissue specimens of patients with glottic squamous cell carcinoma (SCC), supraglottic SCC, and chronic laryngitis: a) expression of MMP-2 in epithelium (MMP-2E); b) expression of MMP-2 in stroma (MMP-2S); c) expression of MMP-9 in epithelium (MMP-9E); d) expression of MMP-9 in stroma (MMP-9S). ***$p < 0.001$ *vs* chronic laryngitis.

immunostaining (r = 0.313, p = 0.05). Also, there was a positive correlation between the epithelial and stromal MMP-9 expression (r = 0.435, p = 0.01). In patients with supraglottic LSCC, there was a statistically significant relationship between the epithelial and stromal MMP-2 expression (r = 0.397, p = 0.05), also between the epithelial MMP-2 and epithelial MMP-9 (r = 0.517, p = 0.01) and stromal MMP-9 expression (r = 0.491, p = 0.05). Also, we found a positive correlation between the epithelial and stromal MMP-9 expression (r = 0.497, p = 0.05) in supraglottic LSCC. All results regarding the correlations between tested marker expressions are presented in Table 2.

The correlations between stromal MMP-2 and MMP-9 expressions and clinicopathological characteristics of both glottic and supraglottic LSCC were not presented due to the statistical insignificances. The statistically significant correlations between the expression of MMP-2 in epithelial cells and clinicopathological features of LSCC are shown in Table 3.

Our study showed that epithelial MMP-2 expression is not in correlation with T or N stages, nuclear grade and clinical stages, disease-free interval and 5-year survival.

In supraglottic LSCC, the MMP-2 epithelial expression was in correlation with the presence of perineural (r = 0.515, p = 0.008) and lymphovascular (r = 0.559, p = 0.004) invasion. A correlation between the epithelial MMP-2 expression and local recurrences of the primary supraglottic cancer (r = 0.415, p = 0.039) was noticed as well. Also, a significant negative correlation was recorded between the MMP-2 epithelial expression and histological stage (r = -0.521, p = 0.008) in supraglottic LSCC. The MMP-2 tumour cell expression was in correlation with the presence of exophytic type of tumour growth in glottic LSCC (r = 0.347, p = 0.020). In supraglottic LSCC, we found no association between the type of tumour growth and the level of MMP-2 expression.

The most important correlations between the MMP-9 epithelial expression and clinicopathological features of LSCC are presented in Table 3.

The present study revealed neither the correlation be-

Table 2

Correlations between MMPs expressions in glottic and supraglottic region of the larynx

Region of larynx			MMP-2E	MMP-2S	MMP-9E	MMP-9S
Glottis	MMP-2E	r	–	0.315	0.313	0.180
		p		0.035	0.037	0.236
	MMP-2S	r			0.259	0.229
		p			0.086	0.131
	MMP-9E	r			–	0.435
		p				0.003
Supraglottis	MMP-2E	r	–	0.397	0.517	0.491
		p		0.049	0.008	0.013
	MMP-2S	r		–	0.110	0.347
		p			0.601	0.089
	MMP-9E	r			–	0.497
		p				0.012

***r* – Spearman's correlation coefficient; *p* – probability; MMP – matrix metalloproteinase; E– expression in epithelium; S – expression in stroma.**

Table 3

Correlations between expressions of MMP-2 and MPP-9 in epithelial cells and clinicopathological features of LSCC

Region of larynx	Clinicopathological features	**MMP-2** (n = 70)		**MMP-9** (n = 70)	
		r	*p*	*r*	*p*
Glottis	Histologic grade	-0.188	0.215	-0.257	0.284
	LV invasion	0.083	0.588	0.031	0.026
	PN invasion	0.074	0.542	0.100	0.409
	Local recurrence	-0.211	0.164	0.178	0.332
	Exophytic growth	0.347	0.020	0.474	0.001
Supraglottis	Histologic grade	-0.521	0.008	-0.358	0.187
	LV invasion	0.559	0.004	0.345	0.092
	PN invasion	0.515	0.008	0.161	0.442
	Local recurrence	0.415	0.039	0.183	0.401
	Exophytic growth	0.265	0.201	-0.228	0.273

***r* – Pearson's correlation coefficient; *p* – probability; MMP – matrix metalloproteinase; LV – lymphovascular; PN – perineural; LSCC – laryngeal squamous cell carcinoma.**

tween the epithelial MMP-9 expression and some clinicopathological characteristics (T stage, N stage, clinical stage, nuclear stage, histological stage), nor the correlation with disease-free interval and 5-year survival.

Epithelial MMP-9 expression significantly correlated with the presence of lymphovascular invasion (r = 0.334 p = 0.026) and with the presence of exophytic type of the tumour growth (r = 0.474, p = 0.001) in glottic LSCC. In patients with supraglottic localization of SCC, there were no similar relationships. We found no statisticaly significant relationship between the expression of MMP-9 in epithelial cells and perineural invasion in the LSCC.

Discussion

Our research, as well as the previous studies [7–18], demonstrated that MMP-2 and MMP-9 are highly expressed in tissue of LSCC in comparison with the mucosa of patients with chronic laryngitis, benign laryngeal pseudotumours (vocal fold polyps, oedemas, granulomas, etc), and normal laryngeal mucosa. MMPs are produced by various types of cells such as epithelial, inflammatory cells, fibroblasts, macrophages in both epithelium and stroma [3–5, 7–11]. As well as in the study of Uloza et al. [8], we demonstrated that MMP-2 and MMP-9 are produced by both epithelial and stromal cells in LSCC. However, Uloza et al. [8] found a significantly higher expression of these markers in stromal than in epithelial cells. We found no statistical differences between stromal and epithelial expression of the examined enzymes. Also, we found a positive relationship between the epithelial and stromal MMP-2 and MMP-9 expression in both glottic and supraglottic region of the larynx.

Earlier studies presented the contradictory results related to expression of the MMPs and their relationships with clinical and pathological features of LSCC. Our study demonstrated no statistically significant relationship between MMP-2 and MMP-9 expression and the clinical stage of LSCC, which is in accordance with the studies presented by Liu et al. [10] and Wael and Manal [11].

The presence of locoregional lymph node metastases is an important prognostic factor. We found no correlation between MMP-2 and MMP-9 expression and the presence of lymph node metastases, which is in accordance with the study performed by Akdeniz et al. [12]. On the other hand, in the studies presented by Sarioglu et al. [13] and by Yuce et al. [14], overexpression of MMP-2 and MMP-9 was found to be significantly higher in cases of lymph node involvement in patients with glottic [13] and supraglottic [14] LSCC.

The histological grade of tumour may be a predictor of cancer behaviour and contribute to the finding of the best modality of the treatment. In order to determine the significance of MMP-2 and MMP-9 expression, many researchers have evaluated the relationship between the MMP tumour expression and the level of tumour histological differentiation. However, the results are not consistent. Sarioglu et al. [13] indicated that MMP-2 expression is not associated with the level of differentiation of LSCC. Our study generally demonstrated no such relation, but in patients with supraglottic carcinoma localisation, a negative correlation was found between the expression of MMP-2 and the histological grade of the tumour. So, our results suggest that lower level of epithelial MMP-2 expression could be associated with the higher level of differentiation of supraglottic LSCC.

The previous studies demonstrated that the higher expression of MMP-2 and MMP-9 is associated with poor outcome of oncological treatment [4, 5, 7]. Many researchers pointed out high MMP-2 and MMP-9 expression as a potential marker of worse prognosis in patients with laryngeal cancer. Although we found no such relationship, Mallis et al. [15] showed a statistically significant difference ($p < 0.05$) for the 5-year overall survival rate between the groups with positive and negative MMP-2 expression in patients with glottic LSCC.

Numerous studies assessed the MMP-2 and MMP-9 immunostaining in the laryngeal cancer. In only few studies, the laryngeal subregions were investigated separately [7, 10, 15]. Our study compared the immunoreactivity of MMP-2 and MMP-9 in patients with supraglottic and glottic LSCC. We found that the level of MMP-2 expression is significantly related to the presence of perineural and lymphovascular invasion, and the presence of local disease recurrence in the supraglottic region. So, according to our results, MMP-2 could be considered as a potential predictive factor for the estimation of spreading the disease in patients with supraglottic LSCC.

Our results demonstrated a positive correlation between epithelial MMP-9 expression and the presence of lymphovascular invasion in patients with glottic LSCC. Wittekindt et al.[17] found a positive correlation between MMP-9 expression and blood vessel density, suggesting that MMP-9 may be a potential target to disrupt tumour neovascularisation during the oncological therapy of LSCC. A recent study by Colovic et al. [18] demonstrated the overexpression of MMP-9 in patients with tumour relapses. MMP-9 could be considered as a potential predictive factor for the assessment of spreading and recurrence of primary disease in glottic region of the larynx. Finally, our results showed a highly significant positive correlation between the level of MMP-2 and MMP-9 immunostaining and the presence of exophytic tumour growth only in glottic LSCC, suggesting the importance of MMP-2 and, especially, MMP-9 expression as potential predictors for the feature of vegetant type of tumour growth in glottic LSCC.

However, our study had some limitations. Due to the financial reasons, we did not perform the measurement of MMP-2 and MMP-9 levels in the serum of patients with LSCC. Also, we did not evaluate the quantitative real-time polymerase chain reaction reactivity for the messenger RNA (mRNA) production for MMP-2 and MMP-9. The previous investigations demonstrated important relationships between the serum level/mRNA tissue immunostaining for MMP-2/MMP-9 and clinical stage, nodal status and survival rates in patients with LSCC [19–21].

Conclusion

This study demonstrated a higher expression of MMP-2 and MMP-9 in both epithelium and stroma of patients with LSCC in comparison to patients with chronic laryngitis. Our results suggest that MMP-2 expression can serve as a potential parameter for the detection of patients with supraglottic LSCC who are at a high risk of perineural and lymphovascular invasion and developing disease recurrence. MMP-9 expression can be a strong predictor of the presence of exophytic type of tumour growth, as well as of the presence of lymphovascular invasion in glottic LSCC.

R E F E R E N C E S

1. *Smee R, Williams JR, Kotevski DP.* Management of locally advanced T3-4 glottic laryngeal carcinomas. J Laryngol Otol 2018; 132(7): 642−50.
2. *Tang D, Tao L, Zhou L, Zhang M, Wu X, Li X,* et al. Retrospective analysis of 659 laryngeal squamous cell carcinoma patients treated with open laryngeal function-preserving operations. Acta Otolaryngol 2018; 138(11): 1043−50.
3. *Whittaker M, Ayscough A.* Matrix metalloproteinases and their inhibitors: current status and future challenges. Celltransmissions 2001; 17(1): 3−14.
4. *Gou X, Chen H, Jin F, Wu W, Li Y, Long J,* et al. Expressions of CD147, MMP-2 and MMP-9 in laryngeal carcinoma and its correlation with poor prognosis. Pathol Oncol Res 2014; 20(2): 475−81.
5. *Rydlova M, Holubec L Jr, Ludvikova M Jr, Kalfert D, Franekova J, Povysil C,* et al. Biological activity and clinical implications of the matrix metalloproteinases. Anticancer Res 2008; 28(2B): 1389−97.
6. *Deschler DG, Moore MG, Smith RV.* Quick Reference Guide to TNM Staging of Head and Neck Cancer and Neck Dissection Classification, 5th ed. Alexandria, VA: American Academy of Otolaryngology – Head and Neck Surgery Foundation; 2014.
7. *Peschos D, Damala C, Stefanou D, Tsanou E, Assimakopoulos D, Vougiouklakis T,* et al. Expression of matrix metalloproteinase-9 (gelatinase B) in benign, premalignant and malignant laryngeal lesions. Histol Histopathol 2006; 21(6): 603−8.
8. *Uloza V, Liutkevicius V, Pangonyte D, Lesauskaite V.* Characteristics of expression of matrix metalloproteinases (MMP-2 and MMP-9) in glottic squamous cell carcinoma and benign vocal fold lesions. Clin Exp Otorhinolaryngol 2015; 8(1): 57−64.
9. *Lotfi A, Mohammadi G, Saniee L, Mousaviagdas M, Chavoshi H, Tavassoli A.* Serum level of matrix metalloproteinase-2 and -9 in patients with laryngeal squamous cell carcinoma and clinical significance. Asian Pac J Cancer Prev 2015; 16(4): 1327−30.
10. *Liu WW, Zeng ZY, Wu QL, Hou JH, Chen YY.* Overexpression of MMP-2 in laryngeal squamous cell carcinoma: a potential indicator for poor prognosis. Otolaryngol Head Neck Surg 2005; 132(3): 395−400.
11. *Wael S, Manal S.* Expression of nm23H1 and MMP2 in laryngeal carcinoma and its role in aggressiveness of the tumour and node metastasis. Egypt J Otolaryngol 2013; 29(2): 86−92.
12. *Akdeniz O, Akduman D, Haksever M, Ozkarakas H, Muezzinoglu B.* Relationships between clinical behavior of laryngeal squamous cell carcinomas and expression of VEGF, MMP-9 and E-cadherin. Asian Pac J Cancer Prev 2013; 14(9): 5301−10.
13. *Sarioglu S, Ozer E, Kirimca F, Sis B, Pabuccuoglu U.* Matrix metalloproteinase-2 expression in laryngeal preneoplastic and neoplastic lesions. Pathol Res Pract 2001; 197(7): 483−6.
14. *Yuce I, Bayram A, Cagli S, Canoz O, Bayram S, Guney E.* The role of CD44 and matrix metalloproteinase-9 expression in predicting neck metastasis of supraglottic laryngeal carcinoma. Am J Otolaryngol 2011; 32(2): 141−6.
15. *Mallis A, Teymoortash A, Mastronikolis NS.* MMP-2 expression in 102 patients with glottic laryngeal cancer. Eur Arch Otorhinolaryngol 2012; 269(2): 639−42.
16. *Cao XL, Xu RJ, Zheng YY.* Expression of type IV collagen, metalloproteinase-2, metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in laryngeal squamous cell carcinomas. Asian Pac J Cancer Prev 2011; 12(12): 3245−9.
17. *Wittekindt C, Jovanovic N, Guntinas-Lichius O.* Expression of matrix metalloproteinase-9 (MMP-9) and blood vessel density in laryngeal squamous cell carcinomas. Acta Otolaryngol 2011; 131(1): 101−6.
18. *Colovic Z, Pesutić-Pisac V, Poljak NK, Racić G, Cikojević D, Kontić M.* Expression of matrix metalloproteinase-9 in patients with squamous cell carcinoma of the larynx. Coll Antropol 2013; 37(1): 151−5.
19. *Kalfert D, Ludvikova M, Topolcan O, Windrickova J, Malirova E, Pesta M,* et al. Analysis of preoperatice serum levels of MMP-1, -2, -9 in patients with site-specific head and neck squamous cell cancer. Anticancer Res 2014; 34(12): 7431−41.
20. *Li JZ, Gao W, Lei WB, Zhao J, Chan JY, Wei WI,* et al. MicroRNA 744-3p promotes MMP-9 mediated metastasis by simultaneously suppressing PDCD4 and PTEN in laryngeal squamous cell carcinoma. Oncotarget 2016; 7(36): 58218−23.
21. *Popov TM, Stancheva G, Goranova TE, Rangachev J, Konov D, Todorov S,* et al. Strong correlation between mRNA expression levels of HIF-2α, VEGFR1, VEGFR2 and MMP2 in laryngeal carcinoma. Pathol Oncol Res 2016; 22(4): 741−6.

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Early initiation of renal replacement therapy improves survival in patients with acute kidney injury

Rani početak primene metoda zamene funkcije bubrega poboljšava preživljavanje bolesnika sa akutnim oštećenjem bubrega

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Abstract

Background/Aim. Defining renal replacement therapy (RRT) initiation in critically ill patients with acute kidney injury (AKI) has become an imperative for nephrologists and intensivists. The aim of this study was to determine 28-day survival and the renal function recovery in patients with AKI. **Methods.** A single-center retrospective study included 385 surgical and non-surgical patients with AKI and episode of AKI in chronic kidney disease who were admitted to the Emergency Center of Clinical Center of Vojvodina (Novi Sad, Serbia) between 2014 and 2017 and received RRT. Patients with the Kidney Disease Improving Global Outcomes (KDIGO) stage 2 AKI and/or volume overload were assigned to the "early" group with RRT (dialysis) start within 24 h of the diagnosis; patients with poor response to conservative treatment or evidence of clinical complications associated with AKI were assigned to the "late" RRT group. **Results.** Based on the retrospective analysis we found that 241 patients (62.6%) received "early" RRT within 24 h. Patients in the "early" RRT group had significantly higher survival compared to the "late" RRT group (63.9% vs. 36.1%; $p = 0.001$). The "early" RRT group had more patients with renal function recovery (56.8%), but without statistical significance ($p = 0.514$). The patients who started RRT within 24 hours with the Sequential Organ Failure Assessment (SOFA) score of 1–3 were twice likely to recover renal function in relation to the patients with the SOFA score of 4 or higher [odds ratio (OR) = 2.01; 95% confidence interval (CI): 1.37–2.95; $p < 0.001$], while septic patients had a 62% lower chance of renal function recovery in relation to non-septic patients (OR = 0.38; 95% CI: 0.18–0.82; $p = 0.013$). In the "late" RRT group, it was found that non-diabetic patients had 3.8 times greater chance for renal function recovery compared to diabetic patients (OR = 3.53; 95% CI: 1.27–9.83; $p = 0.016$). **Conclusions.** Patients with the early initiation of RRT had significantly improved 28-day survival.

Key words:
acute kidney injury; renal dialysis; mortality; risk assessment; survival; time factors.

Apstrakt

Uvod/Cilj. Definisanje početka primene metoda zamene funkcije bubrega kod kritično obolelih sa akutnim oštećenjem bubrega postaje imperativ nefrolozima i intezivistima. Primarni cilj studije je bio ustanoviti preživljavanje bolesnika 28-og dana od prijema, a sekundarni cilj oporavak funkcije bubrega. **Metode.** U Urgentnom centru Kliničkog centra Vojvodine (Novi Sad, Srbija) sprovedeno je retrospektivno ispitivanje koje je uključilo 385 hiruških i nehirurških bolesnika sa akutnim oštećenjem bubrega i akutizacijom hronične bubrežne insuficijencije u periodu od 2014. do 2017. godine, kojima su primenjene metode zamene funkcije bubrega. Bolesnici sa *Kidney Disease Improving Global Outcomes* (KDIGO) stadijumom 2 i/ili hipervolemijom unutar 24 h od potvrđenog akutnog oštećenja bubrega imali su "rani" početak dijalize, dok su "kasni" početak imali bolesnici sa slabijim odgovorom na konzervativnu terapiju ili kliničkim komplikacijama povezanim sa akutnim oštećenjem bubrega. **Rezultati.** Retrospektivnom analizom je utvrđeno da je kod 241 bolesnika (62,6%) dijaliza rano započeta, unutar 24 h. Bolesnici koji su „rano" započeli dijalizu imali su značajno bolje preživljavanje u poređenju sa bolesnicima koji su imali „kasni" početak dijalize (63.9% vs. 36.1%; $p = 0.001$). Kod nešto većeg broja bolesnika sa „ranim" početkom dijalize došlo je do oporavka funkcije bubrega (56,8%), ali razlike nisu bile značajne ($p = 0.514$). Bolesnici sa „ranim" početkom

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dijalize i *Sequential Organ Failure Assessment* (SOFA) skorom 1–3 imali su 2 puta veću šansu da oporave funkciju bubrega u odnosu na bolesnike sa SOFA skorom ≥ 4 [*odds ratio* (OR) = 2,01; 95% *confidence interval* (CI): 1,37–2,95; $p < 0,001$], dok su bolesnici sa sepsom imali za 62% manju šansu oporavka funkcije bubrega u odnosu na bolesnike bez sepse (OR = 0,38; 95% CI: 0,18–0,82; $p = 0,013$). U grupi sa „kasnim" početkom dijalize utvrđeno je da bolesnici koji nemaju dijabetes imaju 3,8 puta veću šansu za oporavak funkcije bubrega u odnosu na obolele od dijabetesa (OR = 3,53; 95% CI: 1,27–9,83; $p = 0,016$). **Zaključak.** Značajno bolje preživljavanje 28-dana imali su bolesnici kojima je zamena funkcije bubrega dijalizom „rano" započeta.

Ključne reči:
bubreg, akutna insuficijencija; bubreg, dijaliza; mortalitet; rizik, procena; preživljavanje; vreme, faktor.

Introduction

In the past decade, acute kidney injury (AKI) has become a well-recognized global occurrence that affects developed and developing countries alike, with initiatives like the Saving Young Lives and the International Society for Nephrology's 0by25 Initiative aiming at reducing the economic, social and healthcare burden imposed by AKI [1]. AKI frequently occurs in critically ill patients and severe AKI is associated with hospital mortality in 60% of the cases [2]. Those that survive the initially high mortality rate associated with dialysis-requiring AKI, mostly become independent of renal replacement therapy (RRT) within a year, but some of them do go on to develop chronic kidney disease (CKD) and even progress to end-stage renal disease [3]. The fundamental principle in the treatment of AKI is to correct the underlying cause, but besides hemodynamic resuscitation and removal of nephrotoxins, we lack any established pharmacotherapy. Although drugs are tested for the prevention and/or treatment of AKI, RRT appears to be our only efficacious option at the time. Thus, the management of AKI is largely limited to preventing further deterioration and the loss of function with the use of temporizing actions in severe cases until RRT is established [4]. Dialysis as the method of RRT, along with mechanical ventilation, vasoactive therapy and nutritional support, is one of the defined life-sustaining technologies in the current treatment of the critically ill. A recent trend suggests an increasing use of RRT in critically ill patients with AKI [5]. Despite the research and growing clinical experience in dialysis, the optimal time to start RRT in a critical disease complicated with AKI is unclear [2]. Heterogeneity in operational definitions of "time", "threshold" or "criteria" in individual observational data (often with variable designs and methodological qualities) have probably interfered with clear conclusions that could guide clinical practice on this issue [6]. It is unclear whether a preventive/early strategy of the initiation of RRT in order to avoid complications associated with AKI leads to better patient outcomes and the use of health services, or a more conservative strategy, in which RRT is started as a response to the development of complications, provides better results. Neither the standard clinical parameters, nor the new biomarkers that have been introduced to clarify the definitive ideal time more precisely, nor the clinical picture have optimized patient outcomes [2]. The primary objective of this study was 28-day patient survival and the secondary objective was renal function recovery in patients with "early" RRT compared with those with "late" RRT.

Methods

We performed a single-center retrospective study of 385 surgical and non-surgical adult patients with AKI and episode of AKI in CKD who were admitted to the Intensive Care Unit (ICU) and Intensive Internal Medicine Unit at the Emergency Center (Clinical Center of Vojvodina, Novi Sad, Serbia) between 2014 and 2017 and received RRT (dialysis). Patients with the Kidney Disease Improving Global Outcomes (KDIGO) stage 2 AKI (serum creatinine 2–2.9 times baseline and urine output < 0.5 mL/kg/h for 12 h) and/or volume overload were assigned to the "early" group with RRT start within 24 h of the diagnosis; patients with a poor response to the conservative treatment or evidence of clinical complications associated with AKI were assigned to the "late" RRT group. Although the condition of certain patients called for the start of RRT in the first 12 hours, they were denied this request at that time due to the organization of the team for starting RRT at weekends or at night, the unavailability of the apparatus and/or difficulty in placing the dialysis catheter. Other reasons for postponement were surgical interventions or radiological tests that were to be performed before the start of the RRT. Some patients started the treatment with intermittent dialysis at the time of hemodynamic stability or the unavailability of the apparatus since "more severe" patients and/or the ones who were occasionally dialyzed with it had the need for it. Patients with an immediate RRT indication having at least one of the following conditions from the beginning were excluded: laboratory analysis at the admission urea > 50 mmol/L, K > 6.5 mmol/L, pH < 7.15 in the context of either pure metabolic acidosis or mixed acidosis despite medical treatment; acute pulmonary edema due to fluid overload causing severe hypoxemia, as well as patients treated with conservative therapy. We analyzed: demographic data, comorbidities, laboratory and clinical data in confirmed AKI [urea, creatinine, C-reactive protein (CRP), procalcitonin (PCT), oliguria/anuria] and before continuous RRT (CRRT) initiation (urea, creatinine, 24 h diuresis (mL); the use of vasopressor therapy and mechanical ventilation; hospital length of stay (days); CRRT modalities: continuous venovenous hemodiafiltration (CVVHDF), continuous venovenous hemofiltration (CVVHF), continuous venovenous hemodialysis (CVVHD), CVVHD combined with CVVHDF; and those achieved ultrafiltration (UF). The choice of RRT modalities (intermittent or continuous) was at the discretion of clinicians, and based on international guidelines [7]. The RRT regimen was daily or every second day, de-

pending on clinical, laboratory parameters and response to the therapy. CRRT was done on the Multifilter and the Prismaflex; standard high-flux filters and membranes/adsorbers were used in septic patient: EMiC2 hemofilter (Fresenius Medical Care, Bad Homburg, Germany, 1.8 m^2 surface area), oXiris [Gambro, AN-69 based membrane, surface treated by polyethyleneimine (PEI) and grafted with heparin] and CytoSorb (total surface of > 40,000 square meters). The CRRT prescription included: treatment modality, blood flow, dilution mode, replacement and dialysis fluid flow, and the patient's weight and heparin anticoagulation, according to clinical practice guideline [7]. Organ dysfunction was quantified using the Sequential Organ Failure Assessment (SOFA) score.

Statistical methods

Descriptive and inferential statistical methods were used for the data analysis. Numerical characteristics are presented by the arithmetic mean, the median with interquartile range (IQR, 25–75 percentiles) and the standard deviation, while the attributive characteristics are expressed by frequency and percentage. The χ^2 test was used to compare the differences between different groups, and the Cox regression model was used to test the predictor of recovery and failure of the renal function, as well as to calculate survival with respect to the selected indicators. Three Cox regression models were made. In the first model, renal function recovery over a period of one month was used for the outcome variable, and the following: gender, age, CRRT 24 h, UF, urea at admission, urea at the start of CRRT, creatinine at admission, creatinine at the start of CRRT, CRP, PCT, surgical patients, sepsis, cardiovascular, cerebrovascular, pulmonary, digestive and other diseases and diabetes mellitus were used as independent indicators. For the formation of the other two models, the sample was selected with regard to the onset of CRRT. The outcome variable in both models is non-recovery of renal function selected with regard to the onset of CRRT (CRRT > 24 h and CRRT < 24 h) over a period of one month, and the independent indicators were the same as in the first model, with the exception of CRRT 24 h. There was a statistical significance if $p < 0.05$, and a high statistical significance if $p < 0.001$. The IBM SPSS Statistical Package for Social Sciences 21 software package was used for statistical data processing.

Results

We found that 241 (62.6%) patients (male 65.4%), mean age 60.6 received "early" RRT within 24 h, and 144 (37.4%) patients (male 70.8%) mean age 63.5 received late RRT after 24 h. All studied comorbidities were more prevalent in "early" RRT, and cardiovascular diseases were the most prevalent comorbidity in both groups of patients. Patients in the "early" RRT group had a higher rate of sepsis and less frequent use of mechanical ventilation and vasopressor therapy compared to the "late" RRT group (54.7% *vs* 41.7%; 58.3% *vs* 70.8%; 56.7% *vs* 75.7%, respectively). The presence of adsorptive membrane/adsorbers according to the type and the number of procedures in both groups was similar. Over 50% in both groups had the SOFA score ≥ 4. Median diuresis (mL) before RRT was smaller in the "early" group compared to the "late" group (150 *vs* 400, respectively); median urea (mmoL) and creatinine (μmoL) were similar in both groups (25.1 *vs* 25 and 449 vs 458, respectively). The most common treatment modality in both groups was CVVHDF and the achieved UF was higher in "early" RRT compared to "late" RRT (2,279 *vs* 2,017 mL). Mean length of hospital stay was similar in both groups (8 *vs* 7 days) (Table 1). The patients in whom CRRT started within 24 hours had significantly better survival ($p < 0.001$) and better recovery of renal function, but without statistical significance ($p = 0.551$) compared to the patients in whom RRT started after 24 h (Figures 1 and 2). The SOFA score and sepsis were differentiated as predictors of renal function recovery. The patients with the SOFA score 1–3 had 1.7 times higher chance for renal recovery in relation to the patients with the SOFA score ≥ 4 [odds ratio (OR) = 1.79; 95% confidence interval (CI): 1.31–2.46; $p < 0.001$], while septic patients had a 53% lower chance to recover their renal function in relation to the patients with no sepsis (OR = 0.47; 95% CI: 0.24–

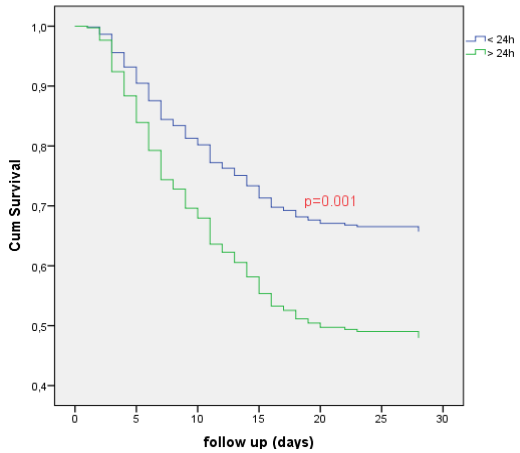

Fig. 1 – Patient survival depending on the time of renal replacement therapy initiation.

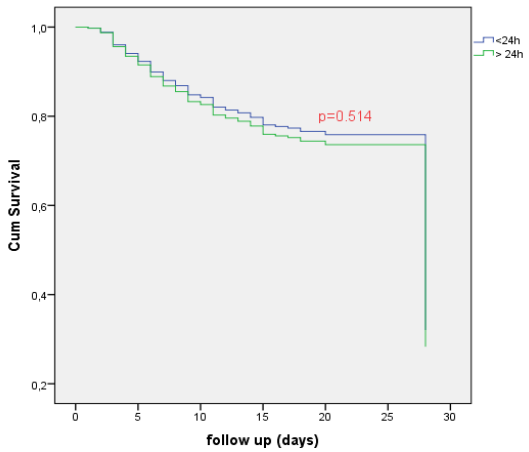

Fig. 2 – Recovery of renal function depending on the time of renal replacement therapy initiation.

Table 1
Demographic and clinical data of patients treated with renal replacement therapy (RRT)

Variable	RRT < 24 h (n = 241) n (%)	RRT > 24 h (n = 144) n (%)
Sex		
male	157 (65.4)	102 (70.8)
female	83 (34.6)	42 (29.2)
Age (years), mean (SD)	60.06 (13.6)	63.5 (13.4)
CRRT modality, n (%)		
CVVHDF	92 (38.3)	57 (39.6)
CVVHD	84 (35.0)	54 (37.5)
CVVH	15 (6.3)	7 (4.9)
CVVHD+CVVHDF	49 (20.4)	26 (18.1)
Comorbidities, n (%)		
cardiovascular diseases	154 (64.2)	69 (47.9)
pulmonary diseases	24 (10.0)	13 (9.0)
gastrointestinal diseases	30 (12.5)	17 (11.8)
diabetes mellitus	56 (23.3)	22 (15.3)
cerebrovascular diseases	23 (9.6)	9 (6.3)
chronic kidney disease	29 (12.1)	6 (4.2)
other	64 (26.7)	41 (28.5)
without comorbidities	29 (12.1)	13 (9.0)
Recovery of renal function, n (%)	137 (56.8)	68 (47.2)
Nonsurvivors, n (%)	87 (57.6)	83 (57.6)
Septic patients, n (%)	131 (54.6)	60 (41.7)
Surgical patients, n (%)	63 (26.3)	43 (29.9)
Oliguric/anuric patients, n (%)	105 (43.3)	47 (32.6)
Diuresis (mL), median (IQR)	150 (0–750)	400 (0–1015)
Physiological support, n (%)		
invasive mechanical ventilation	140 (58.3)	102 (70.8)
vasopressors	136 (56.7)	109 (75.7)
Number of procedures with adsorptive membrane/adsorber, n (%)		
1–2	64 (26.3)	34 (23.9)
≥ 3	31 (12.8)	16 (11.3)
0	148 (60.9)	92 (64.8)
Type adsorptive membrane/adsorber, n (%)		
EMiC2	81 (33.3)	42 (29.6)
oXiris	3 (1.2)	2 (1.4)
CytoSorb	1 (0.4)	-
EMiC2 + oXiris	7 (2.9)	6 (4.2)
oXiris + CytoSorb	1 (0.4)	-
SOFA score, n (%)		
0	2 (0.8)	1 (0.7)
1	32 (13.2)	7 (4.9)
2	25 (10.3)	23 (16.2)
3	35 (14.4)	12 (8.5)
≥ 4	149 (61.3)	99 (69.7)
UF (mL), median (IQR)	2279 (1360 - 2983)	2017 (1310–2734)
Urea at admission (mmoL), median (IQR)	25.1 (18.8–36.7)	25 (18.3–35.2)
Urea at the start of RRT (mmoL), median (IQR)	29.2 (22.2–40.8)	29.05 (20.4–40.0)
Creatinine at admission (μmoL)-median (IQR)	421 (228–585)	330 (222–500)
Creatinine at the start of RRT (μmoL)-median (IQR)	449 (284–603)	458 (278–631)
CRP (mg/L), median (IQR)	120 (37.3–253.8)	121.5 (55.9–244.4)
PCT (ng/L), median (IQR)	4.7 (0.9–22)	5.2 (1.2–19.5)
Length of hospital stay (days), median (IQR)	8 (4.0–13)	7 (5.0–12)

CRRT – continuous renal replacement therapy; CVVHDF – continuous venovenous hemodiafiltration; CVVHD – continuous venovenous hemodialysis; CVVH – continuous venovenous hemofiltration; CVVHD+CVVHDF – CVVHD combined with CVVHDF; UF – ultrafiltration; CRP – C-reactive protein; PCT – procalcitonin; SOFA – Sequential Organ Failure Assessment; SD – standard deviation; IQR – interquartile range.

0.90; $p = 0.024$) (Table 2). The patients who started RRT within 24 hours with the SOFA score of 1–3 were twice likely to recover renal function in relation to the patients with the SOFA score of 4 or higher (OR = 2.01; 95% CI: 1.37–2.95; $p < 0.001$), while septic patients had a 62% lower chance of renal function recovery in relation to non-septic patients (OR = 0.38; 95% CI: 0.18–0.82; $p = 0.013$). In the "late" RRT group, it was found that non-diabetic patients had 3.8 times higher chance for the renal function recovery compared to diabetic patients (OR = 3.81; 95% CI: 1.35–10.76; $p = 0.012$) (Table 3).

Discussion

Numerous organizations have published their studies and guides in order to better inform the clinical practice [8]. Each organization has recognized the limitations of the present evidence and the associated clinical uncertainty, there-

Table 2

Predictors of renal function recovery regardless of the time of renal replacement therapy (RRT) initiation

Variable	B	*p*
Sex	-0.177	0.260
Age	-0.005	0.350
Ultrafiltration	0.000	0.695
Urea at admission	0.003	0.692
Urea at the start of RRT	-0.005	0.544
Creatinine at admission	0.000	0.631
Creatinine at the start of RRT	0.000	0.833
C-reactive protein	0.000	0.859
Procalcitonin	0.001	0.415
Surgical patients	0.072	0.677
Septic patients	0.761	0.024
Cardiovascular diseases	0.188	0.282
Pulmonary diseases	0.221	0.418
Gastrointestinal diseases	0.338	0.165
Diabetes mellitus	0.294	0.140
Cerebrovascular diseases	-0.275	0.314
Other	-0.012	0.941
Number procedures with adsorptive membrane/adsorber	0.518/ -0.038	0.264/ 0.875
Type adsorptive membrane/adsorber	0.228	0.533
SOFA score	0.586	0.000

SOFA – Sequential Organ Failure Assessment.

Table 3

Predictors of renal function recovery depending on the time of renal replacement therapy (RRT) initiation

Variable	RRT < 24 h		RRT > 24 h	
	B	*p*	B	*p*
Sex	-0.241	0.222	-0.258	0.402
Age	-0.005	0.420	0.000	0.995
Ultrafiltration	0.000	0.926	0.000	0.432
Urea at admission	0.008	0.426	-0.002	0.885
Urea at the start of RRT	-0.005	0.609	-0.020	0.129
Creatinine at admission	0.001	0.441	-0.001	0.129
Creatinine at the start of RRT	0.001	0.431	-0.001	0.448
C-reactive protein	0.000	0.659	0.000	0.873
Procalcitonin	-0.001	0.658	0.005	0.119
Surgical patients	0.150	0.511	-0.050	0.866
Septic patients	0.944	0.013	-0.356	0.745
Cardiovascular diseases	0.374	0.096	0.190	0.554
Pulmonary diseases	0.277	0.382	0.095	0.866
Gastrointestinal diseases	0.382	0.199	0.399	0.415
Diabetes mellitus	0.133	0.559	1.337	0.012
Cerebrovascular diseases	-0.220	0.492	-0.172	0.764
Other	-0.037	0.858	-0.074	0.808
Number procedures with adsorptive membrane/adsorber	0.573 -0.038	0.296 0.903	-0.992 -0.307	0.438 0.533
Type adsorptive membrane/adsorber	0.370	0.397	0.348	0.617
SOFA score	0.698	0.000	0.503	0.097

SOFA – Sequential Organ Failure Assessment.

fore each of them recommended that additional high-quality studies should be carried out [9].

Previous studies have shown different results regarding heterogeneity of the population, the definition of the criteria for starting RRT, primary and secondary outcomes. In most studies, for the purpose of analyzing all causes of mortality, clinical indications and AKI classification have been used more frequently than biochemical parameters in order to define "early" and "late" RRT [10]. Our mixed population of predominantly non-surgical ICU patients started "early" RRT, according to the KDIGO practice guides that include statements about the time to start RRT in critically ill patients in the KDIGO 2 stage and/or hypervolemia within 24 hours. On the other hand, "late" RRT started in patients with developed complications related to AKI or the ones who had not responded to the conservative treatment. At the early onset of CRRT there was a higher percentage of oliguric/anuric, septic patients with comorbidities and a lower need for mechanical ventilation and vasoactive support, which were contributory indicators for a faster response in a clinical decision about supportive therapy. In comparison with the patients with "late" RRT, diuresis median was lower and the median of the achieved UF during RRT was higher, which, along with a better recovery of the renal function, indirectly indicates hypervolemia as an important additional criterion for starting RRT. The authors of two previously conducted meta-analyses, which included a total of 38 studies predominantly retrospective and of different quality, came to similar conclusions as ours, reporting a significant improvement in the 28-day mortality with "early" RRT [11, 12]. Other three studies have been conducted. Two of them [the multicenter randomized controlled trial (RCT) and the retrospective study] included septic patients and used different criteria of AKI classification for starting RRT in relation to our study design, but at the same RRT initiation time, showed a significant reduction in mortality in "early" RRT groups, while the third multicenter RCT conducted three years later found that there was no difference in mortality between the "early" and the "late" onset of RRT in 224 postoperative cardiosurgical patients [13–15].

In fact, since 2012, the majority of the published studies have not supported the benefit of "early" RRT in critically ill patients. Conducting a meta-analysis of 36 predominantly retrospective studies, it was established that the early initiation of RRT in critical patients did not improve survival for 28 or 30 days, nor did it reduce the length of stay in the ICU or the overall length of hospitalization. In the abovementioned studies, biochemical markers according to the Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease (RIFLE) classification, Acute Kidney Injury Network (AKIN) stages and time-based cut-offs were used (e.g. within the defined admission time or the development of the biochemical "start time") for defining the early start of RRT. However, the "early" RRT according to one author was the "late" RRT according to another author, which made it difficult to interpret the results. The "late" onset included classical indications of RRT that did not respond to conservative treatment [16].

A meta-analysis which included both the Artificial Kidney Initiation in Kidney Injury trial (AKIKI) and Effect of Early *vs* Delayed Initiation of Renal Replacement Therapy on Mortality in Critically Ill Patients With Acute Kidney Injury trial (ELAIN) was subsequently published, including a total of 10 RCT with 1,672 participants combined. As the authors concluded, there appeared to be no added benefit of an early start of RRT on 30-, 60- and 90-day mortality "with respect to all-cause mortality, dialysis dependence, and recovery of renal functions or hospital stay". It was also found that there was no difference in reported complications: catheter-related infections, bleeding, hypotension, electrolyte disorders, thrombocytopenia, arrhythmias. It should be noted that the ELAIN single-center study analyzed 231 predominantly surgical patients with AKI and the episode of AKI in CKD in which the early criterion was the KDIGO 2 within 8h. It was established that there was no difference between the early and the late onset of RRT compared to the 28-day mortality (30.4 vs. 40.3%, respectively; $p = 0.11$). The exclusion of patients with urgent indications for RRT, as well as the fact that only 3.4% of the patients did not start RRT in the "early" group, were probably some of the reasons for the difference in reducing the 90-day mortality by comparing the "early" and "late" RRTs (39.3% vs. 54.7%, respectively; $p = 0.03$).

Meta-analysis of four RCTs showed similar results, except that a higher risk of catheter-related infections was reported at the early onset of RRT [17, 18]. A meta-analysis with six RCT that provided similar conclusions was conducted the same year, after which 4 additional RCT and 41 observational studies were included (a total of 51 studies) whose results showed that the "early" RRT was associated with a reduced risk of all causes of mortality, although the results were taken with caution, given the variety of design studies.

In our study of the early onset of RRT, we established a better 28-day renal function recovery, although the differences between the groups were not statistically significant. The ELAIN study indicated significant benefit since the early onset of RRT in renal function recovery (53.6% vs. 38.7%, $p = 0.02$), and the last meta-analysis also showed significant renal function recovery in 14 studies with 2,570 patients [10]. When we established a better renal function recovery, we tried to determine which predictors could affect this outcome. By applying multivariate Cox regression analysis in the total sample as well as in patients who started RRT early, SOFA score and sepsis stood out as significant predictors or renal function recovery. Namely, the patients with the 1–3 SOFA score who started RRT early, were two times more likely to recover from renal function in comparison to patients with the SOFA score 4 and above, while septic patients had a 62% lower chance to recover their renal function compared to non-septic patients. Contrary to our results, in a large retrospective study of the critically ill with AKI, older age, heart conditions and admission to ICU were significantly linked to a lower rate of renal function recovery 60–120 days after discharging from ICU [19]. However, in another retrospective study, Pistolesi et al. [20], concluded that older age, oliguria, sepsis and a higher SOFA score in 264 cardio-

surgical patients with severe AKI within the first week of CRRT start were independent prognostic indicators for non-recovery of renal function. However, in the late RRT group it was found that the patients without diabetes had a 3.8 times higher chance of recovering renal function compared to the patients with diabetes. Patschan and Müller [21] suggested that diabetes mellitus potentially increases AKI risk and long-term mortality/morbidity of AKI. Thakar et al. [22] showed that AKI episodes are associated with a cumulative risk of developing progressive CKD in diabetic patients, independently of other risk factors of progression. Unlike our results, the results of Johnson et al. [23] showed that AKI was diagnosed in 403 patients, 20.5% of whom were diabetic patients. Short-term renal function recovery was greater in diabetic patients (87% vs. 63%, $p = 0.001$) and the development of advanced CKD was lower (14%) in comparison with the non-diabetic ones [23]. In our study, a better renal function recovery at the late onset of RRT in non-diabetic patients requires additional testing of long-term renal recovery and the number of episodes of AKI in these patients compared to the patients at the risk of diabetes and the diabetics.

Also, it should be noted that in the studies similar to ours, the predictors of primary and secondary outcomes were less examined, because the main focus was on finding criteria for early/late start of RRT and predicting the outcomes.

Since each center has a limited number of patients for whom supportive therapy at the same time can be provided (in relation to resources, time, staff), the onset of RRT within 12 hours can lead to shortening the duration of RRT initiation or to a delay in starting RRT for other patients. This comes to light if there is at the same time more than one urgent indication for patients of different age, comorbidity, etc. regardless of the defined criteria for the early onset of RRT. The current watchful waiting strategy (in the absence of urgent indications) allows for a greater impact of the doctor's clinical decision based on long-term experience and teamwork. However, if the "real" timing of the onset of RRT is not recognized, the ability to carry out RRT in already developed complications associated with AKI and renal function recovery is reduced.

There are some limitations associated with our study. We performed a single-center retrospective study. All our patients received RRT, there was no control group (due to the limited data availability), nor the possibility that delaying RRT could provide time for renal function recovery. Unlike other studies, we did not obtain the full data on "early" RRT-related complications, or the data on long-term outcomes [17, 18, 24].

Conclusion

The patients who had early started RRT had significantly better 28-day survival. A further prospective research of the primary and secondary outcome predictors is necessary.

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REFERENCES

1. *Smoyer WE, Finkelstein FO, McCulloch MI, Carter M, Brusselmans A, Feehally J.* "Saving Young Lives" with acute kidney injury: the challenge of acute dialysis in low-resource settings. Kidney Int 2016; 89(2): 254−6.
2. *Payen D, Mateo J, Cavaillon JM, Fraisse F, Floriot C, Vicaut E.* Impact of continuous venovenous hemofiltration on organ failure during the early phase of severe sepsis: a randomized controlled trial. Crit Care Med 2009; 37(3): 803–10.
3. *Macedo E, Mehta RL.* Renal recovery after acute kidney injury. Contrib Nephrol 2016; 187: 24−35.
4. *Hertzberg D, Rydén L, Pickering JW, Sartipy U, Holzmann MJ.* Acute kidney injury-an overview of diagnostic methods and clinical management. Clin Kidney J 2017; 10(3): 323−31.
5. *Wald R, McArthur E, Adhikari NK, Bagshaw SM, Burns KE, Garg AX*, et al. Changing incidence and outcomes following dialysis-requiring acute kidney injury among critically ill adults: a population-based cohort study. Am J Kidney Dis 2015; 65(6): 870–7.
6. *Liu Y, Davari-Farid S, Arora P, Porhomayon J, Nader ND.* Early versus late initiation of renal replacement therapy in critically ill patients with acute kidney injury after cardiac surgery: a systematic review and meta-analysis. J Cardiothorac Vasc Anesth 2014; 28(3): 557–63.
7. *Kellum JA, Lameire N, Aspelin P, Barsoum RS, Burdmann EA, Goldstein SL*, et al. Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO clinical practice guideline for acute kidney injury. Kidney Int Suppl 2012; 2(1): 1−138.
8. *Vaara ST, Pettilä V, Kaukonen KM, Bendel S, Korhonen AM, Bellomo R*, et al. The attributable mortality of acute kidney injury: a sequentially matched analysis*. Crit Care Med 2014; 42(4): 878–85.
9. National Institute for Health and Care Excellence (NICE). Acute kidney injury: prevention, detection and management 2013. Available afrom: https://www.nice.org.uk/guidance/cg169/.
10. *Wang, C, Lv LS, Huang H, Guan J, Ye Z, Li S*, et al. Initiation time of renal replacement therapy on patients with acute kidney injury: A systematic review and meta-analysis of 8179 participants. Nephrology (Carlton) 2017; 22(1): 7−18.
11. *Seabra VF, Balk EM, Liangos O, Sosa MA, Cendoroglo M, Jaber BL.* Timing of renal replacement therapy initiation in acute renal failure: a meta-analysis. Am J Kidney Dis 2008; 52(2): 272–84.
12. *Karvellas CJ, Farhat MR, Sajjad I, Mogensen SS, Leung AA, Wald R*, et al. Timing of initiation of renal replacement therapy in acute kidney injury: a meta-analysis [abstract]. Crit Care Med 2010; 38(12 Suppl): A105.
13. *Vaara ST, Korhonen AM, Kaukonen KM, Nisula S, Inkinen O, Hoppu* S, et al. Early initiation of renal replacement therapy is associated with decreased risk for hospital mortality in the critically ill [abstract]. Intensive Care Med 2012; 38(Suppl 1): S9.
14. *Chon GR, Chang JW, Huh JW, Lim CM, Koh Y, Park SK*, et al. A comparison of the time from sepsis to inception of continuous renal replacement therapy versus RIFLE criteria in patients with septic acute kidney injury. Shock 2012; 38(1): 30–6.

15. *Combes A, Bréchot N, Amour J, Cozic N, Lebreton G, Guidon C,* et al. Early high-volume hemofiltration versus standard care for post-cardiac surgery shock: the HEROICS Study. Am J Respir Crit Care Med 2015; 192(10): 1179–90.
16. *Wierstra TB, Kadri S, Alomar S, Burbano X, Barrisford, Kao LC.* R. The impact of "early" versus "late" initiation of renal replacement therapy in critical care patients with acute kidney injury: a systematic review and evidence synthesis. Critical Care 2016; 20(1): 122.
17. *Bhatt GC, Das RR.* Early versus late initiation of renal replacement therapy in patients with acute kidney injury-a systematic review & meta-analysis of randomized controlled trials. BMC Nephrol 2017; 18(1): 78.
18. *Mavrakanas TA, Aurian-Blajeni DE, Charytan DM.* Early versus late initiation of renal replacement therapy in patients with acute kidney injury: a meta-analysis of randomised clinical trials. Swiss Med Wkly 2017; 147: w14507.
19. *Kao CC, Yang JY, Chen L, Chao CT, Peng YS, Chiang CK*, et al. Factors associated with poor outcomes of continuous renal replacement therapy. PLoS One 2017; 12(5): e0177759.
20. *Pistolesi V, Di Napoli A, Fiaccadori E, Zeppilli L, Polistena F, Sacco MI*, et al. Severe acute kidney injury following cardiac surgery: short-term outcomes in patients undergoing continuous renal replacement therapy (CRRT). J Nephrol 2016; 29(2):229-39.
21. *Patschan D, Müller GA.* Acute Kidney Injury in Diabetes Mellitus. Int J Nephrol 2016; 2016: 6232909.
22. *Thakar CV, Christianson A, Himmelfarb J, Leonard AC.* Acute Kidney Injury Episodes and Chronic Kidney Disease Risk in Diabetes Mellitus. Clin J Am Soc Nephrol 2011; 6(11): 2567−72.
23. *Johnson F, Phillips D, Talabani B, Wonnacott A, Meran S, Phillips AO.* The impact of acute kidney injury in diabetes mellitus. Nephrology (Carlton) 2016; 21(6): 506−11.
24. *Libório AB, Leite TT, Neves FM, Teles F, Bezerra CT.* AKI complications in critically ill patients: association with mortality rates and RRT. Clin J Am Soc Nephrol 2015; 10(1): 21–8.

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The use of transpulmonary contrast echocardiography – a first experience in Serbia

Upotreba transpulmonalnog ehokardiografskog kontrasta – prvo iskustvo u Srbiji

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Abstract

Background/Aim. Contrast echocardiography (CE) is an echocardiographic modality where ultrasound contrast echocardiographic agent (CEA) is introduced peripherally for the image enhancement. The aim of this study was to present the initial clinical experience of the use of CEA Optison™ (GE Healthcare, Princeton, NJ) at the Institute for Cardiovascular Diseases of Vojvodina, Serbia and prospectively monitor the occurrence of possible side effects. **Methods.** A total of 357 patients were referred for resting or stress echocardiographic examinations, with an approved indication for CEA administration. The average age of patients was 63.3 years (range, 21–92 years), 62% of them were men. Most of the patients (77.31%) had some form of ischemic heart diseases. Hypertension was the most frequent risk factor (77.03%), but 57 patients had diabetes mellitus and 33 patients had chronic kidney disease as comorbidity. Most (90.5%) of the patients were on beta blocker therapy, 83.5% of them on angiotensin converting enzyme/angiotensin receptor blockers. Majority (80.3%) of the patients received single or dual (49.5%) antiagregation therapy, 74 (26.3%), of them were on anticoagulation therapy, 55.1% of the patients were taking diuretics. The global ejection fraction (EF) was preserved in 39.85% of them, the majority (136 of them), had left ventricle (LV) impairment, with an EF less than 50%. Patients were followed up for 30 minutes after CEA administration for potential side effects. In 118 patients, vital signs (heart rate, oxygen saturation, body temperature, systolic and diastolic blood pressure) were measured before and 30 minutes after CEA administration. **Results.** The administration of CEA was not associated with side effects. Diastolic blood pressure drop and heart rate increase were statistically, but not clinically significant (p = 0.027 and p = 0.028, respectively). **Conclusion**. Changes in analyzed vital signs were clinically non relevant. CE is a safe noninvasive diagnostic modality for patients undergoing rest and stress echocardiography.

Key words:
adverse drug reaction reporting systems; cardiovascular diseases; comorbidity; contrast media; echocardiography.

Apstrakt

Uvod/Cilj. Kontrastna ehokardiografija (CE) je dijagnostička metoda koja podrazumeva aplikaciju kontrastnog agensa (CEA) u perifernu venu u cilju poboljšanja ehokardiografske slike. Cilj rada bio je, da se prikaže inicijalno iskustvo upotrebe Optisona™ (GE Healthcare, Princeton, NJ) kao CEA u Institutu za kardiovaskularne bolesti Vojvodine, Sremska Kamenica, Srbija, kao i prospektivno praćenje pojave eventualnih neželjenih efekata. **Metode.** Procedura CE je urađena kod 357 bolesnika kod kojih je postavljena indikacija za primenu CEA u miru i/ili testu stres ehokardiografije. Prosečna starost ispitanika je bila 63,3 godine (u opsegu 21–92 godine), među kojima je bilo 62% ispitanika muškog pola. Ispitanici su imali različite kliničke dijagnoze, ali najveći broj bolesnika (77,31%) imao je neku formu ishemijske bolesti srca. Hipertenzija je bila najčešći faktor rizika kod ispitanika (77,03%), a od komorbiditeta, šećerna bolest je bila prisutna kod 57 bolesnika, a 33 bolesnika je imalo hroničnu bubrežnu insuficijenciju. Većina bolesnika

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(90,5%) je uzimala beta blokatore, a 83,5% je koristilo inhibitore angiotenzin konvertujućeg enzima (ACE) ili blokatore angiotenzinskih receptora. Monoterapiju je dobijalo 80,3%, a dvojnu antiagregacionu terapiju 49,5% bolesnika, dok je 74 (26,3%) bolesnika dobijalo antikoagulantnu terapiju. Diuretike je koristilo 55,1% bolesnika. Sa očuvanom globalnom ejekcionim frakcijom (EF) leve komore (LK) bilo je 39,85% bolesnika, a većina (njih 136) je imala smanjenu EF LK (manju od 50%). Nakon primene CEA, bolesnici su praćeni još 30 minuta zbog moguće pojave neželjenih efekata. Kod 118 bolesnika su pre i 30 minuta nakon davanja CEA praćeni vitalni parametri (frekvencija srca, saturacija krvi kiseonikom, temperatura tela, sistolni i dijastolni krvni pritisak). **Rezultati.** Nakon primene CEA nisu zabeležene nuspojave. Zabeleženi su statistički značajno, ali ne i klinički značajno, smanjenje dijastolnog krvnog pritiska (p = 0,027), kao i povećanje frekvencije otkucaja srca (p = 0,028). **Zaključak.** Promene praćenih vitalnih parametara nemaju klinički značaj. CE je sigurna neinvazivna ehokardiografska metoda za pacijente podvrgnute CE u miru i testu stres ehokardiografije.

Ključne reči:
lekovi, neželjeno dejstvo, sistemi za izveštavanje; kardiovaskularne bolesti; komorbiditet; kontrastna sredstva; ehokardiografija.

Introduction

Today, echocardiography is growing side by side with modern technology and achievements in the field of other noninvasive modalities. Contrast echocardiography (CE) is a simple method where transpulmonary contrast echocardiographic agent (CEA) is introduced peripherally for the image enhancement. The clinical use of CE is defined both by the European Association of Echocardiography and by the American Society of Echocardiography (ASE) [1, 2].

The initial use of the CE were in technically difficult or uninterpretable echo images [3]. The first indication for the use of CE was to enable the visualisation of the endocardial border of the left ventricle (LV) when two or more contiguous segments were not seen well with native-noncontrast echocardiography [4].

Studies demonstrated the efficiency and safety of CEA improving the diagnostic utility of both rest and stress echocardiography (SE) [5–8].

For transpulmonary CEA, the indication in clinical cardiology is the enhancement of the left ventricule (LV) endocardial border, accurate and repeatable measurements of volumes, global and regional LV function, especially in patients who are candidates for chemotherapy, to establish the diagnosis of apical hypertrophy, LV thrombus or other intracardiac mass evaluation, noncompaction cardiomyopathy (CMP), to assess myocardial perfusion (MP) in rest and in multiparametric SE studies to assess coronary flow reserve (CFR) and/or viability, too [1, 2, 9].

The contraindications in nonpregnant adults are allergic reactions to the components of the CEA, precaution is recommended for patients with pulmonary hypertension (PH) and right to left (R-L) shunts. Side effects are rare and usually not serious [1, 2].

CE reduced intra- and interobserver variability in echocardiography interpretation, medical costs, mortality, and exposure to the ionizing radiation that is associated with other imaging modalities. The applications in research and off-label indications are also growing [1, 2].

The aim of this study was to present the initial experience after application of Optison™ (GE Healthcare, Princeton, NJ) as a CEA in routine medical practice at the Institute for Cardiovascular Diseases of Vojvodina, Sremska Kamenica, Serbia.

Methods

This observational prospective study was conducted from March 2017 to November 2019 at the Institute of Cardiovascular Diseases of Vojvodina in Sremska Kamenica, Serbia. During this period, a total of 357 patients with technically difficult echocardiographic examinations underwent CE. Informed consent was obtained from all patients. Data collected from each subject included demographic characteristics, history of illness, and information on allergies.

Patients with known hypersensitivity to perflutren, blood products, or albumin as well as individuals with previous history of food allergies were not included in the study. The presence of any infection or fever was also excluding criteria. Clinical diagnoses, risk factors and comorbidities, medications of the patients, LV ejection fraction (EF) and indications for CE are shown in Table 1.

CE was performed using ultrasound machines (GE Vivid 9 and VividXPRo) equipped with broadband transducers and low-mechanical index (MI) contrast-specific presets. The recommended MI in this diagnostic procedure is 0.2 or lower, which was used in this study.

In this study, CEA (Optison™), as an injectable sterile suspension, was used which consisted of microspheres filled with perflutren gas with a shell of human serum albumin.

Baseline native echocardiography was always performed before CE.

Preparation and administration of CEA required attention to the storage, preparation and application. The glass vials of the CEA were stored in a refrigerator with temperature between 2–8 °C. The preparation protocol and the administration method followed the instruction given by the manufacturer [3]. Adherence to the prescribed preparation protocol is crucial for good image quality. Optison™ was always applied as a bolus injection in this study, the amount of the CEA in the syringe was gently agitated immediately before the application after it exceeded a room temperature.

Patients were prepared and inserted with i.v. canulla with at least 20 gauge with the 3-way stopcock into the peripheral vein, usually into the right arm. The rate of the iv. bolus did not exceed 1 mL per second, flushed with 10 mL saline.

Table 1

Clinical characteristics of patients underwent contrast echocardiography (CE)

Characteristics	Values
Total number (%)	357 (100)
Gender, n (%)	357 (100)
male	244 (68.3)
female	113 (31.7)
Age (year), mean ± SD	63.28 ± 11.40
BSA (m^2), mean ± SD	1.99 ± 0.22
Clinical diagnoses, n (%)	357 (100)
ischemic heart diseases	276 (77.31)
rhythm disturbances	101 (28.29)
patients with pacemakers, ICD or CRT	21 (5.88)
cardiomyopathies	85 (23.81)
congenital and valvular heart diseases	96 (26.89)
Risk factors and comorbidities, n (%)	357 (100)
hypertension	275 (77.03)
diabetes mellitus	59 (16.53)
chronic kidney disease	33 (9.24)
obstructive lung diseases	17 (4.76)
Medications of the patients receiving CE, n (%)	285 (100)
ACE inhibitors/AT blockers	238 (83.5)
beta blockers	258 (90.5)
statins	210 (73.7)
dual antiagregation therapy	141 (49.5)
aspirin	229 (80.3)
anticoagulant therapy	74 (26.3)
diuretics	157 (55.1)
Ca antagonists	41 (14.4)
EF LV (%), n (%)	332 (100)
< 40	96 (28.9)
40–50	104 (31.3)
> 50	132 (39.8)
Indications for transpulmonary CE, n (%)	357 (100)
better endocardium delineation	82 (22.97)
LV EF estimation	31 (8.68)
apex of the LV (parietal thrombus)	59 (16.53)
hypertrophy of the LV	10 (2.80)
congenital heart diseases with or without bubble test	10 (2.80)
intracavitary mass/other then LV apex	6 (1.68)
suspected aortic dissection	3 (0.84)
transoesophageal echocardiography	4 (1.12)
SE (dobutamine, adenosine and dobutamine, pace maker, exercise)	152 (42.58)

SD – standard deviation; ACE – angiotensin converting enzyme; BSA – body surface area; ICD – implantable cardioverter defibrillator; CRT – cardiac resynchronization therapy; AT – angiotensin receptor; EF – ejection fraction; LV – left ventricle; SE – stress echocardiography .

The administered doses of CEA in our study were not specified in most of the patients, the bolus injection of the CEA was 0.3 or 0.4 mL iv. followed by a 10 mL slow saline flush. The maximum total dose did not exceed 1.5 mL, whenever the image was acceptable; the dose of the Optison™ was repeated, except in SE studies where usually at least two bolus doses were given, during the resting phase and in the peak phase. The administered doses of Optison™ were effective, sufficient to opacify the LV cavity and endocardial border in all cases of resting and SE.

Statistical analysis

Continuous variables were presented as mean ± standard deviation. Categorical variables were presented as frequencies in percentages. Statistical significance was calcu-

lated by the Student's *t*-test and $p < 0.05$ and was considered statistically significant.

Results

The average age of patients was 63.28 ± 11.40 years, the youngest patient was 21 and the oldest one 92 years old. More than two thirds of patients were men (68.3%).

The patients who were referred for routine resting or SE examinations with an approved indication for CEA administration, fulfilled at least one of the indications listed in Table 1.

Most of the patients (77.31%) had some form of ischemic heart diseases (IHD). Rhythm disturbances, CMPs, congenital and valvular heart diseases were also present among the tested patients. Since CE was introduced, apical hypertrophic CMP was newly diagnosed in 3 of them.

Patients were with a large number of comorbidities (Table 1).

The first line indications were: better delineation of the LV endocardium, estimation of the LVEF and better evaluation of the apex of the LV.

In 3 of the patients, when CE was introduced, apical hypertrophic CMP was newly diagnosed. One patient was diagnosed with CE successfully with LV diverticulum.

Most of SE studies were indicated for patients with previous history of IHD and chest pain. Exercise, pace maker (PM), dobutamin and adenosine SE were performed with Optison™. There were 2 CE exercise SE for valve diseases-mild aortic stenosis, and for congenital heart diseases, by which two patients had corrected transposition of the great arteries. In 4 patients the indication for Optison™ administration was the left auricle exploration before electroconversion. In patients with suspected aortic dissection, the diagnoses were excluded 3 times with CE, and in one patient the dissection was confirmed with CE.

Patients were followed up for 30 minutes for any side effects and symptoms as flushing, headache, chest pain, back pain, skin rash, palpitations, dyspnea, nausea, vomiting, dizziness or vertigo. None of these or other adverse effects (AE) or side effects were present in our group. No allergic or anaphylactoid reactions occurred.

In 118 patients, vital signs (heart rate, oxygen saturation, body temperature, systolic and diastolic blood pressure) were measured before and 30 minutes after the CEA administration (Table 2).

The average systolic and diastolic blood pressure was lower after the administration of Optison™. The diastolic blood pressure drop and the heart rate increase for 4.7 beat/min (on average) were statistically significant ($p = 0.027$ and $p = 0.028$, respectively) but clinically irrelevant.

The other followed up parameters were not significantly different after the 30 minutes monitoring time in this patients subgroup.

Figure 1 shows CE in a patient with large parietal thrombus which is presented as avascular, black formation at the LV apex, on transthoracic four chamber view.

Table 2

Vital parameters before and after the administration of contrast echocardiographic agents in 118 patients

Parameters	Before (mean ± SD)	After (mean ± SD)	*p*
Systolic BP (mmHg)	125.8 ± 14.93	123.5 ± 15.41	0.208
Diastolic BP (mmHg)	72.5 ± 10.05	69.9 ± 8.88	0.027
Heart rate (beat/min)	74.9 ± 12.27	78.6 ± 16.76	0.028
Oxygen saturation (%)	96.8 ± 1.95	96.7 ± 3.10	0.373
Body temperature (°C)	35.8 ± 0.55	36.3 ± 0.47	0.434

SD – standard deviation; BP – blood pressure.

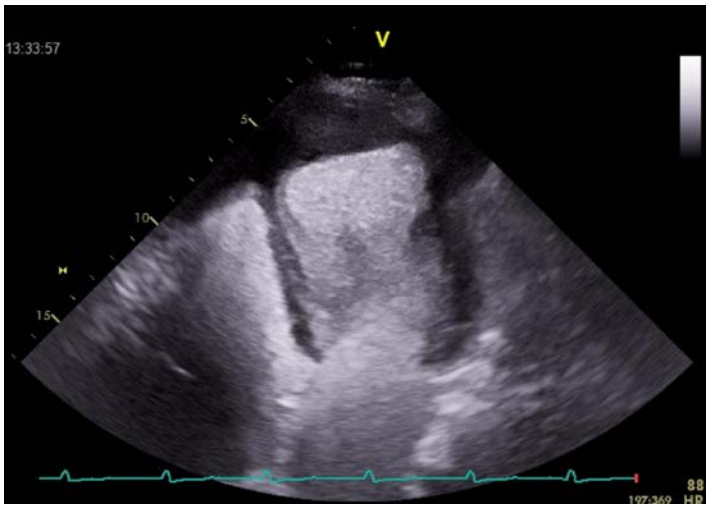

Fig. 1 – Contrast echocardiography shows large parietal thrombosis which is shown as avascular (block) formation at the left ventricle apex on transthoracic four chamber view.

In our investigated cohort, in 3 of patients, apical hypertrophic CMP was newly diagnosed by using CE (Figure 2). Two of them were more than once underwent coronary angiography for the previous IHD suspicion.

With the use of CE, the LV diverticulum was successfully diagnosed in one patient (Figure 3). It was done in dextrocardiac patients and with corrected transposion of the great arteries but none of them had a R-L shunt.

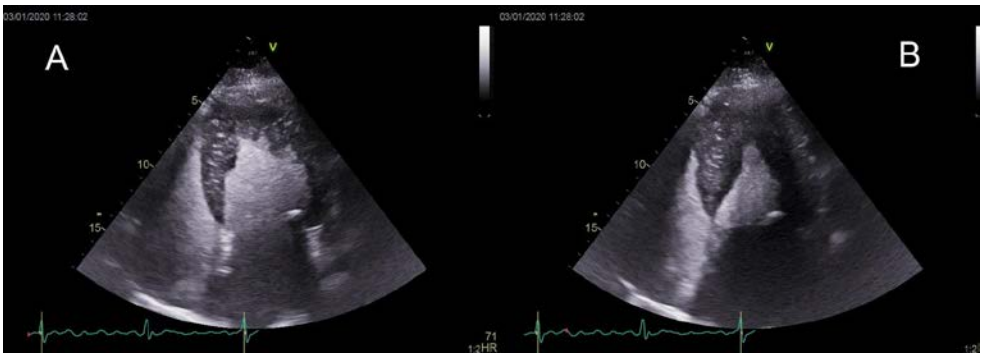

Fig. 2 – Contrast echocardiographic finding of newly diagnosed apical hypertrophic cardiomyopathy.

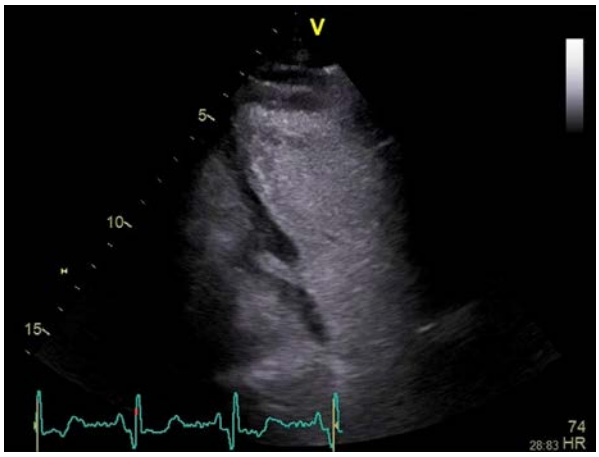

Fig. 3 – Left ventricle diverticulum in dextrocardiac patient diagnosed using contrast echocardiography.

Discussion

Today, there are three new, commercially available echocardiographic contrast agents (ECA): Optison™ (GE Healthcare Princeton, NJ), Lumason™ (Bracco) and Definity™ (Lantheus) in Europe and North America, and all of them are approved for use by the Food and Drug Administration (FDA) for the indication of LV opacification (LVO) in adults [1, 2].

The Levovist™ (Schering AG, Berlin, Germany) was the first commercially available ECA [10]. Initially, the idea was to use it in the patients with poor acoustic window or uninterpretable images [4, 5].

Currently, Optison™ is the only available CEA in Serbia. Its routine administration started at the Institute of Cardiovascular Diseases of Vojvodina, Sremska Kamenica in 2017, after the project was accepted and approved by the Local Government [12]. The regulatory documents and the permission for this method were obtained from the Republic Ministry of Health.

Optison™ is a sterile, nonpyrogenic suspension of microspheres or microbubles-filled with perflutren gas in albumin shell, that are small and stable enough to pass the pulmonary circulation during the ultrasound imaging procedures. The microbubles create an echogenic contrast effect in the blood, so this imaging modality is called transpulmonary CE [3].

It is used mostly for the LVO, for segmental or global LV wall motion analysis and for identification of cardiac masses. The indications of CE are defined by the European Society of Echocardiography and ASE [1, 2, 9, 11].

A review on the safety of CEA compared to the other commonly used radiology contrast agents pronounced them safe, reliable and radiation-free diagnostic modality [7, 8].

To perform CE, in addition to CEA it is necessary to possess an ultrasound machine equipped with adequate, low MI contrast software. The MI stands for the measure of the power generated by the transducer during the echocardiographic examination.

Side effects of CEAs are rare and usually not serious, but the administration can be associated with flushing, headache, nausea, vomiting, dyspnea, chest or back pain. The albumin component of the Optison™ is a derivative of human blood, so allergic or anaphylactoid reactions can be expected although very rarely [3].

The incidence of an anaphylactoid reaction from CEA exposure was estimated at about one in 15,000 [13], so our sample size could not be able to detect such rare events.

According to the recommendations, both "physicians and sonographers who wish to perform CE, should receive training in interpretation and operational details". The antiallergic drugs and the resuscitation equipment have to be available in case of emergency [1, 2].

In our patients, systematic preprocedural detailed history was taken with special care to those allergic to proteins (blood products, food or some medications). With positive history data or even in case of a suspected allergy or elevated temperature, the patients were not given CEA, what probably increased safety.

We were eager to experience the advantage of CE in the real clinical work, but safety was our great concern in various clinical settings, having in mind that Optison™ has been available since 2017, but it is still not registered in Serbia. For this reason safety was, if not more important, but as important as the diagnostic efficacy of the CEA.

The investigated patients were indicated in accordance with the latest recommendations [1, 2]. More than two thirds of them had low EF LV, some of them had acute IHD and congestive heart failure, but all of them tolerated Optison™ well. In other reports [14, 15], a low serious side effects rate of 0.01% was noted in those patients that received CEAs.

Patients included in our study represent those who are seen daily in echocardiographic laboratories, with a high frequency of cardiac risk factors and comorbidities. CE in pregnant women and in children under 5 years are however not recommended for CE. Chronic renal insufficiency is not, but liver insufficiency is an important issue in CE [3].

Although R-L shunts and PH are not a contraindication for CE anymore, we did not administer CEA in patients with R-L shunts or Eisenmenger syndrome. Whenever a suspicion occurred on a shunt, prior to CEA administration, bubble test was done with agitated saline.

The updated focused guidelines in 2014 for contrast use about AE or side effects denounced the risk of iv. commercial contrast agents in patients with small R-L shunts through a patent *foramen* ovale [16].

Perflutren gas, a component of Optison™, was eliminated through the lungs within 10 minutes after administration, but the interaction of Optison™ and other drugs were not studied and reported [3]. That is why we monitored patients for oxygen saturation during rest and stress CE, but AE never occurred. Wever-Pinzon et al. [6] published a study on 1,513 patients with PH who had received CE and were under control for 24 hours after the administration, but no respiratory decompensation, hypotension, arrhythmias, syncope, convulsions, anaphylactic reactions, or death was registered among them.

The preparation and the administration of CEA is an important part of the imaging. The administered doses of CEA in our study were not specified by any protocol. The injected doses of Optison™ were sufficient to opacify the LV cavity and endocardial border for several minutes in all cases of resting and SE. No patients received a total of 5.7 mL of CEA which is the highest dose proposed by the manufacturer [3].

The first indication for CEA according to the ASE guidelines was that it can "be used for improved endocardial visualization (ie., when two contiguous endocardial segments of the LV are not observed or to improve Doppler evaluations if the initial spectral signals are inadequate)" [4].

Today, inadequate segment visualisation is a first class recommendation, even one segment of the LV is not visualised [17].

The latest guideline for chronic coronary syndrome, pointed out that this imaging modality with CEA should precede cardiac magnetic resonance [18].

CE can accurately detect LV regional wall motion disturbances, even in technically challenging and obese patients [19]. Wall motion and MP analysis improved coronary artery disease (CAD) detection during SE with this imaging modality.

Wall motion analysis and MP defect detection were attempted in our patients not only with dobutamine or exercise SE, but PM SE as well, where an accelerated contrast SE was conducted in 25 of our patients, which is, according to our knowledge, the first group of patients with this kind of imaging modality.

In 2014, the contraindication was removed for the use of CEA in patients with recent acute coronary syndrome (ACS) or clinically unstable IHD [13]. Optison™ may also be used in ACS, what was presented in Galiuto et al. [20] paper.

Most resting and SE studies were performed with this imaging modality to evaluate LV endocardial border delineation for regional and segmental wall motion analysis and accurate measurement of the LV volumes and function [21].

The quantitative assessment of the EF of the LV is an important parameter, and CE measurements can provide similar values as cardiac magnetic resonance which is a "golden standard". It is well known that LV volumes obtained by CE are generally larger than by native echocardiography. CE can reduce interobserver variability [21, 22].

Our big concern was the LV apex visualisation on native echocardiography, what was the subject of our earlier research [12]. Apexes are often incompletely visualised, or trabeculations of the apical region can make the examination difficult [23].

It is clinically important to identify otherwise unrecognised thrombus in the apex of the LV. CE can improve the interpretation not only for the presence, but for the absence of an apical thrombus, because anticoagulation therapy will be introduced to prevent embolic event if the thrombus is detected, otherwise patients would be restricted from the unnecessary anticoagulation therapy if thrombus was excluded. Guidelines are still non uniform for the treatment of patients with parietal thrombi. With CE, the shape, the size and the embologenity of the thrombi can be more accurately assessed then with native echocardiography. Preventive checkups with CE would be necessary in patients prone to develop thrombi with large hypocontractile LV, or with an akinetic segment or aneurysms of the LV [9]. CE should be an integral part of the individualized

follow up and monitoring of anticoagulation therapy for registered thrombi in the heart chambers.

Cardiac masses in all heart chambers are an indication for CEA use, not only to determine the presence of the mass, but the vascularisation with perfusion imaging to determine the etiology of the questionable formation. Hyperenhancement of the mass would raise suspicion on its malign etiology [24].

In routine use of CE in patients with anterior MI, thrombi were reported in more than 20%, when there was even no suspicion at all with native echocardiography [25].

For the left appendage thrombus detection we performed transesophageal echocardiography (TEE) with CE in four of our patients.

We experienced the advantage of the CE in patients with suspected aortic dissection, since the diagnoses of aortic dissection by using CE were excluded three times and in one patient the dissection was confirmed.

There were a large number of patients in our investigated group with arrhythmias or PM, resinchronisation therapy, or cardioverter defibrilators, with valvular but also with congenital heart diseases.

The CE should not be withheld on the bases of any diagnoses or comorbidity [1] and may reduce health care cost because Optison™ helped define abnormalities that required appropriate hospitalization for further management [26].

During SE, CE was an option not only for IHD but for valve disesase for better evaluation of the highest velocity in mild stenotic lesions. Regional wall motion disturbances were registered with CE in rest and SE. The accuracy of CE was not compared to noncontrast study results in our patients, but according to the published papers of other investigators, there is a significantly higher accuracy in SE with CE for the detection of CAD, especially if they are done as multiparametric SE, not only for endocardial enhancement and wall motion analysis, but for coronary flow registration and MP and viability assessment as well [11, 27]. CE can improve interobserver agreement for wall motion analysis [28]. MP is a promising indication in CE, which can give us diagnostic and prognostic information. The use of ECA improved not only image quality, but the reader confidence of interpretation as well [29]. Comprehensive evaluation in IHD is the optimal approach for noninvasive assessment of the coronary artery lesions [30]. Our great concern was the interaction of the CEA with other therapy and medications. Some of the patients were on anticoagulation therapy and had chronic kidney disease (CKD) or obstructive pulmonary diseases. Interactions with medications and Optison™ were not investigated or referred to in the previous studies. Our patients were on a large variety of medications and had different diagnoses and that is important and encouraging for the routine clinical use of the CEA.

In 2007, after 4 deaths and several severe cardiopulmonary reactions occurred after the use of Definity™ and Optison™, the FDA issued a black box warning, which turned out to be unjustified [31], but added new contraindication for patients with PH and unstable chronic pulmonary disease and required a 30 minutes post-procedure monitoring period after the use of ECA.

We decided to follow these instructions and precautions, although the warnings were later withdrawn. The 30 minutes follow up time after the administration of Optison™ was conducted while heart rate, blood pressure, oxygen saturation and body temperature monitoring was completed. Systolic and diastolic blood pressure was slightly lower in patients after the administration of Optison™. Heart rate increased after the application of the CEA but it was clinically irrelevant.

Slightly higher temperature was registered in patients, in average, after the administration of Optison™, but the values were never above 37 °C. We have to point out that patients with a suspicion of infectious diseases or fever would not be given CEA. The minor change of the body temperature is clinically irrelevant after the administration of CEA. Patients with infectious diseases should avoid Optison™, but such observation was not a subject of previous reports although the manufacturer mentioned it [3].

The follow up was not continued after this monitoring period, thus, it is possible that some events were missed. Previous reports found that serious AEs to CEAs (allergic or anaphylactoid reactions) occurred early after administration, usually within 30 minutes [3], so it is unlikely that significant later AEs were missed.

There have been several published articles and reviews arguing both the safety and efficacy of CEAs in several large variety of patients, with PH followed up for 24 hours after the administration of CEA but no respiratory decompensation, hypotension, arrhythmias, syncope, convulsions, anaphylactic reactions, or death were registered among these patients [6, 15].

We think that patients taking cardiovascular medication and/or been undergoing SE with or without pharmacological stressor are a challenging group to follow up the AEs for CEAs, since the interaction of all these medications and CEA are difficult to analyse even in randomised circumstances.

For safety reasons, other authors followed up patients for 30 minutes after dobutamine or exercise stress testing with CEA. Among the reported symptoms, there were chest pain, arrhythmias such as premature atrial contractions, premature ventricular contractions, nonsustained ventricular tachycardia, hypertension, tachycardia, electrocardiographic changes, dyspnea, nausea, vomiting, tremor, and dizziness. None of these AEs were attributed to Optison™. There were no anaphylactoid reactions or deaths during or after studies conducted [8, 34].

Publishing on the safety and improved efficacy of CEAs in the retrospective studies [32] showed that propensity-matched patients who underwent a CE were 24% less likely to die within 1 day than patients who did not receive an CEA. Similar result was obtained in another study where 2,518 patients who received CEA had less overall one day mortality than patients who did not receive CEA [7].

Several authors also noted the safety of these agents in SE as well as the lack of AEs in long-term follow-up [31, 32].

In a retrospective study including 5,956 patients who received CE and were monitored for AEs, back pain and rash were registered in only 0.27% of the observed patients, but

there were no cases of serious anaphylaxis or death within 30 minutes of the contrast administration [8].

In prospective safety study of Optison™ [33] which included 203 patients, 37% of the patients had dilated CMP with diminished LV EF (20%–40%). There were no changes in the monitored vital signs. Patients were also followed up for AEs, but none of them were noted. Similar results were obtained in our study in patients with dilated CMP (Figure 4).

There is a trend toward improvement in outcomes when such patients undergo contrast-enhanced rather than unenhanced echocardiography [26, 38].

Extracardiac application of a CEA, for carotid, femoral, aortic endografts, peripheral perfusion is also recommanded. Among others, emerging applications are molecular imaging, targeted drugs-gene therapy and thrombolysis [1, 2].

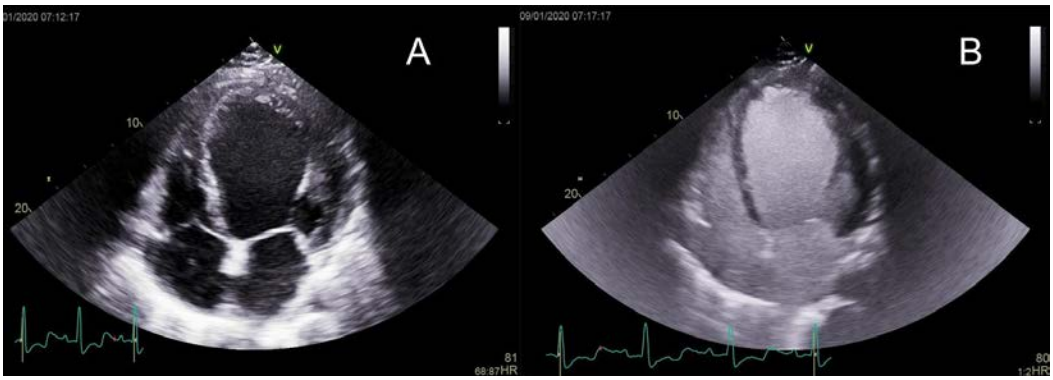

Fig. 4 – Dilated cardiomyopathy is shown using contrast echocardiography.

A prospective randomized trial showed that an abnormal MP with CE was more often observed than in conventional SE, and more frequently resulted in revascularization [29]. Significantly more cases of ischemia were diagnosed with MP CE and detected a greater ischemic burden than in the case of wall motion analysis in patients undergoing native SE [34].

Since 2012, the FDA has removed the need for monitoring of patients with PH, unstable chronic pulmonary diseases and stress testing [32]. In October 2016 shunt contraindications were removed [35]; since then this modality in patients with PH and shunts have not been a contraindication any more. Accordingly, the monitoring of vital signs can be practiced only in selected cases of patients with PH or R-L shunts [1, 2].

CE is a minimally invasive technique for perfusion analysis [36], by which sometimes other diagnostic modality can be avoided. When comparing noninvasive diagnostic methods in a study conducted by Senior et al. [37], CE demonstrated superior sensitivity but lower specificity for the detection of CAD as compared to scintigraphy, when results were confirmed by coronary angiography.

The CE can be and should be routinely used, not only in clinics and hospitals, but in every local outpatient office with an appropriate echocardiographic facility, since it is a safe and cost effective diagnostic modality.

Limitations of the study

The main limitation of this study was the sample size.

Conclusion

Contrast echocardiography with Optison™ as a CEA, is a very safe, noninvasive diagnostic modality, useful in a large variety of clinical settings, in patients being on medical treatment and undergoing resting and SE in the routine everyday clinical practice. It is important to check all the issues before performing CE concerning the patient selection which should be individualized, to exclude persons with allergy and to strictly follow the administration methodology. Vital parameter changes after Optison™ administration were clinically irrelevant.

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REFERENCES

1. *Porter TR, Mulvagh SL, Abdelmoneim SS, Becher H, Belcik JT, Bierig M*, et al. Clinical application of ultrasonic enhancing agents in echocardiography: 2018 American Society of Echocardiography Guidelines Update. J Am Soc Echocardiogr 2018; 31(3): 241−74.
2. *Senior R, Becher H, Monaghan P, Agati L, Zamorano J, Vanoverschelde J*, et al. Clinical practice of contrast echocardiography: recommendation by the European Association of Cardiovascular Imaging (EACVI)2017. Eur Heart J Cardiovasc Imaging 2017; 18(11): 1205−1205af.
3. GE Healthcare. OPTISON Prescribing Information. http://www.optisonimaging. Available from: com/us/wp-content/uploads/2013/10/optison_pi.pdf
4. *Montalescot G, Sechtem U, Achenbach S, Andreotti F, Arden C, Budaj A*, et al. ESC guidelines on the management of stable coronary artery disease. Eur Heart J 2013; 34(38): 2949−3003.
5. *Douglas PS, Weyman AE, Lindner JR, Wei K.* Contrast echocardiography: past, present, and future? JACC Cardiovasc Imaging 2008; 1(1):107–10.

6. *Wever-Pinzon O, Suma V, Ahuja A, Romero J, Sareen N, Henry SA*, et al. Safety of echocardiographic contrast in hospitalized patients with pulmonary hypertension: a multi-center study. Eur Heart J Cardiovasc Imag 2012; 13(10): 857–62.
7. *Goldberg YH, Ginelli P, Siegel R, Ostfeld RJ, Schaefer M, Spevack DM.* Administration of perflutren contrast agents during transthoracic echocardiography is not associated with a significant increase in acute mortality risk. Cardiology 2012; 122(2): 119–25.
8. *Platts DG, Luis SA, Roper D, Burstow D, Call T, Forshaw A*, et al. The safety profile of perflutren microsphere contrast echocardiography during rest and stress imaging: results from an Australian multicentre cohort. Heart Lung Circ 2013; 22(12): 996–1002.
9. *Saric M, Armour A, Arnaoutet S, Chaudhry F, Grimm RA, Kronzon I,* et al. Guidelines for the Use of Echocardiography in the Evaluation of a Cardiac Source of Embolism. J Am Soc Echocardiogr 2016; 29(1): 1−42.
10. *van der Meer SM*. Ultrasound Contrast Agents: Resonances of Coated Bubbles. [thesis]. Enschede, Netherlands: University of Twente; 2007.
11. *Shah BN, Chahal NS, Bhattacharyya S, Li W, Roussin I, Khattar RS,* et al. The feasibility and clinical utility of myocardial contrast echocardiography in clinical practice: results from the incorporation of myocardial perfusion assessment into clinical testing with stress echocardiography study. J Am Soc Echocardiogr 2014; 27(5): 520–30.
12. *Stojšić-Milosavljević A.* Importance of contrast echocardiography in left ventricle parietal thrombus. Novi Sad: Faculty of Medicine, University of Novi Sad; 2015. (Serbian)
13. EMA, Assessment Report 2 May 2014, SonoVue Procedure No. EMEA/H/C/000303/II/0025. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000303/WC500170218.pdf [accessed 2014 December 16].
14. *Wei K, Mulvagh SL, Carson L, Davidoff R, Gabriel R, Grimm RA*, et al. The safety of Definity and Optison for ultrasound image enhancement: a retrospective analysis of 78,383 administered contrast doses. J Am Soc Echocardiogr 2008; 21(11): 1202−6.
15. *Exuzides A, Main ML, Colby C, Grayburn PA, Feinstein SB, Goldman JH.* A restrospective comparison of mortality in critically ill hospitalized patients undergoing echocardiography with and without an ultrasound contrast agent. JACC Cardiovasc Imaging 2010; 3(6): 578−85.
16. *Porter TR, Abdelmoneim S, Belcik JT, McCulloch ML, Mulvagh SL, Olson JJ,* et al. Guidelines for the cardiac sonographer in the performance of contrast echocardiography: a focused update from the American Society of Echocardiography. J Am Soc Echocardiogr 2014; 27(8): 797–810.
17. *Mulvagh SL, Rakowski H, Vannan MA, Abdelmoneim SS, Becher H, Bierig SM,* et al. American Society of Echocardiography consensus statement on the clinical applications of ultrasonic contrast agents in echocardiography. J Am Soc Echocardiogr 2008; 21(11): 1179−201; quiz 1281.
18. *Knuuti J, WijnsW, Saraste A, Capodanno D, Barbato E, Funck-Brentano C*, et al. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. Eur Heart J 2020; 41(3): 407−77.
19. *Hoffmann R, von Bardeleben S, Barletta G, Pasques A, Kasprzak J, Greis C,* et al Comparison of two- and three-dimensional unenhanced and contrast-enhanced echocardiographies versus cineventriculography versus cardiac magnetic resonance for determination of left ventricular function. Am J Cardiol 2014; 113(2): 395−401.
20. *Galiuto L, Garramone B, Scarà A, Rebuzzi AG, Crea F, La Torre G*, et al. The extent of microvascular damage during myocardial contrast echocardiography is superior to other known indexes of post-infarct reperfusion in predicting left ventricular remodeling results of the multicenter AMICI Study. J Am Coll Cardiol 2008; 51(5): 552−9.
21. *Hoffmann R, Barletta G, von Bardeleben S, Vanoverschelde JL, Kasprzak J, Greis C,* et al. Analysis of left ventricular volumes and function–a multicenter comparison of cineventriculography, cardiac magnetic resonance imaging, unenhanced and contrast enhanced 2D and 3D echocardiography. J Am Soc Echocardiogr 2014; 27(3): 292−301.
22. *Hoffmann R, von Bardeleben S, ten Cate R, Borges AS, Kasprzak J, Firschke C,* et al. Assessment of systolic left ventricular function: a multi-centre comparison of cineventriculography, cardiac magnetic resonance imaging, unenhanced and contrast-enhanced echocardiography. Eur Heart J 2005; 26(6): 607−16.
23. *Mansencal N, Nasr IA, Pillière R, Farcot JC, Joseph T, Lacombe P,* et al. Usefulness of contrast echocardiography for assesment of lft ventriclular thrombus after acute myocardial infarction. Am J Cardiol 2007; 99(12): 1667−70.
24. *Kirkpatrick JN, Wong T, Bednarz JE, Spencer KT, Sugeng L, Ward RP*, et al. Differential diagnosis of cardiac masses using contrast echocardiographic perfusion imaging. J Am Coll Cardiol 2004; 43(8): 1412−9.
25. *Staab W, Bergau L, Schuster A, Hinojar R, Dorenkamp M, Obenauer S,* et al. Detection of intracardiac masses in patients with coronary artery disease using cardiac magnetic resonance imaging: a comparison with transthoracic echocardiography. Int J Cardiovasc Imaging 2014; 30(3): 647−57.
26. *Kurt M, Shaikh KA, Peterson L, Kurrelmeyer KM, Shah G, Nagueh SF*, et al. Impact of contrast echocardiography on evaluation of ventricular function and clinical management in a large prospective cohort. J Am Coll Cardiol 2009; 53(9): 802−10.
27. *Gaibazzi N, Rigo F, Reverberi C.* Detection of Coronary Artery Disease by Combined Assessment of Wall Motion, Myocardial Perfusion and Coronary Flow Reserve: A Multiparametric Contrast Stress-Echocardiography Study. J Am Soc Echocardiogr 2010; 23(12): 1242−50.
28. *Nayyar S, Magalski A, Khumri TM, Idupulapati M, Stoner CN, Kusnetzky LL*, et al. Contrast administration reduces interobserver variability in determination of left ventricular ejection fraction in patients with left ventricular dysfunction and good baseline endocardial border delineation. Am J Cardiol 2006; 98(8): 1110–4.
29. *Pellikka P, Arruda-Olson A, Chaudhry F, Chen M, Marshall J, Porter T*, et al. Guidelines for performance, interpretation, and application of stress echocardiography in ischemic heart disease: from the American Society of Echocardiography. J Am Soc Echocardiogr 2020; 33(1): 1−41.e8.
30. *Mladenovic Z, Djordjevic -Dikic A, Djuric P, Angelkov Ristic A, Dzudovic B, Jovic Z.* Diagnostic value of noninvasive comprehensive morphological and functional assesment of coronary artery disease. Vojnosanitet Pregl 2019; 76(2): 136−43.
31. *Grayburn PA.* Product safety compromises patient safety (an unjustified black box warning on ultrasound contrast agents by the Food and Drug Administration). Am J Cardiol 2008; 101(6): 892–3.
32. *Main ML.* Ultrasound contrast agent safety: from anecdote to evidence. JACC Cardiovasc Imaging 2009; 2(9): 1057–9.
33. *Cohen L, Cheirif J, Segar D, Gillam L, Gottdiener J, Hausnerova E*, et al. Improved left ventricular endocardial border delineation and opacification with OPTISON (FS069), a new echocardiographic contrast agent results of a phase III multicenter trial. J Am Coll Cardiol 1998; 32(3): 746–52.
34. *Porter TR, Smith LM, Wu J, Thomas D, Haas JT, Mathers DH,* et al. Patient outcome following 2 different stress imaging approaches: a prospective randomized comparison. J Am Coll Cardiol 2013; 61(24): 2446−55.
35. *Parker JM, Weller MW, Feinstein LM, Adams RJ, Main ML, Grayburn PA,* et al. Safety of ultrasound contrast agents in pa-

tients with known or suspected cardiac shunts. Am J Cardiol 2013; 112(7): 1039−45.

36. *Emanuel AL, Meijer RI, van Poelgeest E, Spoor P, Serné EH, Eringa EC.* Contrast-enhanced ultrasound for quantification of tissue perfusion in humans. Microcirculation 2020; 27(1): e12588.
37. *Senior R, Moreo A, Gaibazzi N, Agati L, Tiemann K, Shivalkar B*, et al. Comparison of sulfur hexafluoride microbubble (SonoVue)-enhanced myocardial contrast echocardiography with gated single-photon emission computed tomography for detection of significant coronary artery disease: a large European multicenter study. J Am Coll Cardiol 2013; 62(15): 1353−61.
38. *Main ML, Ryan AC, Davis TE, Albano MP, Kusnetzky LL, Hiberd M.* Acute mortality in hospitalized patients undergoing echocardiography with and without an ultrasound contrast agent (multicenter registry results in 4,300,966 consecutive patients). Am J Cardiol 2008; 102(12): 1742−6.

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Recognition of emotions and affective attitudes in children with attention-deficit hyperactivity disorder

Prepoznavanje emocija i afektivnih stavova kod dece sa poremećajem pažnje i hiperaktivnošću

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Abstract

Background/Aim. Children with attention-deficit hyperactivity disorder (ADHD) show lower degree of recognition of their own emotions and greater behavioral difficulties than children who do not have this disorder. This also affects the recognition of other people's emotions that are expressed not only by their language content, but also by their facial expression and the way they express the emotional message through speech. Most research in this area focuses on recognizing emotions based on facial expressions rather than on emotions in speech. The aim of this study was to examine how children with ADHD recognize emotions in speech (joy, anger, fear, sadness) and affective attitudes (threat and commandment) in relation to children of typical development. **Methods.** The study included 31 children with ADHD and 29 typical developmental children aged 6 to 13. To assess the ability to recognize emotions and affective attitudes, a corpus of Speech Emotional Expression and Attitudes (*Govorna emocionalna ekspresija i stavovi* – GEES, in Serbian) was used. This corpus included sentences that expressed emotions of anger, joy, fear, and sadness, and of affective attitudes, threat and command. **Results.** The results obtained showed that children with ADHD had worse recognition of emotions and affective attitudes in speech than children of typical development ($t = 8.81$; $p = 0.000$). Joy was the only emotion where no statistically significant difference was found. In all other emotions and affective attitudes, statistically significant differences were found ($p < 0.01$). Also, the results showed that there was a statistically significant association between age and recognition of emotion and affective attitudes in both groups, but this association was more pronounced in children of typical development. **Conclusion.** The results of this study provided important clues about the perception of emotions and affective attitudes in speech in children with ADHD. These results are very important for the conception of therapeutic procedures, especially in the development of strategies for modeling the behavior of children with ADHD.

Key words:
affective symptoms; attention deficit disorder with hyperactivity; emotions; speech.

Apstrakt

Uvod/Cilj. Deca sa poremećajem pažnje i hiperaktivnošću (ADHD) pokazuju slabije prepoznavanje sopstvenih emocija i veće ispoljavanje teškoća u ponašanju od dece koja nemaju tu smetnju. Samim tim je pogođeno i prepoznavanje tuđih emocija koje se ispoljavaju ne samo jezičkim sadržajem već i izrazom lica i načinom izražavanja emotivne poruke govorom. Cilj ovog istraživanja je bio da se ispita kako deca sa ADHD prepoznaju emocije u govoru (radost, ljutnju, strah, tugu) i afektivne stavove (pretnju i zapovest) u odnosu na decu sa tipičnim razvojem. Većina istraživanja iz ove oblasti fokusirana je na prepoznavanje emocija na osnovu izraza lica, a ne na osnovu emocija u govoru. **Metode.** Istraživanje je obuhvatilo 31 dete sa ADHD i 29 dece sa tipičnim razvojem uzrasta od 6 do 13 godina. Za procenu sposobnosti prepoznavanja emocija i afektivnih stavova korišćen je korpus za procenu Govorne emocionalne ekspresije i stavova (GEES). U tom korpusu su bile rečenice koje su izražavale emocije ljutnje, radosti, straha i tuge, a od afektivnih stavova pretnju i zapovest. **Rezultati.** Deca sa ADHD imala su lošije prepoznavanje emocija i afektivnih stavova u govoru u odnosu na decu sa tipičnim razvojem ($t = 8.81$; $p = 0.000$). Radost je bila jedina emocija gde nije utvrđena statistički značajna razlika. Za druge emocije i afektivne stavove utvrđene su statistički značajne razlike ($p < 0.01$). Utvrđena je statistički značajna povezanost između uzrasta i prepoznavanja emocija i afektivnih stavova u obe grupe, ali je ta povezanost bila izraženija kod dece sa tipičnim razvojem. **Zaključak.** Rezultati istraživanja su dali važne pokazatelje o percepciji emocija i afektivnih stavova u go-

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voru kod dece sa ADHD. Ovi rezultati su veoma bitni za osmišljavanje terapijskih procedura, posebno u razvoju strategija za modeliranje ponašanja dece sa ADHD.

Ključne reči:
afektivni simptomi; hiperkinetički sindrom; emocije; govor.

Introduction

Attention deficit hyperactivity disorder (ADHD) is diagnosed in childhood or adolescence and is characterized by three groups of persistent symptoms: hyperactivity, attention deficit and impulsiveness [1]. In addition, children with ADHD can have a number of comorbid externalizing and internalizing psychiatric conditions such as anger, aggression, behavioral disorders, delaying responsibilities, anxiety, depression, guilt [2]. ADHD affects optimal children's development, their self-esteem, weakens social contacts with parents and teachers [3, 4], but it also affects the occurrence of school skills difficulties and academic failure [5, 6]. Worldwide prevalence of ADHD ranges from 5% to 7% [7, 8]. The high prevalence of this disorder has attracted a great deal of attention from researchers and clinicians trying to understand the causes and mechanisms leading to the onset of characteristic symptoms of ADHD, as well as finding the best therapy for it. ADHD is believed to be a neurobehavioral developmental disorder but its pathophysiology has not been fully known yet. So far, the investigated causes of ADHD can be classified into two categories: environmental and molecular-genetic. In the category of environmental causes, those arising from obstetric complications [9], fetal or infantile exposure to various agents [10], as well as the conditions in which children grow and develop, have been studied [11–13]. Molecular genetic causes have been extensively investigated in the field of dopamine transmission [14], or catecholaminergic dysregulation [15].

Studies conducted in recent years show that children with ADHD have specific social deficits such as: poor recognition of role-playing behaviors, inflexibility in responses, inability to modify their own behavior in response to changes in environmental demands, and unconscious subtle, but very important social cues [16–18]. Research findings up till now have suggested that impaired social skills and present behavioral problems in children and adolescents with ADHD are not fully explained by additional comorbidities or secondary consequences in the form of executive function deficits [19–21]. A number of authors believe that answers related to social skills disorders and behavior in children and adolescents with ADHD can be found in understanding possible deficits in emotion recognition. Studying the emotional competence of children with ADHD can be an important complement to these children's social skills research. In order for children to engage in appropriate social interactions, they must be able to recognize and make judgments about the emotions of others. The ability to interpret and respond appropriately to the emotions of others is crucial for interpersonal interactions. Evidence to support this finding can be found in studies of the social functioning of children with autism and their poor recognition and processing of emotions [22]. Based on this, it can be assumed that in children with ADHD, there is an association between poor social skills and difficulties in recognizing emotions.

Research to date has shown that children with ADHD show poorer results in recognizing facial emotions than the typical population [17, 23, 24]. For these children, tasks that required the interpretation of emotional cues from face photos were difficult and they made more mistakes solving those tasks than the typical population [25]. Also, it was difficult for children and adolescents with ADHD to reconcile the primary emotion face expression with the emotional message of the story read to them [26]. There is evidence that children with ADHD were less successful in recognizing their own emotions [27].

It is important to note that most of the research performed so far has been examining the recognition of facial emotions and contextual cues in children with ADHD. The general impression is that there is no research related to recognizing emotions in the voice. Through research done on typical developmental children, it has been concluded that the emotional properties of vocal cues can influence the infant's focus on objects and the exploratory behavior of the world [28–30]. Typical developmental children have been shown to be able to accurately classify prosody as joy or sadness at a very young age [31]. A survey of typical developmental children aged 5 to 10 has shown that children as young as five are able to easily and accurately recognize and interpret a range of emotional cues from the voice [32]. Also, this research has shown that there is improvement in results with advancing age. There is a very interesting study comparing the recognition of emotions on the basis of visual (facial expressions), auditory (speech) and audio-visual (facial expressions and speech) modalities of information transmission. Children between the ages of 5 and 18 were exposed to: only auditory, only visual or audio-visual modalities of parent-child communication. For younger children (up to 8), the auditory canal was more important than the visual. Older children, on the other hand, relied more on visual cues [33].

In their interactions with others, children, just like adults, need to interpret a wide range of social signals to understand the intentions and feelings of others. The ability to distinguish social signals is thought to develop very early, as early as about the fifth month of life. Babies at that age are able to respond to approvals and bans [34], even when spoken in an unfamiliar language [35]. These discriminations can be made on the basis of differences in lower cognitive stimulus classes, such as Fo's height, while connecting auditory cues to social circumstances and events require more sophisticated cognitive abilities [36]. These findings highlight the im-

portance of auditory modality in recognizing emotion in speech as an important component of understanding social communicative context.

One of the reasons for performing our study was the small number of studies that dealt with examining the abilities of children with ADHD as well as of the typical population children to understand and recognize the emotional forms of spoken expression. Based on the evidence that this form of obtaining information about the emotional background of spoken expression plays an important role in children's behavior and directing attention to important components of the environment, it can be assumed that children with ADHD have altered patterns of processing emotions in speech relative to the typical population. This was the starting point of this research.

The aim of the study was to evaluate the ability to recognize emotions and affective orders in speech, and to examine whether there were differences in their recognition in children with ADHD compared to the typical population children. It has been hypothesized that children with ADHD perform worse in recognizing emotions and affective speech orders than the typical population children, and that there is a difference in the ability to recognize different types of emotions and affective speech orders in this population of children.

Methods

The research was approved by the Academic Council and Ethics Committee of the Faculty of Special Education and Rehabilitation, University of Belgrade, Serbia and the consent was also signed by the parents of children who participated in this research. All participants were examined individually in the Speech Therapy Cabinet "Plećević" in Belgrade, Serbia. Speech and language status of children was assessed by the Speech and Language Test Battery of the Institute for Experimental Phonetics and Speech Pathology in Belgrade, Serbia which is standardly used in the Serbian speaking language. The diagnosis of ADHD was made at the Institute of Mental Health by competent neurologists and psychiatrists and the children sent for rehabilitation to the Speech Therapy Cabinet "Plećević". Based on the received medical records, information was obtained on the type and severity of the disorder present, and this institution followed the protocol recommended by the American Pediatric Academy [1]. The protocol includes: detailed medical history, general and neurological medical examination, parent-child interview, Swanson, Nolan and Pelham Teacher and Parent Rating Scale (SNAP IV scale), child observation, psychological tests for measuring intelligence quotient (IQ) as well as social and emotional adaptability tests and neuropsychological tests for diagnosing specific learning disorders. At the time of testing, children with ADHD did not use pharmacotherapy, and the rehabilitation program they attended included metacognitive strategies, psychomotor reeducation, neurofeedback training, and sports and recreational therapy.

The criteria for determining whether children would be included in the sample of this study were as follows: children had no other disorders or disorders, no other neurological or psychiatric illnesses, did not use pharmacological therapy, all children on the Raven matrices had scores above 80 and all children on the SNAP IV scale had over 70% expression of combined type ADHD symptoms. The experimental group was selected from the population of children who, due to ADHD, were included in the rehabilitation program of the Speech Therapy Cabinet "Plećević". The control group also met the above criteria (except those related to the severity of ADHD) and was selected from a population of children who attended regular schools and were never included in defectology or speech therapy programs.

Assessment of emotion recognition and affective attitudes was performed using the Speech Emotional Expression and Attitude Assessment Corps (Govorna ekspresija emocija i stavova − GEES) [37], which was accepted by the Institute for Experimental Phonetics and Speech Pathology and the Center for the Advancement of Life Activities, Belgrade, Serbia. The speech materials were uttered by six actors (three women and three men) who are final year students of the Faculty of Dramatic Arts (FDU). The recording of the voice base was performed using professional digital audio equipment in the antisonor room of the FDU studio. The choice of recorded spoken content respected the criteria of the phonetic and linguistic proportions of the Serbian language. For the purposes of this research, a portion of the GEES corpus was used, namely: 3 short sentences for 4 primary emotions, totaling 12 sentences, and 3 sentences for 2 affective attitudes, totaling 6 sentences uttered by a male speaker. Accurately recognized emotions and affective attitudes were scored with 1 point, so the maximum score for each task group was 3 points and the maximum total score for GEES was 18 points. Inaccurate answers and no responses were scored with 0 points, so the minimum score could be 0 points for each task group and also for the total score at GEES. The test was performed during one encounter and the children were presented with the pronunciation of selected sentences in a randomized schedule. The task was for the children to recognize the emotion and affective attitude in the spoken material, without paying attention to the linguistic content, and to verbalize their observations. To help them, they were provided with a list of selected emotions in writing. The recorded material was presented using computer equipment and the participants used headphones. Children were expected to respond within 300 to 6,000 ms and the next task was set 1,200 ms after the registered response. If no response was given, it was automatically switched to the next task. The GEES internal consistency was good ($\alpha = 0.831$).

A total of 60 children were included in the study.

Descriptive statistics, 95% confidence interval, effect size (Cohen's d), *t* test for independent samples, *t* test for dependent samples and Pearson's correlation coefficient were used in the analysis of the results. Statistical analysis was performed using the IBM SPSS 25 software package.

Results

The study involved 60 children, which were divided into two groups: 31 (51.7%) of children with ADHD and 29 (48.3%) typical population children. The children were from 6 to 13 years old [mean age ± standard deviation (SD) = 9.25 ± 1.97]. Based on the *t* test for independent samples, it was concluded that the two groups did not differ significantly in terms of age [t (58) = -0.974, p = 0.332]. There were 51 (85%) boys and 9 (15%) girls in relation to gender in the sample. The *t* test for two independent samples showed no statistically significant difference with respect to gender [t (58) = 1.913, p = 0.061]. Speech and language assessment was performed prior to the GEES examination and the results showed that children from the two observed groups did not differ statistically in terms of achievement [t (58) = 0.942, p = 0.327].

Table 1 shows the arithmetic mean, standard deviation, and 95% confidence interval and statistical significance of differences in arithmetic means for recognizing emotions and affective speech orders in ADHD and the typical population. Children with ADHD best recognized joy and typical children sadness. The affective threat order was the least recognized in children with ADHD and in the typical population, fear emotions and the affective command order.

The typical population of children was better in recognizing most emotions and affective orders with a statistically higher significant difference ($p < 0.01$) than children with ADHD, except for the joy emotion, where statistical significance was not established. Based on the analyzed effect size (Cohen's d) for all statistically significant differences found in recognizing emotions and affective orders, it can be concluded that there was a large difference in effect for emotions: fear (d = 1.13), sadness (d = 1.23) and anger (d = 1.42) as well as for affective orders threat (d = 2.01) and command (d = 0.98). The effect size for the overall results was also large (d = 2.28).

The results presented in Table 2 indicated that children with ADHD recognize emotions and affective orders with different success, whereas this was not the case with the typical population. Children with ADHD had a statistically significantly better recognition of joy than all other emotions and affective orders, and the statistical significance of differences also appears in fear–threat and sadness–threat. The typical population children differently recognized only the emotion of sadness in relation to the affective command order. Both groups of children had statistically significant higher recognition of emotions than affective accounts.

Table 1

Recognition of emotions and affective speech orders in ADHD patients relative to a typical population

Parameter	ADHD patients (n = 31)			Typical population (n = 29)			*t*-test	
	mean ± SD	95% confidence interval		mean ± SD	95% confidence interval			
		lower	upper		lower	upper		
Emotion							*t*	*p*
joy	2.48 ± 0.77	2.20	2.77	2.72 ± 0.53	2.52	2.92	1.41	0.167
fear	1.61 ± 0.84	1.30	1.92	2.48 ± 0.69	2.22	2.74	4.36	0.000
sadness	1.81 ± 0.98	1.45	2.17	2.76 ± 0.51	2.56	2.95	4.76	0.000
anger	1.48 ± 0.99	1.12	1.85	2.62 ± 0.56	2.41	2.83	5.49	0.000
Affective orders								
threat	1.19 ± 0.91	0.86	1.53	2.69 ± 0.54	2.48	2.90	7.79	0.000
command	1.58 ± 1.12	1.17	1.99	2.48 ± 0.69	2.22	2.74	3.78	0.000
Total	10.13 ± 2.96	9.04	11.22	15.76 ± 1.80	15.07	16.45	8.81	0.000

ADHD – affective orders in attention-deficit hyperactive disorder; SD – standard deviation.

Table 2

Differences in recognition of emotions and ADHD patients and typical population

Pairs	ADHD patients			Typical population		
	mean ± SD	*t*-test		mean ± SD	*t*-test	
		t	*p*		*t*	*p*
Joy – fear	0.87 ± 1.09	0.44	0.000	0.24 ± 0.95	1.37	0.182
Joy – sadness	0.68 ± 1.17	3.23	0.000	-0.03 ± 0.57	-0.33	0.744
Joy – anger	1.00 ± 1.32	4.23	0.001	0.10 ± 0.86	0.65	0.522
Joy – threat	1.29 ± 1.07	6.71	0.003	0.03 ± 0.78	0.24	0.816
Joy – command	0.90 1.33	3.79	0.001	0.24 ± 0.83	1.56	0.126
Fear – sadness	-0.19 ± 1.22	-0.88	0.383	-0.28 ± 0.75	-1.98	0.062
Fear – anger	0.13 ± 1.41	0.51	0.612	-0.17 ± 0.83	-0.89	0.387
Fear – threat	0.42 ± 1.02	2.28	0.000	-0.21 ± 0.73	-1.53	0.133
Fear – command	0.03 ± 1.33	0.13	0.892	0.00 ± 0.96	0.00	0.902
Sadness – anger	0.32 ± 1.27	1.40	0.163	0.13 ± 0.74	1.00	0.322
Sadness – threat	0.61 ± 1.12	3.06	0.000	0.07 ± 0.59	0.63	0.533
Sadness – command	0.23 ± 1.31	0.97	0.345	0.28 ± 0.65	2.29	0.032
Anger – threat	0.29 ± 1.21	1.33	0.193	-0.07 ± 0.84	-0.44	0.662
Anger – command	-0.09 ± 1.51	-0.36	0.721	0.24 ± 0.83	0.89	0.383
Threat – command	-0.39 ± 1.17	-1.84	0.082	-0.03 ± 0.77	1.44	0.162
Affective orders – emotion	-4.61 ± 2.01	-12.77	0.000	-5.41 ± 1.29	-22.49	0.000

ADHD – affective orders in attention-deficit hyperactive disorder; SD – standard deviation.

This study also examined the association between age and recognition of emotions and affective speech orders in children with ADHD and children of typical development. Pearson's correlation coefficient and confidence interval (CI) for linear correlation were used for this analysis. In children with ADHD, a mean association between age and command recognition was found r (31) = 0.481, $p < 0.01$ with 95% CI: 0.15–0.71; age and overall scores r (31) = 0.373, $p < 0.05$, with 95% CI: 0.02–0.64; and age and overall recognition scores of affective orders r (31) = 0.434, $p < 0.05$, with a 95% CI: 0.09–0.68. In the typical development children, a greater association between age and recognition of emotions and affective speech orders was found compared to children with ADHD. Mean correlation with age in typical population children was found in fear recognition r (29) = 0.441, $p < 0.05$, with 95% CI: 0.08–0.69; sadness r (29) = 0.401, $p < 0.05$, with 95% CI: 0.04–0.67; and command r (29) = 0.445, $p < 0.05$ with 95% CI: 0.08–0.69. High correlation with age was recorded in overall scores r (29) = 0.512, $p < 0.01$ with 95% CI: 0.17–0.74. In contrast to children with ADHD, in the typical population children mean correlation between the age and total scores in the recognition of affective orders r (29) = 0.443, $p < 0.05$ with 95% CI: 0.08–0.69 and overall scores in the recognition of emotions r (29) = 0.401, $p < 0.05$ with 95% CI: 0.04–0.67 were recorded. All reported correlations were statistically significant.

Discussion

Considering that there is not much research that has examined emotion recognition solely on the basis of speech prosody labels in children with ADHD, the discussion on this research is limited to the small number of available papers.

The analyzed results indicate a statistically significant poorer recognition of the emotions and affective attitudes in speech of children with ADHD compared to the typical population. Differences were observed in quantitative indicators but also in structure because the typical population children were most sensitive to recognizing sadness, then joy, threat, anger and least sensitive to recognizing fear and command. ADHD children recognized joy best, followed by sadness, fear, command and anger, while threat was the worst. The only emotion in speech that children with ADHD recognized similarly to the typical population children was joy (positive emotion) and there was no significant difference.

Similar results were obtained by Shapiro et al. [38] who used an alternative model of emotion representation, that is, tasks related to recognizing emotions from the face and in prosody. The results of their study showed that children with ADHD were less likely to recognize emotion in prosody and in cross-modal tasks where they were required to match that emotion with the appropriate facial expression. In the face recognition tasks, children with ADHD achieved very similar results to those of the typical population [38]. Corbett and Glidden [39] also found in their study mild to moderate difficulties in recognizing emotions in prosody. Based on such results, many studies supported the thesis that the right cerebral hemisphere correlates with the emotion recognition deficit [39–41].

Children with ADHD were significantly more likely to recognize joy over all other emotions and in relation to threat and command. A significant difference was still present in the better recognition of the fear and sadness in relation to the threat. In the typical population, a significant difference appeared only in the better recognition of sadness in relation to the command, while all other emotions and affective attitude threat were equally well recognized. Most studies that dealt with emotion recognition support the result of this research, which indicates that positive emotions are better recognized than negative ones in children with ADHD [17, 23, 42]. Some authors explain this difference in recognition of different emotions by deficits in verbal and nonverbal attention, which may contribute to incorrect or incompletely processed labels of speech stimuli. Based on their opinion, children with ADHD generally pay attention to the most prominent features of speech impulse from the environment, while subtle information does not come into their focus and thus remain unrecognized [39].

However, the question arises as to why children with ADHD successfully recognize joy (positive emotion) rather than, for example, anger (negative emotion), although both emotions are strong and usually emphasized through speech prosody. These results cannot be fully explained by attention deficit such as brevity, selectivity, poor focus etc. Consideration should also be given to the possible altered patterns and mechanisms of processing the emotional cues in speech primarily due to the lack of recognition of its own anger and fear [43] or as a result of unconsciously ignoring such emotions. This interpretation can be substantiated by the result that children with ADHD were the least sensitive in recognizing anger, and threats, which, despite carrying a strong message, remain poorly recognized.

Interestingly, emotions were better recognized than affective attitudes in both groups of children. This is important information for all significant persons from the child's environment: parents, educators, teachers as well as professionals involved in the treatment and rehabilitation of children with ADHD. Affective attitudes are usually used to warn of the danger, inhibition and interruption or to control children's negative behavior. The command and threat are clearly not adequate speech patterns through which the child can recognize the information conveyed to them from the environment. This is an important finding that needs to be considered in an educational and therapeutic context when it comes to determining approaches for working with this population of children.

The association between age and recognition of emotion and affective attitudes was much more pronounced in the typical population than in children with ADHD. In this study, growing up has been shown to improve overall outcomes, overall affective attitudes, and command recognition. However, this influence was not recorded on the indi-

vidual emotion recognition results as in the typical population. This means that with age, children with ADHD have not made significant progress in recognizing individual emotions in speech, but significant improvement has been observed in recognizing threat and command. Over the years, children seem to learn patterns of behavior that stem from command and threats, but they still do not rely on the truly recognized and experienced emotions that underlie those affective attitudes (such as anger, fear). It would be very interesting to focus future research on exploring the relationship between primary emotions and affective attitudes, as well as exploring mechanisms for recognizing and understanding affective attitudes in children with ADHD.

The limitations of this research are related to a relatively small sample, especially when it comes to the population of girls. Also, a dilemma was raised regarding the language corpus, which was filmed with actors and objectively represented the played emotional roles. It is certain that real situations would give a better emotional expression, but this is questionable approach since it touches the intimacy of the people who would be filmed.

Conclusion

Hyperactivity, impulsivity, and attention deficit disorder are certainly key factors contributing to the difficulties of children with ADHD. However, the results of this study suggest that deficits in understanding the emotional information may also be another critical factor affecting the problems that occur in the daily functioning of children with ADHD. One consequence of these difficulties is the avoidance or inappropriate response to social situations that require recognition of emotional information. Also, understanding and accepting the fact that children with ADHD have objective impediments to the processing of emotional voice messages and affective orders will contribute to better acceptance of these children by the loved ones and the social environment, as well as by the professionals involved in the treatment and education of these children.

Conflict of interest

None.

R E F E R E N C E S

1. *American Psychiatric Association.* Diagnostic and Statistical Manual of Mental Disorders - DSM-IV-TR. New York: American Psychiatric Publishing, 2000. Available from: https://dsm.psychiatryonline.org/doi/book/10.1176/appi.books.9781585629992
2. *Young S, Sedgwic O, Fridman M, Gudjonsson G, Hodgkins P, Lantigua M, González R.* Co-morbid psychiatric disorders among incarcerated ADHD populations: a meta-analysis. Psychol Med 2015; 45(12): 2499−510.
3. *Schachar R, Taylor E, Wieselberg M, Thorley G, Rutter M.* Changes in family function and relationships in children who respond to methylphenidate. J Am Acad Child Adolesc Psychiatry 1987; 26(5): 728−32.
4. *Greene RW, Biederman J, Faraone SV, Monuteaux MC, Mick E, DuPre EP,* et al. Social impairment in girls with ADHD: patterns, gender comparisons, and correlates. J Am Acad Child Adolesc Psychiatry 2001; 40(6): 704−10.
5. *Loe IM, Feldman HM.* Academic and educational outcomes of children with ADHD. J Pediatr Psychol 2007; 32(6): 643−54.
6. *Daley D, Birchwood J.* ADHD and academic performance: why does ADHD impact on academic performance and what can be done to support ADHD children in the classroom? Child Care Health Dev 2010; 36(4): 455−64.
7. *Polanczyk G, De Lima MS, Horta BL, Biederman J, Rohde LA.* The worldwide prevalence of ADHD: a systematic review and metaregression analysis. Am J Psychiatry 2007; 164(6): 942−8.
8. *Willcutt EG.* The prevalence of DSM-IV attention-deficit/hyperactivity disorder: a meta-analytic review. Neurotherapeutics 2012; 9(3): 490−9.
9. *Mill J, Petronis A.* Pre-and peri-natal environmental risks for attention-deficit hyperactivity disorder (ADHD): The potential role of epigenetic processes in mediating susceptibility. J Child Psychol Psychiatry 2008; 49(10): 1020−30.
10. *Banerjee TD, Middleton F, Faraone SV.* Environmental risk factors for attention-deficit hyperactivity disorder. Acta Paediatr 2007; 96(9): 1269−74.
11. *Audet K, Le Mare L.* Mitigating effects of the adoptive caregiving environment on inattention/overactivity in children adopted from Romanian orphanages. Int J Behav Dev 2011; 35(2): 107−15.
12. *Crittenden PM, Kulbotton GR.* Familial contributions to ADHD: An attachment perspective. Tidsskr Nor Psykologoren 2007; 44(10): 1220−9.
13. *Dallos R, Smart C.* An exploration of family dynamics and attachment strategies in a family with ADHD/conduct problems. Clin Child Psychol Psychiatry 2011; 16(4): 535−50.
14. *Swanson JM, Kinsbourne M, Nigg J, Lanphear B, Stefanatos GA, Volkow N et al.* Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. Neuropsychol Rev 2007; 17(1): 39−59.
15. *Aboitiz F, Ossandón T, Zamorano F, Palma B, Carrasco X.* Irrelevant stimulus processing in ADHD: catecholamine dynamics and attentional networks. Front Psychol 2014; 5: 183.
16. *Landau S, Milich R.* Social communication patterns of attention-deficit disorder boys. J Abnorm Child Psychol 1988; 16(1): 69–81.
17. *Aspan N, Bozsik C, Gadoros J, Nagy P, Inantsy-Pap J, Vida P,* et al. Emotion Recognition Pattern in Adolescent Boys with Attention-Deficit/Hyperactivity Disorder. Biomed Res Int 2014; 2014: e761340.
18. *Aduen PA, Day TN, Kofler MJ, Harmon SL, Wells EL, Sarver DE.* Social Problems in ADHD: Is it a Skills Acquisition or Performance Problem? J Psychopathol Behav Assess 2018; 40(3): 440−51.
19. *Nijmeijer JS, Minderaa RB, Buitelaar JK, Mulligan A, Hartman CA, Hoekstra PJ.* Attention-deficit/hyperactivity disorder and social dysfunctioning. Clin Psychol Rev 2008; 28(4): 692−708.
20. *Uekermann J, Kraemer M, Abdel-Hamid M, Schimmelmann BG, Hebebrand J, Daum I,* et al. Social cognition in attention-deficit hyperactivity disorder (ADHD). Neurosci Biobehav Rev 2010; 34(5): 734−43.

21. *Nixon E.* The social competence of children with attention deficit hyperactivity disorder: A review of the literature. J Child Psychol Psychiatry 2001; 6(4): 172–80.
22. *Lindner JL, Rosén LA.* Decoding of emotion through facial expression, prosody and verbal content in children and adolescents with Asperger's syndrome. J Autism Dev Disord 2006; 36(6): 769−77.
23. *Pelc K, Kornreich C, Foisy ML, Dan B.* Recognition of emotional facial expressions in attention-deficit hyperactivity disorder. Pediatr Neurol 2006; 35(2): 93−7.
24. *Kats-Gold I, Besser A, Priel B.* The role of simple emotion recognition skills among school aged boys at risk of ADHD. J Abnorm Child Psychol 2007; 35(3): 363−78.
25. *Cadesky EB, Mota VL, Schachar RJ.* Beyond words: how do children with ADHD and/or conduct problems process nonverbal information about affect? J Am Acad Child Adolesc Psychiatry 2000; 39(9): 1160−7.
26. *Yuill N, Lyon J.* Selective difficulty in recognising facial expressions of emotion in boys with ADHD. Eur Child Adolesc Psychiatry 2007; 16(6): 398−404.
27. *Casey RJ.* Emotional competence in children with externalizing and internalizing disorders. In: *Lewis M, Sullivan MW* editors. Emotional development in atypical children. New York, London: Taylor & Francis; 1996; p. 161−83.
28. *Moses LJ, Baldwin DA, Rosicky JG, Tidball G.* Evidence for referential understanding in the emotions domain at twelve and eighteen months. Child Dev 2001; 72(3): 718−35.
29. *Parise E, Cleveland A, Costabile A, Striano T.* Influence of vocal cues on learning about objects in joint attention contexts. Infant Behav Dev 2007; 30(2): 380−4.
30. *Vaish A, Striano T.* Is visual reference necessary? Contributions of facial versus vocal cues in 12-month-olds' social referencing behavior. Dev Sci 2004; 7(3): 261−9.
31. *Morton JB, Trehub SE.* Children's understanding of emotion in speech. Child Dev 2001; 72(3): 834−43.
32. *Sauter DA, Panattoni C, Happé F.* Children's recognition of emotions from vocal cues. Brit J Dev Psychol 2013; 31(1): 97−113.
33. *Bugental DE, Kaswan JW, Love LR, Fox MN.* Child versus adult perception of evaluative messages in verbal, vocal, and visual channels. Dev Psychol 1970; 2(3): 367−75.
34. *Mumme DL, Fernald A, Herrera C.* Infants' responses to facial and vocal emotional signals in a social referencing paradigm. Child Dev 1996; 67(6): 3219−37.
35. *Fernald A.* Approval and disapproval: Infant responsiveness to vocal affect in familiar and unfamiliar languages. Child Dev 1993; 64(3): 657−74.
36. *Walker-Andrews AS.* Infants' perception of expressive behaviors: differentiation of multimodal information. Psychol Bull 1997; 121(3): 437−56.
37. *Jovičić S, Kašić Z, Đorđević M, Vojnović M, Rajković M, Savković J.* Corpus creating of speech expression ofemotions and attitudes in serbian language. In: *Sovilj M,* editor. Speech and language. Beograd: IEFPG; 2004; p. 36−62. (Serbian)
38. *Shapiro EG, Hughes SJ, August GJ, Bloomquist ML.* Processing of emotional information in children with attention-deficit hyperactivity disorder. Dev Neuropsychol 1993; 9(3−4): 207−24.
39. *Corbett B, Glidden H.* Processing affective stimuli in children with attention-deficit hyperactivity disorder. Child Neuropsychol 2000; 6(2): 144−55.
40. *Ahern GL, Schomer DL, Kleefield J, Blume H, Cosgrove GR, Weintraub S,* et al. Right hemisphere advantage for evaluating emotional facial expressions. Cortex 1991; 27(2): 193−202.
41. *George MS, Parekh PI, Rosinsky N, Ketter TA, Kimbrell TA, Heilman KM,* et al. Understanding emotional prosody activates right hemisphere regions. Arch Neurol 1996; 53(7): 665−70.
42. *Da Fonseca D, Seguier V, Santos A, Poinso F, Deruelle C.* Emotion understanding in children with ADHD. Child Psychiatry Hum Dev 2009; 40(1): 111−21.
43. *Karnik NS.* Categories of control: Foster children and ADHD. Child Youth Serv Rev 2001; 23(9−10): 761−80.

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Application of two types of suspensory fixation in reconstruction of anterior cruciate ligament with a semitendinosus-gracilis graft – A randomized prospective study

Primena dva tipa suspenzione fiksacije kod rekonstrukcije prednje ukrštene veze semitendinozus-gracilis graftom – randomizirana prospektivna studija

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Abstract

Background/Aim. Injury to the anterior cruciate ligament (ACL) of the knee is the most common ligament injury that requires operative treatment. So far, multiple ACL reconstruction (ACLR) techniques using a variety of graft types and implants that fixate the grafts have been described. The aim of the study was to compare two different ACLR techniques using two implant types for suspensory graft fixation in the femoral tunnel. **Methods.** This randomized-prospective study encompassed 60 patients/subjects who underwent ACLR in the period between January 2015 and December 2017 at the Department of Orthopaedics and Traumatology of Military Hospital "Dr Vladan Djordjević" Niš. The ACLR in all patients included in the study was performed using a quadruple semitendinosus-gracilis (STG) graft with two types of suspensory fixation on the lateral femoral cortex, whereas the graft fixation in the tibial tunnel was performed using an osteoconductive bioresorbable screw. The post-operative knee stability was assessed 24 months after surgery using the Lachman test and the lateral pivot shift test, as well as the KT-1000 arthrometer test. **Results.** In patients whose graft was fixated using a fixed-length loop implant, the mean post-surgery knee stability, measured with the KT-1000, was 1.167 ± 0.780; in patients whose graft was fixated using an adjustable-length loop implant, the mean value of the KT-1000 was 1.100 ± 0.894 (p = 0.605). The mean post-surgery International Knee Documentation Committee (IKDC) score for the fixed-length loop group was 84.887 ± 9.0207, while for the adjustable-length loop the score was 88.327 ± 7.302. The mean Lysholm score was 93.50 ± 6.872 for the fixed-length loop group of patients and 94.00 ± 5.527 for the adjustable-length loop group of patients. **Conclusion.** Both types of implants can be used with success during ACLR, because the functional results of operative treatment using both implants were identical after surgery.

Key words:
anterior cruciate ligament; anterior cruciate ligament reconstruction; orthopedic procedures; treatment outcome; transplants.

Apstrakt

Uvod/Cilj. Oštećenje prednje ukrštene veze kolena je najčešća povreda ligamenata koje zahteva operativno lečenje. Do sada je opisano više tehnika rekonstrukcije prednje ukrštene veze raznim tipovima graftova i implantanata kojima se graftovi fiksiraju. Cilj rada bio je da se uporede dve različite tenike rekonstrukcije prednje ukrštene veze korišćenjem dva tipa implantata za suspenzionu fiksaciju grafta u femoralnom tunelu. **Metode.** Ovom randomizovanom, prospektivnom studijom obuhvaćeno je 60 pacijenata podvrgnutih rekonstrukciji prednje ukrštene veze u periodu januar 2015–decembar 2017. godine, na Odeljenju za ortopediju i traumatolgiju Vojne bolnice "Doktor Vladan Đorđević" u Nišu. Jednoj polovini pacijenata urađena je fiksacija grafta u femoralnom tunelu implantatom sa fiksnom, a drugoj polovini sa promenljivom dužinom omče. Postoperativna stabilnost kolena procenjivana je 24 meseci posle operativnog zahvata Lachman, Lateral Pivot Shift testom, kao i merenjem artrometrom KT 1000. **Rezultati.** Kod pacijenata kojima je izvršena fiksacija grafta implantatom sa fiksnom dužinom omče srednja vrednost stabilnosti kolena posle operativnog zahvata merena artrometrom KT 1000 iznosila je 1,167 ± 0,780, dok je kod pacijenata sa varijabilnom dužinom omče ista iznosila 1,100 ± 0,894 (p = 0,605). Srednja vrednost *International Knee Documentation*

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Committee (IKDC) skora postoperativno za grupu sa fiksnom omčom iznosila je 84,887 ± 9,0207, a kod onih sa promenljivom omčom 88,327 ± 7,302. Srednja vrednost Lysholm skora za grupu pacijenata sa fiksnom omčom je bila 93,50 ± 6,872, a za grupu sa promenljivom dužinom omče 94,00 ± 5,527. **Zaključak.** Oba implantata se mogu uspešno koristiti prilikom rekonstrukcije prednje ukrštene veze jer su funkcinalni rezultati operativnog lečenja uz njihovo korišćenje pokazala identičan postoperativni rezultat.

Ključne reči:
ligament, prednji, ukršteni; rekonstrukcija; ortopedske procedure; lečenje, ishod; graftovi.

Introduction

Injury to the anterior cruciate ligament (ACL) of the knee is the most common ligament injury that requires operative treatment. It is usually a non-contact injury, occurring due to dynamic knee valgus [1]. Untreated injuries can progress to early osteoarthrosis, which is why prompt and proper treatment is required [2]. Patient treatment can be nonoperative and operative. Nonoperative treatment is prescribed for patients who do not have demanding functional requirements and who are prepared to accept certain functional limitations [3]. The goals of operative treatment are to achieve complete functional recovery of the injured knee and to reduce the risk of early osteoarthrosis and damage of other knee structures [4]. So far, multiple ACL reconstruction (ACLR) techniques using a variety of graft types and implants that fixate the grafts have been described. The functional result of treatment depends on graft type and quality, tunnel position, and graft fixation stability [5, 6]. Since 1995, titanium buttons with a loop have been used as implants for suspensory fixation of the graft in the femoral tunnel during ACLR. There are two types of titanium buttons in use: one with a fixed-length loop and the other with an intraoperative adjustable-length loop [7].

The aim of the study was to compare two different techniques of ACLR using two types of implants for suspensory fixation of the semitendinosus-gracilis (STG) graft in the femoral tunnel.

Methods

This randomized-prospective study encompassed 60 patients who underwent ACLR in the period between January 2015 and December 2017 at the Department of Orthopaedics and Traumatology of Military Hospital "Dr. Vladan Djordjević" Niš. The ACLR in all patients included in the study was performed using a quadruple STG graft with two types of suspensory fixation on the lateral femoral cortex, whereas the graft fixation in the tibial tunnel was performed using an osteoconductive bioresorbable screw. One half of the patients had their femoral tunnel graft fixated using an implant with adjustable-length loop, while the other half had their graft fixated with a adjustable-length loop implant (Figure 1). The choice of implant for the purpose of graft fixation in the femoral tunnel, with either a fixed-length or an adjustable-length loop, was made based on randomization using a table generated by a random number by means of Stat Trek random number generator [8]. Only the scrub nurse was familiar with the randomized list. On the day of each surgery, she would inform the surgeon about which implant for graft fixation in the femoral tunnel should be used according to the randomized table. The fixed-length loop implant used was the VersiTomic G-Lok (Stryker, Kalamazoo, Michigan, USA), while the adjustable-length loop used was the ACL TightRope RT (Arthrex, Naples, Florida, USA). The anatomic ACLR in each patient from both groups was performed identically, by creating three portals, and the graft fixation in the tibial tunnel was performed by means of a bioresorbable screw. The femoral portion of the graft was 25 mm long for both groups of subjects, whereas the graft socket length was determined differently for each group [9].

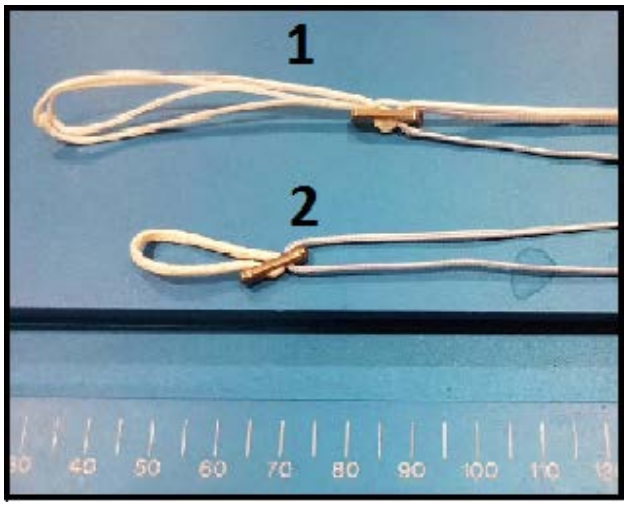

Fig. 1 – Femoral cortical suspension devices:
1) Adjustable-length loop device;
2) Fixed-length loop device.

The length of the femoral graft socket in the femoral tunnel during fixation with a titanium button with a fixed-length loop was determined according to the following formula: planned graft length in the femoral tunnel + 10 mm. Implant loop length was determined according to the formula: total tunnel length (TTL) – socket length (SL). The first loop that was longer than the value obtained by applying the formula was used to fixate the graft (Figure 2).

In titanium implants with an adjustable-length loop, the femoral tunnel length was fixed at 27 mm. The implant was introduced up to 25 mm, while 2 mm were left for additional graft tensioning after fixation in the tibial tunnel. The tunnel position was verified postoperatively through X-ray imaging, which encompassed knee images, tunnel images during a 40-degree knee flexion, and a lateral image of a fully extended knee. The femoral tunnel position was determined according

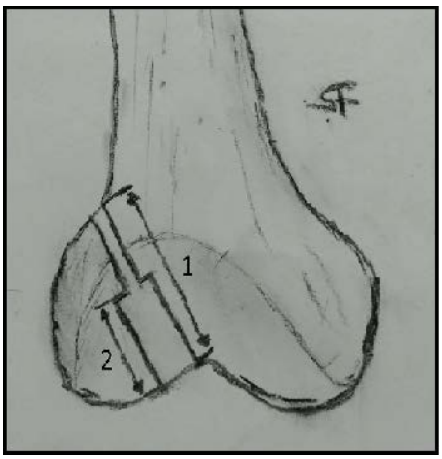

Fig. 2 – Calculation for femoral cortical suspension devices with fixed-loop length: 1) Total tunnel length (TTL); 2) Socket length (SL). Loop length = TTL−SL (use first larger size of loop).

to the method by Sommer et al. [10], who designed the ACL ruler for use in clinical practice. The ruler consists of a template for femoral tunnel verification in anterior-posterior (AP) and lateral knee X-rays. The lateral X-ray is used to identify and draw the Blumensaat line (B-line) and the line that cuts it perpendicularly and is drawn over the final curve of the intercondylar notch roof. The ruler is placed on the lateral X-ray such that the horizontal line follows the Blumensaat line and the ruler marker follows the perpendicular line, after which the values from the schematic representation on the ruler are read (Figure 3). To assess the femoral tunnel position in the frontal plane, another part of the ruler is placed over the AP radiograph. It is necessary to position the template horizontally by placing the ruler circle over the middle of the notch and then to read the tunnel position, which is schematically divided into four types (Figure 4).

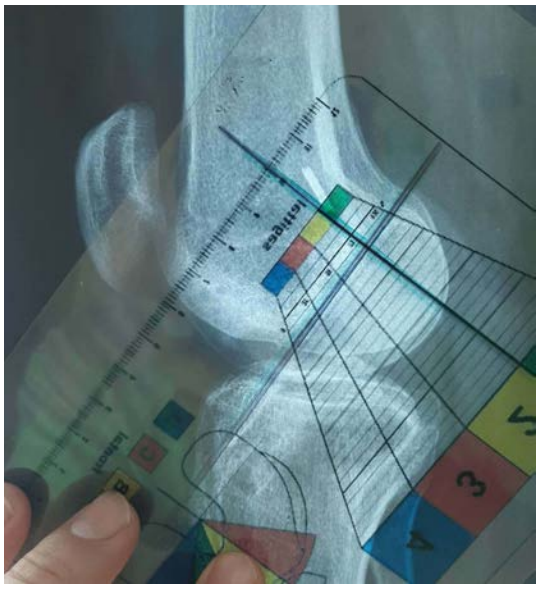

Fig. 3 – Femoral tunnel placement measurement on the lateral plane with anterior cruciate ligament (ACL) ruler.

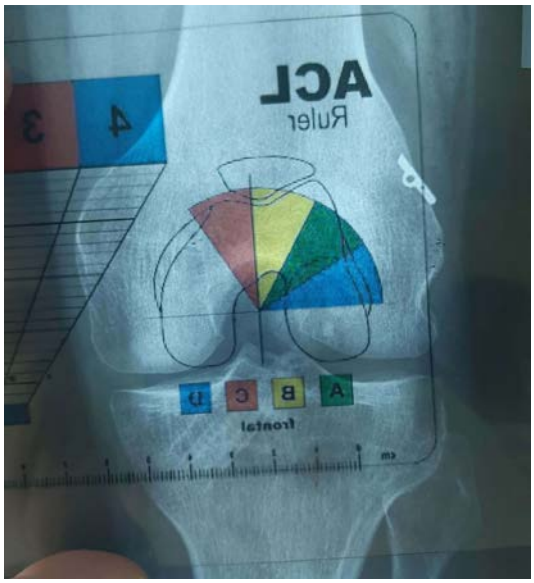

Fig. 4 – Femoral tunnel placement measurement on the anterior-posterior plane with a anterior cruciate ligament (ACL) ruler.

Tunnel position in the tibia was determined in relation to the M-point in the central plane (Figure 5) and in relation to the B-line during full knee extension (Figure 6). The M-point is the cross point between the vertical line that starts from the medial intercondylar tubercle and the horizontal joint line of the tibia. The tunnel position on the AP X-ray is divided into three types: Type A – medial position of the tunnel in relation to the M-point; Type B – the tunnel passes through the M-point; and Type C – lateral position of the tunnel in relation to the M-point [11].

The B-line is a straight line drawn through the roof of the femoral intercondylar notch on the lateral X-ray. The tunnel position was classified into four types: Type I – the tibial tunnel was entirely placed in front of the B-line; Type II – the tibial tunnel axis was anterior to the B-line; Type III – the tibial tunnel axis was behind the B-line; and Type IV –

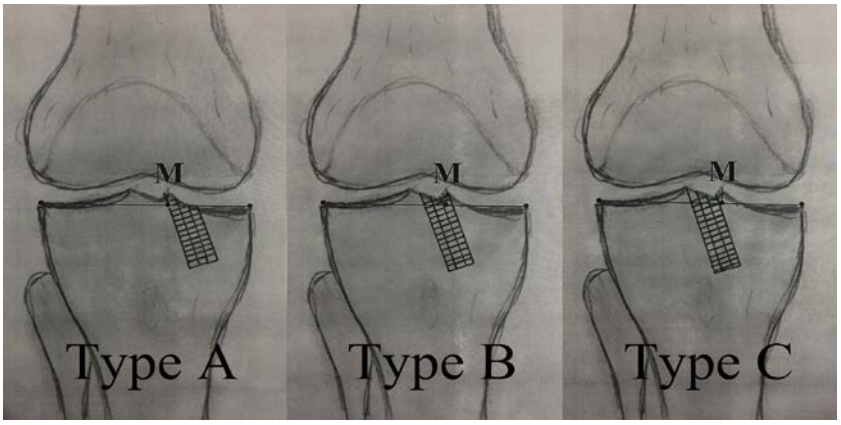

Fig. 5 – Types of tibial tunnel position in the frontal plane in relation to the M-point.

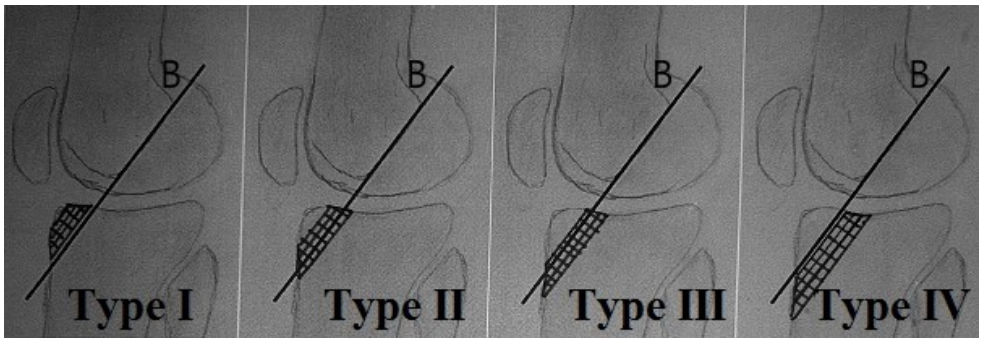

Fig. 6 – Type of tibial tunnel position in the sagittal plane in relation to the B-line.

the tibial tunnel was entirely placed behind the B-line (Figure 6).

All patients included in the study had identical tunnel positions. The following criteria needed to be met for inclusion in the study: patients with a unilateral lesion of the knee ACL that occurred no more than 10 months prior to reconstruction; with or without a minor lesion of the medial or lateral meniscus (up to 50% of the surface); without arthrotic changes and neuromuscular diseases; with 1A femoral tunnel position, with 4B tibial tunnel position; with a willingness to participate in the study and to adhere to the rules for clinical and functional evaluation and for rehabilitation.

The postoperative rehabilitation treatment was conducted according to a previously devised plan and program, and it began the first day after surgery with certain limitations. The most notable limitations include not allowing the patients to lean on the operated leg one month after the surgery and allowing them to run only in a straight-line three months after and with direction change six months after the surgery. Complete return to the preinjury physical activities was allowed nine months after the surgery, while the functional knee testing was performed 24 months after the surgery.

Both groups of patients had identical grafts, tibial fixation, tunnel position, and postoperative rehabilitation treatment, the only difference being the manner of fixation in the femoral tunnel. There were no reports of postoperative complications among patients included in our study.

A special form to be filled out was designed for the purpose of this study. One portion of the form, pertaining to sociodemographic characteristics, was filled out by the patients themselves. For the purpose of a more reliable analysis of the functional results, the patients also filled out authorized scoring tools for the functional assessment of the knee: the International Knee Documentation Committee (IKDC) Subjective Knee Evaluation Form and the Lysholm Knee Scoring Scale. The other portion of the form was filled out by a given physician based on the interview with the patient and the physical examination. KT-1000 arthrometer (MEDmetric, San Diego, California, USA) was used to measure objective knee stability and the results were then written in the form.

The Mann-Whitney *U* test, the Fisher's exact test, and the *t*-test were used for statistical analysis of the results. All the results were statistically processed by means of SPSS software (SPSS for Windows release 12.0; IBM Corp). The values of $p < 0.05$ were considered statistically significant.

Results

The sociodemographic characteristics of the patients included in the study, shown in Table 1, indicate that there were no statistically significant differences between the two groups of patients with regard to sex, age, left/right leg, and the time from injury to ACLR surgery.

Table 1

Sociodemographic characteristics of the patients underwent to anterior cruciate ligament construction (ACLR)

Parameter	Fixed loop (n = 30)	Adjustable loop (n = 30)	*p*
Age (years), mean ± SD	27.87 ± 6.902	26.87 ± 6.388	0.563[1]
Sex (male/female), n	26/4	27/3	1.00[2]
Side (left/right), n	12/18	14/16	1.00[2]
Time from injury to ACLR (months), mean	4.173	4.7	1.00[2]

SD – standard deviation;
[1]Mann-Whitney *U* test; [2]Fisher's Exact Test.

Table 2

Postoperative knee stability and functional results for both group of patients

Parameter	Fixed loop (n = 30)	Adjustable loop (n = 30)	*p*
KT-1000 arthrometer* measurement (mm), mean ± SD	1.167 ± 0.781	1.100 ± 0.894	0.605[1]
Lysholm Score, mean ± SD	93.50 ± 6.872	94.00 ± 5.527	0.994[1]
2000 IKDC score, mean ± SD	84.887 ± 9.021	88.327 ± 7.303	0.123[1]
Lachman test, n (%)			1.00[2]
stable	23 (76.66)	25 (83.33)	
gradus 1	7 (23.34)	5 (16.67)	
gradus 2	0 (0)	0 (0)	
Pivot-shift test, n (%)			1.00[2]
stable	28 (93.33)	30 (100)	
gradus 1	1 (3.33)	0 (0)	
gradus 2	1 (3.33)	0 (0)	

***KT-1000 knee ligament artrometer (MEDmetric, San Diego, California); IKDC – International Knee Documentation Committee.**
[1]Mann-Whitney *U* test; [2]Fisher's Exact Test.

Table 2 shows the postoperative knee stability and functional results for both groups of patients, again with no established statistically significant differences between the groups. For the subjects whose graft was fixated with a fixed-length loop implant, the mean value of postoperative knee stability measured using the KT-1000 arthrometer was 1.167 ± 0.780, whereas the same value for the subjects with adjustable-length loop was 1.100 ± 0.894. The mean value of the postoperative IKDC score was 84.887 ± 9.0207 for the fixed-loop group and 88.327 ±7.302 for the adjustable-loop group. The mean Lysholm score was 93.50 ± 6.872 for the fixed-loop group and 94.00 ± 5.527 for the adjustable-loop group.

Discussion

There have been numerous studies attempting to determine which graft type and fixation method is the best, but there is yet to be a consensus among researchers, which is why there is still a broad range of options when choosing a suitable graft type and fixation method [12]. The time that has passed from injury to the ACLR surgery is a factor that significantly impacts the postoperative result [13].

Shelbourne et al. [14] state that at least three weeks should pass between the injury and ACLR to reduce the risk of arthrofibrosis. On the other hand, Bottoni et al. [15] believe that satisfactory clinical results can also be achieved if the ACLR is performed soon after the injury, although they do not claim that all reconstructions should be performed in the acute stage. There is currently no consensus about how much time needs to pass between the injury and the ACLR nor about when the ACLR should be considered "early" and when "delayed" [16]. There is also no consensus about the definition of early and delayed ACLR. Meighan et al. [17] define early ACLR as one performed within two weeks from injury, whereas Hur et al. [18] define it as one performed within the first three weeks from injury. For Church and Keating [19], however, early ACLR is performed up to 12 months from injury, and they believe that this is the optimal period to perform the surgery. The mean time from injury to ACLR in the present study was different between the two considered groups: 4.125 months for the fixed-loop group and 4.7 months for the adjustable-loop group. The surgery was performed after at least one month after injury for both groups, and the longest periods between injury and surgery were 9 months in the fixed-loop group and 10 months in the adjustable-loop group.

Investigation of the preferences regarding graft type and surgical technique used by the surgeons from the Magellan Society revealed that they most often choose STG graft (58%) for primary ACLR, form the tunnel anatomically (62%), prevalently as a single bundle (75%), and use suspensory fixation as the graft fixation method (52%) [20]. This corresponds to the surgical technique as well as graft and implant selection discussed in the present study. Pokharel et al. [21] as well as Boyle et al. [22], independently compared the

treatment outcome for two groups of patients who had undergone ACLR with fixed- and adjustable-length loop implants. They concluded that both patient groups showed significant improvements in their functional results after the surgery without any statistically significant differences between the groups. The analysis of the results obtained in the present study led to the same conclusion: there was a significant improvement in the functional results across both groups and there was no statistically significant difference regarding knee stability and functional results between the two groups.

Similarly, Mariscalco et al. [9] also did not find any statistically significant differences in functional results between the group of patients who underwent ACLR with graft length in the femoral tunnel less than 25 mm and the group whose graft length in the femoral tunnel was 25 mm or longer. In the present study, the minimum intratunnel graft length in the femoral tunnel for the ACLR was 25 mm.

Standard suspensory technique of titanium button fixation with a fixed-length loop requires a specific tunnel length that exceeds the length of the intratunnel portion of the graft [23, 24]. The extra space is necessary for the button to go through the entire tunnel and back in order to fixate the graft; however, it also creates conditions for the so-called "bungee cord" effect, which is considered to be a major cause of graft tunnel dilatation and graft loosening, which in turn diminishes the functional result. With the said implant, there is no compression to the tunnel walls, which negatively affects both the primary tightness of fixation and the biological incorporation of the graft. In contrast to the fixed-length loop implant, a new generation implant with intraoperatively adjustable loop length potentially resolves the aforementioned issues by completely filling the formed femoral tunnel with the graft, which provides compression to all tunnel walls including the tunnel roof, leaving no empty space to be filled with synovial fluid and ultimately enabling a faster and more secure graft incorporation [25–27]. In addition, when the entire tunnel length is filled by the graft, the graft-tunnel interface increases, which in turn increases the surface area of collagen that anchors the graft in the tunnel, thus reducing the probability of graft slippage – this directly impacts later functional result [28]. In the present authors' opinion, this is one of the more relevant issues, which requires further investigation. It was not possible to conduct postoperative multislice computed tomography (MSCT) monitoring of the patients included in this study, but they nevertheless showed no clinical signs of graft loosening. The patients were monitored clinically 24 months after the surgery.

Eguchi et al. [29] suspect that when implants with an adjustable-length loop are used, the loop can loosen postoperatively, which could later cause graft loosening and consequently diminish functional result. This postoperative complication was not registered in the present study. As opposed to Eguchi et al. [29], Smith et al. [30] conducted a controlled *in-vitro* biomechanical study comparing multiple types of implants and concluded that the initial strength and elongation of implants with a fixed-length and adjustable-length loops are equal. Wise et al. [28] also assessed the results of ACLR with fixed- and adjustable-length loops. Their study showed that the clinical laxity, or the measure of anterior tibial translation in the injured knee obtained from the KT-1000, which was 3 mm larger than in a healthy knee, was found in 6.1% of the adjustable-loop patients and in 12.5% of the fixed-loop patients. The present study did not include any patient with a postoperative laxity larger than 3 mm compared to a healthy knee.

Based on the results from numerous studies, including the present one, it can be concluded that an implant with an intraoperatively adjustable loop length is more advantageous than the fixed-length loop device, as it provides more freedom to the surgeon to form the femoral tunnel by eliminating the need to calculate the length of the femoral tunnel and the loop [12]. Furthermore, implants with adjustable-length loops enable intraoperative graft retensioning after fixation in the tibial tunnel, which allows poor graft tension to be corrected [27].

Accordingly, in addition to making a decision on which graft type and tunnel position to choose, the surgeon can also control graft tension during the entire surgical procedure and thus put the entire preoperative plan into effect.

Limitations of the study

The minimum postoperative time for patient monitoring in this study was only two years. Knee computed tomography was not performed, even though it is the most reliable method for assessing tunnel dilatation, because the rules of the healthcare institution where the study was conducted prohibit this diagnostic method for postoperative monitoring of patients who do not suffer from any other health issues.

Conclusion

Based on the results obtained in the course of the presented study, it can be concluded that both types of implants discussed can be used with success in ACLR, because the functional results after operative treatment of ACLR with both implants were identical. After knee stability measurements and the assessment of functional results by means of scores and tests, the study did not establish any statistically significant differences in the results of anterior crucial ligament reconstruction between the patients with fixed-length loop and those with adjustable-length loop titanium implants. This study focused on knee stability assessment after anterior crucial ligament reconstruction using two different implant types, but there is ample room for further research in terms of the stability of the implant itself and tunnel dilatation, which can be conducted with the aid of additional diagnostic methods and over a longer monitoring period.

Conflict of interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial, non-financial interest in the subject matter or materials discussed in this manuscript.

R E F E R E N C E S

1. *Kobayashi H, Kanamura T, Koshida S, Miyashita K, Okado T, Shimizu T, Yokoe K,* et al. Mechanisms of the Anterior Cruciate Ligament Injury in Sports Activities: A Twenty-Year Clinical Research of 1,700 Athletes. J Sport Sci Med 2010; 9(4): 669−75.
2. *Papathanasiou I, Michalitsis S, Hantes ME, Vlychou M, Anastasopoulou L, Malizos KN,* et al. Molecular changes indicative of cartilage degeneration and osteoarthritis development in patients with anterior cruciate ligament injury. BMC Musculoskelet Disord 2016; 17: 21.
3. *Grindem H, Logerstedt D, Eitzen I, Moksnes H, Axe MJ, Snyder-Mackler L,* et al. Single-legged hop tests as predictors of self-reported knee function in non-operatively treated individuals with ACL injury. Am J Sports Med 2011; 39(11): 2347−54.
4. *Renstron P.* Eight clinical conundrums relating to anterior cruciate ligament (ACL) injury in sports: recent evidence and personal reflections. Br J Sports Med 2013; 47(6): 367−72.
5. *Reed M.* Anterior cruciate ligament reconstruction with patellar or hamstring tendon grafts: A review of the evidence. WLMJ 2009; 1(2): 1−15.
6. *Buller LT, Best MJ, Baraga MG, Kaplan LD.* Trends in Anterior Cruciate Ligament Reconstruction in the United States. Orthop J Sports Med 2015; 3(1): 2325967114563664.
7. *Zeng C, Lei G, Gao S, Luo W.* Methods and devices for graft fixation in anterior cruciate ligament reconstruction (Protocol). Cochrane Database Syst Rev 2018; 2018(6): CD010730.
8. StatTrek.com. Cumulative normal distribution [calculator]. Available at: http://stattrek.com/Tables/Normal.aspx. [accessed 2011 May 13].
9. *Mariscalco MW, Magnussen RA, Mitchell J, Pedroza AD, Jones MH, Andrish JT*, et al. How much hamstring graft needs to be in the femoral tunnel? A MOON cohort study. Eur Orthop Traumatol 2015; 6(1): 9−13.
10. *Sommer C, Friederich NF, Muller W.* Improperly placed anterior cruciate ligament grafts: correlation between radiological parameters and clinical results. Knee Surg Sports Traumatol Arthrosc 2000; 8(4): 207−13.
11. *Muneta T, Yamamoto H, Ishibashi T, Asahina S, Murakami S, Furuya K.* The effects of tibial tunnel placement and roofplasty on reconstructed anterior cruciate ligament knees. Arthroscopy 1995; 11(1): 57−62.
12. *Nag HL, Gupta H.* Seating of tight rope RT button under direct arthroscopic visualization in anterior cruciate ligament reconstruction to prevent potential complications. Arthrosc Tech 2012; 1(1): e 83−5.
13. *Evans S, Shaginaw J, Bartolozzi A.* Acl reconstruction – it's all about timing. Int J Sports Phys Ther 2014; 9(2): 268−73.
14. *Shelbourne KD, Wilckens JH, Mollabashy A, DeCarlo M.* Arthrofibrosis in acute anterior cruciate ligament reconstruction. The effect of timing of reconstruction and rehabilitation. Am J Sports Med 1991; 19(4): 332−6.
15. *Bottoni CR, Liddell TR, Trainor TJ, Freccero DM, Lindell KK.* Postoperative range of motion following anterior cruciate ligament reconstruction using autograft hamstrings: A prospective, randomized clinical trial of early versus delayed reconstructions. Am J Sports Med 2008; 36(4): 656−62.
16. *Smith TO, Davies L, Hing CB.* Early versus delayed surgery for anterior cruciate ligament reconstruction: a systematic review and metaanalysis. Knee Surg Sports Traumatol Arthrosc 2010; 18(3): 304−11.
17. *Meighan AA, Keating JF, Will E.* Outcome after reconstruction of the anterior cruciate ligament in athletic patients. A comparison of early versus delayed surgery. J Bone Joint Surg Br 2003; 85(4): 521−4.
18. *Hur CI, Song EK, Kim SK, Lee SH, Seon JK.* Early anterior cruciate ligament reconstruction can save meniscus without any complications. Indian J Orthop 2017; 51(2): 168–73.
19. *Church S, Keating JF.* Reconstruction of the anterior cruciate ligament: timing of surgery and the incidence of meniscal tears and degenerative change. J Bone Joint Surg Br 2005; 87(12): 1639−42.
20. *Lee YH, Kuroda R, Chan KM.* Anterior Cruciate Ligament Reconstruction: A 2015 global perspective of the Magellan Society. Asia Pac J Sports Med Arthrosc Rehabil Technol 2015; 2(4): 122−8.
21. *Pokharel B, Bhalodia M, Raut A, Gajjar SM.* Comparative study on fixed versus adjustable-length loop device for femoral fixation of graft in anterior cruciate ligament reconstruction. Int J Orthod Sci 2018; 4(1): 889−92.
22. *Boyle MJ, Vovos TJ, Walker CG, Stabile KJ, Roth JM, Garrett WE Jr.* Does adjustable-loop femoral cortical suspension loosen after anterior cruciate ligament reconstruction? A retrospective comparative study. Knee 2015; 22(4): 304−8.
23. *Turnbull TL, LaPrade CM, Smith SD, LaPrade RF, Wijdicks CA.* Dimensional assessment of continuous loop cortical suspension devices and clinical implications for intraoperative button flipping and intratunnel graft length. J Orthop Res 2015; 33(9): 1327–31.
24. *Houck DA, Kraeutler MJ, McCarty EC, Bravman JT.* Fixed- Versus Adjustable-Loop Femoral Cortical Suspension Devices for Anterior Cruciate Ligament Reconstruction: A Systematic Review and Meta-analysis of Biomechanical Studies. Orthop J Sports Med 2018; 6(10): 2325967118801762.
25. *Yoshiya S.* Editorial commentary: Controversy on fixation properties of the adjustable-loop cortical suspension fixation device used for anterior cruciate ligament reconstruction. Arthroscopy 2016; 32(2): 262.
26. *Barrow AE, Pilia M, Guda T, Kadrmas WR, Burns TC.* Femoral suspension devices for anterior cruciate ligament reconstruction: Do adjustable loops lengthen? Am J Sports Med 2014; 42(2): 343−9.
27. *Petre BM, Smith SD, Jansson KS, de Meijer PP, Hackett TR, LaPrade RF,* et al. Femoral cortical suspension devices for soft tissue anterior cruciate ligament reconstruction: a comparative biomechanical study. Am J Sports Med 2013; 41(2): 416−22.
28. *Wise BT, Patel NN, Wier G, Labib SA.* Outcomes of ACL reconstruction with fixed versus variable loop button fixation. Orthopedics 2017; 40(2): e275−80.
29. *Eguchi A, Ochi M, Adachi N, Deie M, Nakamae A, Usman MA.* Mechanical properties of suspensory fixation devices for anterior cruciate ligament reconstruction: Comparison of the fixed-length loop device versus the adjustable-length loop device. Knee 2014; 21(3):743−8.
30. *Smith PA, Piepenbrink M, Smith SK, Bachmaier S, Bedi A, Wijdicks CA.* Adjustable- versus fixed-loop devices for femoral fixation in ACL reconstruction: An in vitro full-construct biomechanical study of surgical technique-based tibial fixation and graft preparation. Orthop J Sports Med 2018; 6(4): 2325967118768743.

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Anatomically high division of sciatic nerve and its clinical significance

Anatomski visoka podela sedalnog živca i njen klinički značaj

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Abstract

Background / Aim. The sciatic nerve (SN) is a mixed nerve formed in pelvis by joining of L4 - S3 anterior spinal nerve roots. SN can be under the pressure in different regions throughout its course. However, the most frequent site of impingement is under the *piriformis* muscle which causes the occurrence of *piriformis* syndrome. High division of SN has its relevance considering the fact that it leads to the compression of nerve resulting in *piriformis* syndrome. The aim of this study was to determine the connection between anatomical parameters of pelvis and high division of SN, which is considered to be one of the most common causes of *piriformis* syndrome in both genders. **Methods.** This study was conducted on 28 formalin fixed cadavers of both genders at the Institute of Anatomy, Faculty of Medicine, University of Belgrade (Belgrade, Serbia) and the Department of Anatomy, School of Medicine, University of St Andrews (St Andrews, United Kingdom). For the measuring of required dimensions, we used a ruler and a caliper. **Results.** A statistically significant difference in the values of bispinal and bituberal lines was observed. A high division of SN was found on 58.33% of the male cadavers and 80% of the female cadavers. A statistically significant difference in the mean value of the bituberal line between the male and female sex was also recognised. **Conclusion**. The connection between the anatomical parameters of the pelvis and the level of division of the SN is confirmed. Although on the basis of the results it could be assumed that people with smaller pelvic dimensions would have greater likelihood of developing a *piriformis* syndrome, the other factors, such as biomechanics related to a wider „Q angle" in women that could result in a higher incidence of *piriformis* syndrome, should also be considered.

Key words:
anatomy, regional; cadaver; piriformis muscle syndrome; sciatic nerve; sex characteristics.

Apstrakt

Uvod/Cilj. Sedalni živac (*n. ischiadicus*) je mešoviti živac koji nastaje u karlici od prednjih grana kičmenih (spinalnih) živaca od L4-S3. Tokom svog puta, n. *ischiadicus* može da bude podložan pritisku u različitim regionima, a naročito u predelu zgloba kuka od strane piriformnog mišića što prouzrokuje simptome piriformnog sindroma. Visoka podela nerva klinički je značajna zbog toga što ona najčešće ima za posledicu kompresiju nerva, odnosno piriformni sindrom. Cilj rada je bio ispitivanje povezanosti anatomskih parametara karlice sa visinom podele *n. ischiadicusa* kod oba pola, uz pretpostavku da ispitivani parametri utiču na samu podelu nerva koja se smatra jednim od glavnih uzročnika piriformnog sindroma. **Metode.** Studija je sprovedena na 28 formalinskih kadavera oba pola na Institutu za anatomiju Medicinskog fakulteta Univerziteta u Beogradu (Beoigrad, Srbija) i Institutu za anatomiju Medicinskog fakulteta Univerziteta u St Andrews-u (St Andrews, Velika Britanija). Za merenje parametara dimenzija karlice korišćeni su lenjir i kaliper **Rezultati.** Uočena je statistički značajna razlika u vrednosti bispinalne i bituberalne linije. Visoka podela je pronađena u 58.33% uzoraka muškog pola i 80% uzoraka ženskog pola. Takođe, ustanovljena je statistički značajna razlika u srednjoj vrednosti bituberalne linije između muškog i ženskog pola. **Zaključak.** Veza između anatomskih parametara karlice sa nivoom podele *n. ischiadicusa* je potvrđena. Iako bi se na osnovu rezultata moglo pretpostaviti da će osobe sa manjim dimenzijama karlice imati veću verovatnoću da se kod njih razvije piriformni sindrom, trebalo bi uzeti u obzir i druge faktore kao što je biomehanika koja je povezana sa širim uglom *m. quadriceps femoris-a* („Q angle") kod žena s posledičnom većom učestalošću piriformnog sindroma.

Ključne reči:
anatomija, regionalna; kadaver; n. ischiadicus; sindrom, piriformni; pol, karakteristike.

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Introduction

Sciatic nerve (SN) is the longest and the widest nerve of the human body. It is a mixed nerve formed in pelvis by joining of L4-S3 anterior spinal nerve roots. At the level of the sacroiliac joint, the SN can be almost 2 cm wide. The main reason for the high division of the SN is its size [1].

The SN exits the pelvic cavity wrapped with a single epineurium through the greater sciatic notch under the *piriformis* muscle. Most often, at the level of superior angle of popliteal cavity, the SN is divided into the terminal branches, medial tibial nerve and lateral common fibular nerve [2]. Once it enters gluteal region through the infrapirifom opening, it moves over the pelvitrochanteric muscles, covered with *gluteus maximus* muscle and soft tissue [3]. The SN then descends between tuberosity of ischium inside, and the greater trochanter of femur, which is outside. In the thigh, the SN is located posterior from the *adductor magnus* muscle and anterior from the long head of *biceps femoris* muscle[3]. In this area, the nerve is particularly vulnerable to injury during the administration of intramuscular injections [4]. Motor branches of the SN are responsible for the innervation of hip and knee joint, while the sensitive branches innervate almost the entire lower leg, with the exception of the anterior inner part of the lower leg and the medial edge of the foot [2]. By achieving a close relationship with the *piriformis* muscle, the SN in gluteal region can cause the occurrence of the "piriformis syndrome" [4]. Because of the common distal attachment with the *piriformis*, the muscle on the greater trochanter of femur, *obturator internus* muscle, as well as *superior* and *inferior gemellus* muscle, have the ability to compensate for the loss of its function [3].

According to the classification of Beaton and Anson [5], anatomical variations of the SN relative to the pelvitrochanteric *piriformis* muscle can be classified into several types: type 1 (undivided nerve below undivided muscle); type 2 (division of the nerve between and below undivided muscle); type 3 (division above and below undivided muscle); type 4 (undivided nerve between heads); type 5 (division between and above heads); type 6 (undivided nerve above undivided muscle) (Figure 1).

Babinski et al. [6] also described the new anatomical variation in which the common fibular nerve extends above and the tibial nerve below, relative to the *superior gemellus* muscle. All these classifications are important in surgery to determine the cause and location of nerve compression and the appropriate treatment. It is believed that divided *piriformis* muscle is an important cause of the piriform syndrome, because it contributes to the compression and irritation of the SN that runs between its fibers [2]. Besides the *piriformis* muscle, it is thought that nerve compression can also be performed by the *obturator internus* muscle (Figure 2), which belongs to the group of external rotators in hip joint, located below the SN [7]. The close contact between the tendon of the *obturator internus* muscle and SN causes similar symptoms, such as those occurring in the piriform syndrome [7].

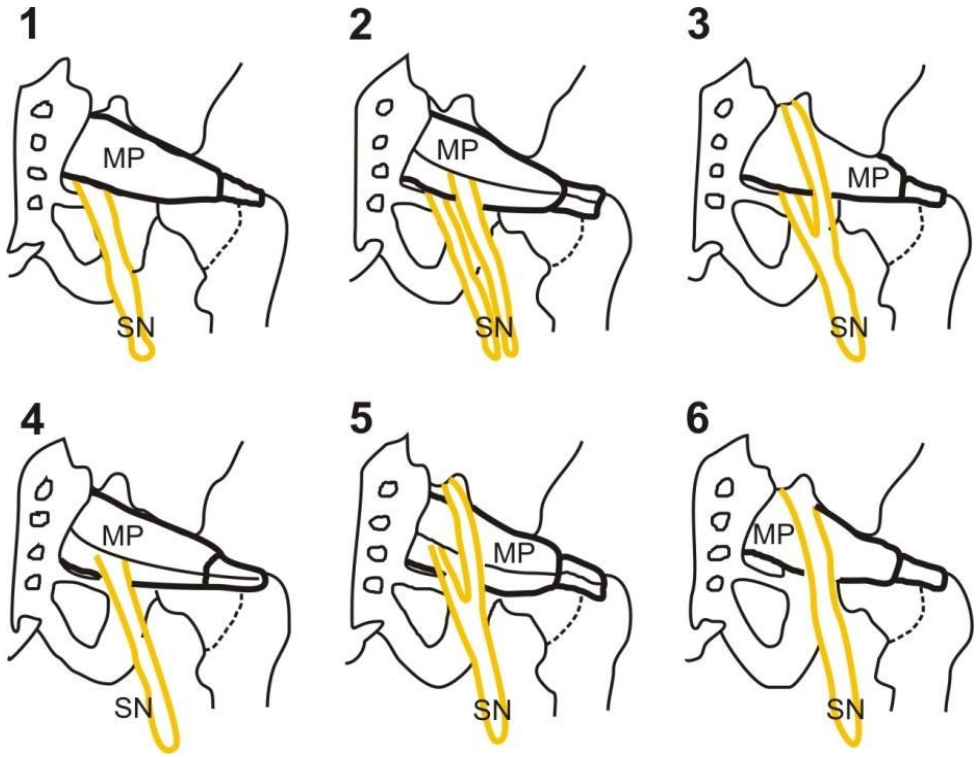

Fig. 1 - Anatomical variations of the sciatic nerve (SN) (Beaton's and Anson's classification [5]).
MP – muscle *piriformis*.
1) Undivided nerve below undivided muscle; 2) Division of nerve between and below undivided muscle; 3) Division above and below undivided muscle; 4) Undivided nerve between heads; 5) Division between and above heads; 6) Undivided nerve above undivided muscle.

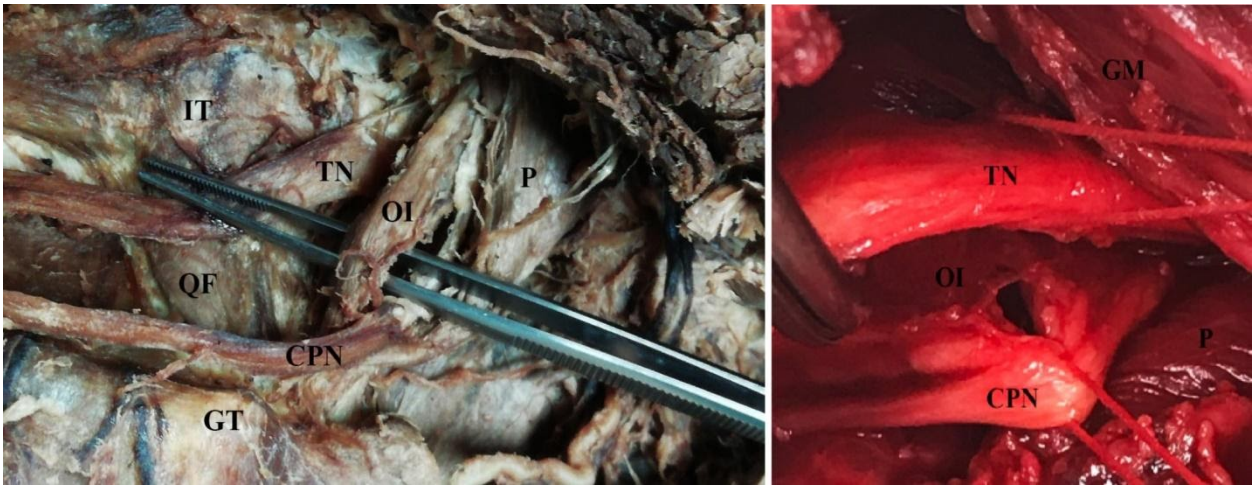

Fig. 2 - High division of the sciatic nerve, above the *obturator internus* muscle. The sciatic nerve exits in the pelvis from the infrapiriform portion of the greater sciatic *foramen* divided by attachment of the *obturator internus* muscle in the left gluteal region.
IT – tuberosity of ischium ; GT – great trochanter of femur; QF – *quadratus femoris* muscle; CPN – common peroneal (fibular) nerve; TN- tibial nerve; OI – *obturator internus* muscle; P- *piriformis* muscle, GM – *gluteus medius* muscle.

The aim of this study is to examine the relationship between the anatomical parameters of the bony pelvis and the height of SN division in both sexes, assuming that the analyzed parameters affect the SN division itself, which is considered to be one of the main causes of the piriform syndrome.

Methods

Our study was conducted at the Faculty of Medicine, University of Belgrade (Serbia) and School of Medicine, University of St Andrews (United Kingdom). The study was conducted on 28 cadavers, out of which 18 cadavers were male and 10 female, aged 74–86 years. These cadavers were embalmed and fixed with 10% formalin. We formed two study groups, which together comprised 56 lower extremities, with the aim of defining the high and low division of the SN. Distal attachment of the *piriformis* muscle on the greater *trochanter* of the femur was taken for boundary of division of the SN. All divisions above the distal attachment of the *piriformis* muscle are defined as high, while divisions below are defined as low. For the parameters of the bony pelvis dimensions, we took the distance between the right and left superior anterior iliac spine which we defined as a bispinal line, as well as the distance between two tuberosities of *ischium* that we defined as a bituberal line (Figure 3). For measuring of required dimensions, we used a ruler and a caliper.

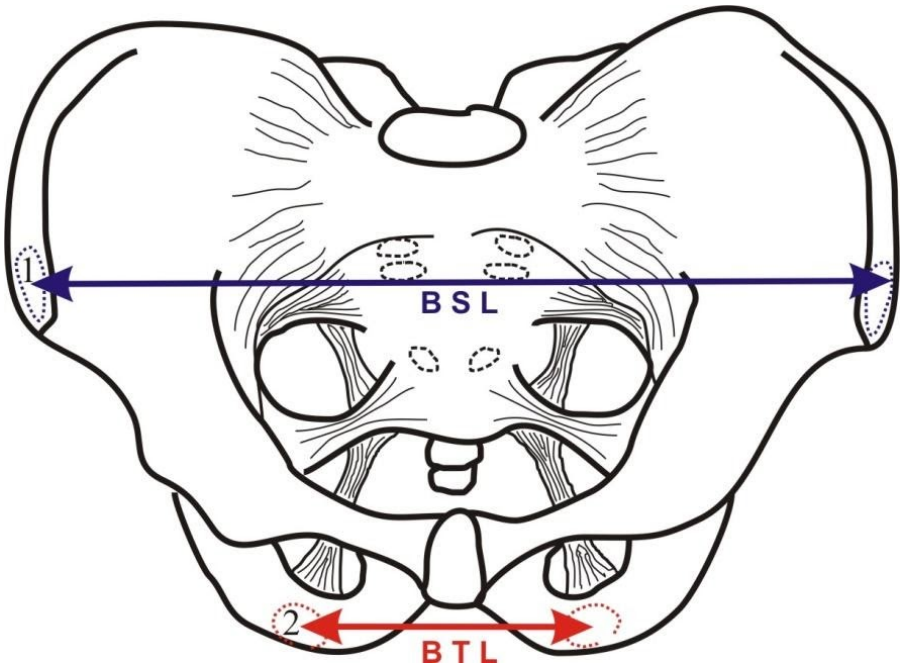

Fig. 3 –Anatomical parameters of the pelvis.
1 – superior anterior iliac spine; 2 – tuberosity of ischium; BSL – bispinal line; BTL – bituberal line.

Statistical data processing was performed in SPSS 11.0 using the Mann-Whitney *U* test, with an accepted level of statistical significance was $p < 0.05$, and $p < 0.001$ for highly statistically significant result.

Results

The results of our research are presented in Tables 1 and 2. We analyzed 56 lower extremities of 36 male and 20 female cadavers.

Table 1

Pelvic parameters of cadavers analyzed

Parameters	Mean ± SD
High division of SN (67.86%)	
bispinal line (cm)	24.38 ± 1.69*
bituberal line (cm)	14.56 ± 1.05*
Low division of SN (32.14%)	
bispinal line (cm)	30.01 ± 2.27
bituberal line (cm)	16.38 ± 0.25

SN – sciatic nerve; SD – standard deviation. *statistically significant difference versus low division of SN (p< 0.05).

Table 2

Pelvic parameters in cadavers with high division of SN

Parameters	Mean ± SD
High division of SN in male (58.33%)	
bispinal line (cm)	24.75 ± 2.36
bituberal line (cm)	15.38 ± 0.63
High division of SN in female (80%)	
bispinal line (cm)	24.00 ± 0.82
bituberal line (cm)	13.75 ± 0.65*

SN – sciatic nerve; SD – standard deviation. *statistically significant difference in decrease of length of bituberal line in female (p< 0.05).

High and low division of the SN were found in 38 (67.86%) and 18 (32.14%) of total sample, respectively. The high division of the SN was found in 21/36 (58.33%) of the male specimens and 16/20 (80%) of the female specimens. The mean values of the length of bispinal line on the total cadaver sample with the high and low division of the SN were 24.38 ± 1.69 and 30.01 ± 2.27, respectively. Comparing the two formed groups (with the high and low division of the SN), we demonstrated a statistically significant difference of mean value of the bispinal line between high and low divisions ($p = 0.003$ and $p < 0.05$, respectively). The mean values of the length of bituberal line in cadavers with the high and low division of the SN were 14.56 ± 1.05 and 16.38 ± 0.25 ($p = 0.004$ and $p < 0.05$, respectively). The mean values of the length of bispinal line in the male and female cadaveric groups with the high division of the SN were 24.75 ± 2.36 and 24.00 ± 0.82, without statistical significance ($p = 0.88$ and $p > 0.05$, respectively). Mean value of the bituberal line in the male cadaveric group was 15.38 ± 0.63, while the same parameter in the female cadaveric group was 13.75 ± 0.65 showing statistically significant diference between two groups ($p = 0.028$ and $p < 0.05$, respectively).

Discussion

The SN represents the nerve which is due to anatomical characteristics particularly susceptible to various forms of injury [3]. The neuropathy of this nerve is one of the most common diseases of lower extremities [3]. The SN may be susceptible to the pressure in different regions, but it most commonly occurs in the area of hip joint by the *piriformis* muscle, causing the symptoms of "piriformis syndrome" in patients [4]. The "piriformis syndrome" is characterized by severe pain in the gluteal region, lower part of back, and worsening of symptoms due to prolonged sitting [9]. The reason of the "piriformis syndrome" can be caused by impaired anatomical structures during growth and development, as well as congenital anomalies [4]. In the study [4], the researches analyzed topographic characteristics on fetuses that are thought to vary during the fetal development of the organs. When they followed distances between the infrapiriform opening and individual anatomical structures on the hip bone, it was observed that distances increased with age. However, it was not possible to make the correlation between nerve thickness in the level of the infrapiriform opening and age, which is explained by the faster development of the musculoskeletal system versus nervous system [4]. It was also noted that the distance between tuberosity of the *ischium* and SN, as well as the distance between the greater trochanter of the femur and SN at the point where the SN transitions from the gluteal region into the posterior femoral compartment, remained unchanged relative to age [4]. The significance of these results is the fact that the SN position in relation to the femur and pelvic bone does not change depending on age [4]. The high division of the SN is clinically significant because it most often results as nerve compression which is the cause of "piriformis syndrome" [2]. In our study, we followed the relationship between the high and low division of the SN and pelvic parameters (bispinal and bituberal line). Division of the SN into the end branches at a higher level than usual can happen anywhere in a thigh or pelvis and it is a relatively common phenomenon [3]. This was confirmed by our results which showed that in 67.86% of the total sample, the SN was highly divided above the distal attachment of the *piriformis* muscle on the greater trochanter of femur, which we have designated as the criterion for determining the level of the division. Analyzing the pelvic parameters of cadavers with the high division of the SN, it can be observed that the mean value of both parameters, bispinal line (24.38 ± 1.69) and bituberal line (14.56 ± 1.05) is less than mean value of bispinal line (30.01 ± 2.27) and bituberal line (16.38 ± 0.25) obtained in cadavers with the low division of the SN, which proved to be statistically significant ($p < 0.05$). Further analyzing the obtained results by gender, we got the result indicating statistically significant ($p < 0.05$) decrease in the length of bituberal line in the female cadavers. These results indicate the relationship between the anatomical parameters of the pelvis and the level of SN division, so that the high division is associated with smaller pelvic dimensions. It could be assumed that, due to frequent high division, the person with the smaller size of the pelvis will have a higher probability to develop "piriformis

syndrome". However, other factors, such as biomechanics, should be considered. The "piriformis syndrome" is up to six times more common in women than in men, due to the biomechanics which is associated with a wider angle *m. quadriceps femoris* ("Q angle") in women [8]. Our results may indicate the cause of the "piriformis syndrome", primarily in individuals of the female population, because the conducted cadaveric study showed a more frequent high division of the SN in women (80% of the total sample), with a statistically significant difference in the length of bituberal line, which is certainly smaller in females. The results of one study showed the relationship between the anatomical structures of the hip bone and SN, first in the anatomical position, and then in the positions which are simulating the *piriformis* muscle elongation test (such as 60° flexion, 30° adduction and 10° medial rotation in the hip joint) [10]. In the mentioned study, the results showed that during biomechanics stretching tests the infrapiriform opening becomes closer to the spine of *ischium* and the angle between transversal plane and the SN becomes larger, so that it makes the SN more susceptible to being „stuck".

Conclusion

The results of our study show that there is a relationship between the anatomical parameters of the bony pelvis with the SN division level. A high division of the SN is associated with the smaller length of bispinal and bituberal line in both sexes. In females, there is a more frequent high division of the SN and statistically significant difference in the length of bituberal line, which is smaller.

Although the results would suggest that people with smaller pelvic dimensions are more likely to develop the "piriformis syndrome", other factors, such as biomechanics, associated with the wider "Q angle" in women, result in a higher incidence of this syndrome.

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R E F E R E N C E S

1. *Patel S, Shah M, Vora R, Zalawadia A, Rathod SP*. A variation in the high division of the sciatic nerve and its relation with piriformis muscle. Natl J Med Res 2011; 1(2): 27−30.
2. *Adibatti M, Sangeetha V*. Study on variant anatomy of sciatic nerve. J Clin Diagn Res 2014; 8(8): AC07−9.
3. *Distad BJ, Weiss MD*. Clinical and electrodiagnostic features of sciatic neuropathies. Phys Med Rehabil Clin N Am 2013; 24(1): 107−20.
4. *Davut O, Yakup G, Sevgi B, Senoglu M, Kalender AM, Calik M*. The topographical features and variations of nervus ischiadicus in human fetuses. Bratisl Lek Listy 2011; 112(8): 475−8.
5. *Beaton LE, Anson BJ*. The relation of the sciatic nerve and of its subdivisions to the piriformis muscle. *The Anat Rec* 1937; 70(1): 1–5. doi: 10.1002/ar.1090700102.
6. *Babinski MA, Machado FA, Costa WS*. A rare variation in the high division of the sciatic nerve surrounding the superior gemellus muscle. Eur J Morphol 2003; 41(1): 41–42.
7. *Meknas K, Christensen A, Johansen O*. The internal obturator muscle may cause sciatic pain. Pain 2003; 104(1−2): 375−80.
8. *Boyajian-O'Neill LA, McClain RL, Coleman MK, Thomas PP*. Diagnosis and management of piriformis syndrome: an osteopathic approach. J Am Osteopath Assoc 2008; 108(11): 657−64.
9. *Miller TA, White KP, Ross DC*. The diagnosis and management of Piriformis Syndrome: myths and facts. Can J Neurol Sci 2012; 39(5): 577–83.
10. *Güvençer M, Akyer P, Iyem C, Tetik S, Naderi S*. Anatomic considerations and the relationship between the piriformis muscle and the sciatic nerve. Surg Radiol Anat 2008; 30(6): 467−74.

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Pharmacological characterisation of *Seseli gracile* Waldst. & Kit. (Apiaceae) essential oil

Farmakološka karakterizacija etarskog ulja *Seseli gracile* Waldst. & Kit. (Apiaceae)

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Abstract

Background/Aim. Phytochemical and pharmacological investigations of essential oils isolated from plant species of the genus *Seseli* have been intensified recently. These plant species have long-term use in nutrition and traditional medicine in the treatment of various disorders. Volatile secondary metabolites of *Seseli gracile* Waldst. & Kit (*Apiaceae*) have not been pharmacologically examined so far. The aim of the conducted research was to assess the antiradical, antimicrobial and spasmolytic activities of *S. gracile* essential oil isolated from the aerial parts of the plant. **Methods.** The antiradical activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test, antimicrobial activity using broth microdilution method with standard strains of Gram (+), Gram (-) bacteria and yeast, while spasmolytic activity was evaluated on isolated rat ileum, pre-contracted with a high concentration of potassium. **Results.** The results showed moderate antiradical (SC_{50} = 78.60 μg/mL) and good spasmolytic activity (IC_{50} = 271.4 nL/mL) of *S. gracile* essential oil. In the range of tested concentrations, minimal inhibitory concentration (MIC) was determined only for the strain of *Escherichia coli* (500 μg/mL). **Conclusion.** Results obtained in this study justify the need for further studies to elucidate exact molecular mechanism underlay this spasmolytic effect of *S. gracile* herb essential oil.

Key words:
apiaceae; oils, volatile; pharmacology; plant extracts; muscle relaxation; antioxidants; anti-infective agents.

Apstrakt

Uvod/Cilj. Etarska ulja izolovana iz vrsta roda *Seseli* su u poslednje vreme intenzivno farmakološki i fitohemijski proučavana. Biljke iz ovog roda se koriste već duži niz godina u ishrani i tradicionalnoj terapiji različitih oboljenja. Farmakološka aktivnost etarskog ulja izolovanog iz *Seseli gracile* do sada nije ispitivana. Stoga je cilj ovog istraživanja bio da se ispita antiradikalska, antimikrobna i spazmolitična aktivnost etarskog ulja izolovanog iz nadzemnih delova biljne vrste *S. gracile*. **Metode.** Antiradikalska aktivnost je utvrđena testom neutralizacije 2.2-difenil-1-pikrilhidrazil (DPPH) radikala, antimikrobna, bujon-mikrodilucionom metodom uz upotrebu standardnih sojeva Gram (+), Gram (-) bakterija i gljivica, a spazmolitična na izolovanom ileumu pacova, prethodno kontrahovanom visokom koncentarcijom kalijuma. **Rezultati.** Rezultati pokazuju umerenu antiradikalsku (SC_{50} = 78.60 μg/mL) i dobru spazmolitičnu aktivnost (IC_{50} = 271.4 nL/mL) etarskog ulja *S. gracile*. U opsegu testiranih koncentracija utvrđena je minimalna inhibitorna koncentracija (MIC) samo za soj *Escherichia coli* (500 μg/mL). **Zaključak.** Dobijeni rezultati opravdavaju potrebu za budućim istraživanjima koja bi razjasnila tačne mehanizme kojima se ostvaruje spazmolitički efekat etarskog ulja nadzemnog dela *S. gracile*.

Ključne reči:
apiaceae; etarsko ulje; farmakologija; ekstrakti, biljni; mišić, relaksacija; antioksidanti; antiinfektivni agensi.

Introduction

Although many species from genus *Seseli* (Apiaceae) are distributed worldwide, there are some endemic species that are narrowly distributed, sometimes only at the locations where the taxon was first described [1, 2]. Different *Seseli* species contain various pharmacologically active compounds: coumarins [3], flavonoids [4], polyacetylenes [5], sesquiterpene

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compounds [6]. Many plants of this genus species are traditionally used worldwide as food [7], spices [8] and remedies for treating various disorders [9] including gastrointestinal [10] and even neurological [11] ones. They also contain secretory structures in different plant organs for essential oil (EO) deposition. The chemical composition of EOs isolated from different plant organs greatly varies [12]. Until recently, EO contribution to total demonstrated pharmacological effects of the isolates from various *Seseli* species was unknown [13]. A possible explanation for this was pronounced pharmacological effects and promising therapeutic potential of coumarins as the main group of secondary metabolites in *Seseli* species [14]. *Seseli (S.) gracile* Waldst. & Kit. (Apiaceae*)* is endemic species present in Serbian and Romanian flora [15]. The first study of *S. gracile* volatile fraction showed that it was not a rich source of EO like some other species from the same genus [12, 16]. This could be the reason why its chemical composition was unknown until recently [17]. According to the literature, none of the previous studies investigated the pharmacological effects of *S. gracile* EO.

Methods

Chemicals

Chemicals used were: sodium chloride, potassium chloride, calcium chloride, magnesium chloride, magnesium sulphate, potassium dihydrogen phosphate, sodium bicarbonate, glucose and boric acid from Lach-Ner, s.r.o (Brno, Czech Republic); acetylcholine chloride, phenylephrine hydrochloride, *n*-hexane (CHROMASOLV®, for HPLC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,3,5-triphenyltetrazolium chloride (TTC) and dimethyl sulfoxide (DMSO) from Sigma-Aldrich (St. Louis, U.S.A.); ethanol absolute (for HPLC) from Fisher Scientific (Loughborough, UK) and carboxymethyl cellulose, sodium salt from Across Organics (Geel, Belgium). Ampicillin, amikacin and nystatin were purchased from Galenika, Belgrade, Serbia; Müeller-Hinton broth and Sabouraud dextrose broth were purchased from Institute of Immunology and Virology Torlak, Belgrade, Serbia.

Plant material

Aerial parts of *S. gracile* were collected in 2015 from the natural habitat on Mali Štrbac in the Danube/Đerdap gorge (Serbia) in a flowering phase (July) and identified by Professor Branislava Lakušić. A voucher specimen was deposited in the Herbarium of the Department of Botany, Faculty of Pharmacy, Belgrade under the accession number HFF3702.

EO isolation

The EO from air-dried and powdered plant material was isolated by hydro distillation in a Clevenger-type apparatus, using 1 mL of *n*-hexane as a collecting solvent according to the European Pharmacopoeia 7.0 procedure.

Chemical analysis

Equipment and operating conditions of essential oil chemical analysis are presented in Table 1.

The linear retention indices (RI) were determined in relation to a homologous series of n-alkanes (C_9–C_{24}) under the same operating conditions.

Identification of the compounds was based on comparison of their RI, retention times (RT), mass spectra (MS) and flame ionization detector (FID) values with those obtained from authentic samples and/or the NIST/NBS, Wiley libraries and the literature [18].

Relative percentages of the identified compounds were computed from the gass chromatography (GC)-FID peak area.

Animals

Male normotensive Wistar rats (200–300 g) were housed in standard laboratory conditions (4–8 animals in a cage), at room temperature and 12-hour light-dark cycle with unrestricted access to food and water. All procedures conducted in this pharmacological test were approved by the local Ethics Committee and are in compliance with the Euro-

Table 1

Equipment and operating conditions of essential oil chemical analysis

Equipment	Operating conditions
GC system	Agilent 6890N
Detectors	5975 MSD and FID
Column	HP-5 MS column (30 m x 0.25 mm x 0.25 μm)
Operating conditions	
Injection volume	2 μL
Injection temperature	200 °C
Split ratio	10 : 1
Carrier gas	Helium
Gas flow rate	1.0 mL/min (constant flow mode)
Column temperature	60–280 °C (linearly programmed) with 3 °C/min rate; held at 280 °C for 5 min
Transfer line temperature	250 °C
FID detector temperature	300 °C
m/z range of EI mass spectra	35–550

GC – gas chromatography; FID – flame ionization detector; EI – electron ionization.

pean Council Directive of November 24, 1986 (86/609/EEC).

Antioxidant activity (DPPH radical assay)

Three aliquots of the EOs were mixed with 0.4 mL of 0.5 mM DPPH in absolute ethanol, and the final volume was adjusted to 2 mL. All mixtures were vigorously stirred for 30 s and left for 30 min in dark at room temperature. Zero point four millilitres of 0.5 mM DPPH diluted up to 2 mL of absolute ethanol was used as the control. The absorbance of samples and controls were measured at 517 nm immediately after incubation. Scavenging (SC) of DPPH radical was calculated using the equation: SC (%) = 100 (A_0 - As)/A_0, where A_0 is the absorbance of the control, and As is the absorbance of the tested sample. The SC_{50} value represented the concentration of the EO that caused 50% of DPPH radical scavenging. Results were compared with the activity of L-ascorbic acid [19].

Antimicrobial activity

Antimicrobial activity was tested by the broth microdilution method and expressed as the minimal inhibitory concentrations (MIC). Standard strains of three Gram (+) bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 and *Bacillus subtilis* ATCC 6633), four strains of Gram (−) bacteria (*Escherichia (E.) coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Salmonella enterica* subsp. *enterica* serovar *Abony* NCTC 6017 and *Pseudomonas aeruginosa* ATCC 27853) and two strains of yeasts *Candida (C.) albicans* ATCC 10231 and *C. albicans* ATCC 10259 were used in this study.

The assay was performed in 96-well microtiter plates with test strains suspended in Müller-Hinton and Sabouraud broth for the bacteria and yeast, respectively, to make the final density of 5×10^5 cfu/mL. 2,3,5-triphenyltetrazolium chloride (TTC) was added as the indicator of bacterial growth, while the growth of *C. albicans* was estimated by monitoring the formation of a precipitate or opalescence. Microorganisms and TTC were used as a positive control. Serial doubling dilutions from 31.25 to 500.00 μg/mL of the investigated EO were prepared and tested in duplicate against each organism. First EO samples were dissolved in DMSO)and then diluted with Müller-Hinton and Sabouraud broth. The final concentration of DMSO in the tested samples was lower than 1%. The plates were incubated at 37 °C for 24 h for the bacteria and 48 h for *C. albicans*. As standards, antibiotics ampicillin, amikacin and nystatin were used [19].

Spasmolytic activity

To avoid any anaesthetic substances impact on the further pharmacological assay, animals were sacrificed by stunning and exsanguination. Ileum was isolated, cleaned from perivascular tissues, and quickly cut into rings (3–5 mm) [20]. These rings were suspended by triangle stainless steel hooks connected to a force transducer (Ugo Bazile model) in organ baths containing 10 mL Tyrode's solution (mmoL/L: NaCl, 136.89; KCl, 2.68; $CaCl_2$, 1.80; $MgSO_4$, 1.05; NaH_2PO4, 0.42; $NaHCO_3$, 11.90; glucose, 5.5). This solution was continuously gassed with a mixture of 95% O_2 and 5% CO_2 at 36 °C. The rings were incubated for 60 minutes (stabilization period) and after that, they were gradually stretched to the tension of 1 g. Tonic contractions of isolated ileum segments, elicited by depolarizing KCl solution (80 mM) were registered using an isotonic transducer (Ugo Basile S.R.R. model 7003) and displayed on data acquisition program LabScribe2.

Sample preparation

EO dissolved in 0.5% carboxymethyl cellulose sodium salt (Na-CMC) water solution was added directly to the organ bath in a cumulative manner (0.1–400 nL/mL). Higher EO concentrations were added only after achieving maximum response effect of the following lower concentration or 10 minutes after an absence of the response. The spasmolytic effect was expressed as the concentration (IC_{50}) which causes 50% relaxation of contraction induced by KCl (100% contraction). The EO-induced relaxation was compared with the vehicle effect.

Statistics

The one-way analysis of variance (ANOVA) with post-hoc Bonferroni test was used for determination of statistically significant differences between the spasmolytic effects of tested sample and vehiculum.

Results

Chemical analysis

S. gracile essential oil chemical analysis results are presented in Table 2. Twenty-two compounds were identified in the analysed sample representing 99.31% of total essential oil. Monoterpenes, with a content of 94.1%, were the mayor constituents. *S. gracile* essential oil is characterized by high amount of terpinolene (40.55%) followed by γ-terpinene (23.34%) and *p*-cymene (9.62%).

Antioxidant activity (DPPH radical assay)

Antioxidant activity of *S. gracile* EO was determined using DPPH radical assay. The results of DPPH antiradical activity of analysed EO and L-ascorbic acid were presented in Figure 1. The concentration of the sample that caused 50% of DPPH radical scavenging (SC_{50}) was 78.6 μg/mL. Although *S. gracile* EO exhibited lower antioxidant activity than L-ascorbic acid (referent antioxidant substance), it could be considered as significant.

Antimicrobial activity

S. gracile EO has exhibited none or weak antimicrobial potential (Table 3). In the range of tested concentrations,

Table 2

***Seseli gracile* essential oil tested sample chemical composition**

Compound	KI	%
α -Thujene	929.0	0.30
α - Pinene	936.2	2.17
Sabinene	975.8	1.67
ß- Pinene	980.8	6.26
Myrcene	992.4	1.24
α -Phellandrene	1007.3	0.28
p-Cymene	1028.0	9.62
Limonene	1031.1	3.65
ß-*(Z)*-Ocimene	1038.1	2.86
ß-*(E)*-Ocimene	1048.2	0.59
γ-Terpinene	1063.4	23.34
Terpinolene	1095.9	40.55
1,3,8-*p*-Menthatriene	1136.4	0.37
trans-*p*-Mentha-2,8-dien-1-ol	1145.8	0.74
cis-*p*-Mentha-2,8-dien-1-ol	1167.8	0.53
cis-Carveol	1177.4	0.29
p-Cymen-9-ol	1186.3	1.19
α-Copaene	1375.9	0.20
(E)-Caryophyllene	1419.9	0.63
ß-*(E)*-Farnesene	1456.1	1.28
trans-Muurola-4(14),5-diene	1481.5	0.32
δ-Cadinene	1523.8	1.22
Total identified		99.31
Monoterpenes		94.10
Sesquiterpenes		3.66
Other		1.55

KI – Kovat's retention indices determined relative to two series of n-alcanes on HP-5 MS column.

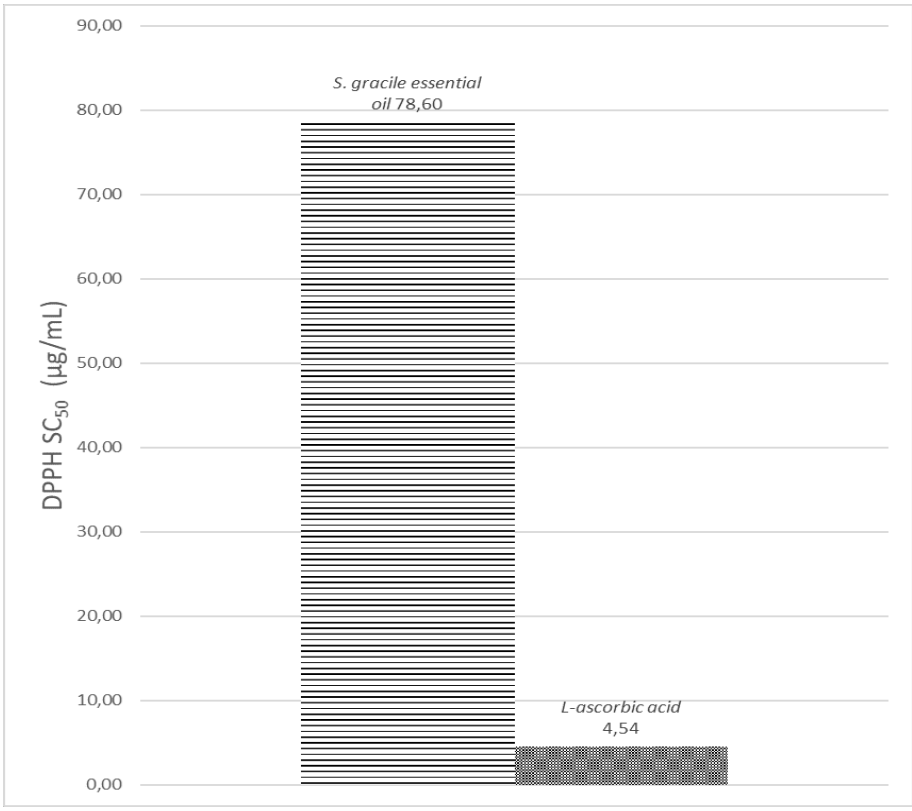

Fig. 1 – Antioxidant activity of *S. gracile* essential oil compared to L-ascorbic acid. DPPH – 2,2-diphenyl-1-picrylhydrazyl; SC_{50} – scavenging concentration 50%.

Table 3

Antimicrobial effects of *Seseli gracile* essential oil (SGEO) and standard antibiotics on tested strains of bacteria and yeasts

Microorganisms		(SGEO)	Ampicillin	Amikacin	Nystatin
		MIC (μg/mL)			
Gram (+)	*Staphylococcus aureus* ATCC 25923	> 500	0.5	2	n.t.
	Enterococcus faecalis ATCC 29212	> 500	0.5	n.t.	n.t.
	Bacillus subtilis ATCC 6633	> 500	n.t.	n.t.	n.t.
Gram (-)	*Escherichia coli* ATCC 25922	500	2	5	n.t.
	Klebsiella pneumoniae ATCC 13883	> 500	4	n.t.	n.t.
	Salmonella abony NCTC 6017	> 500	n.t.	n.t.	n.t.
	Pseudomonas aeruginosa ATCC 27853	> 500	3	0.5	n.t.
Yeast	*Candida albicans* ATCC 10231	> 500	n.t.	n.t.	3
	Candida albicans ATCC 10259	> 500	n.t.	n.t.	5

MIC – minimal inhibitory concentrations; n. t. – not tested.

MIC was determined only for the strain of *E. coli* (500 μg/mL). The EO did not affect the growth of other microorganisms (MIC > 500 μg/mL).

Spasmolytic activity

Essential oil of *S. gracile* showed dose-dependent relaxation effect on isolated ileum, previously contracted with a high concentration of K^+ (C_{KCl}= 80 mM). EO concentration which caused 50% of inhibition was 271.4 nL/mL. Concentration-response curves of the relaxation effect of EO and vehiculum are presented in Figure 2 and show that the relaxation effect originates from the EO.

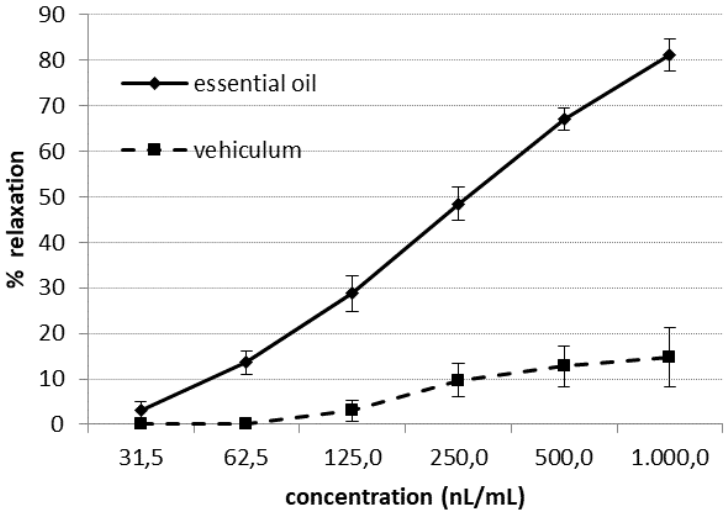

Fig. 2 – Concentration-response curve for relaxation of induced effect by increasing concentrations *(*0.1–400 nL/mL*)* of essential oil (solid line curve) and by vehiculum (0.5% carboxymethyl cellulose sodium salt) added in the same manner as an essential oil (dashed line curve). Data are given as the mean ± standard error of the mean. The concentration curves are significantly different (one-way ANOVA, $p < 0.001$; *post-hoc* Bonferonni test, $p < 0.001$).

Discussion

As previously shown, EO isolated from aerial parts of *S. gracile* is characterized by a high amount of monoterpenes (with content over 90%). Its major constituents were terpinolene (> 40.0%) followed by γ-terpinene (> 20.0%) and *p*-cymene (9.62%) [17].

Some similarities regarding monoterpenes content were observed when comparing the chemical composition of EOs isolated from aerial parts between *S. gracile* and other members of *Seseli* genus. Monoterpenes are major constituents of EOs isolated from aerial parts of *S. rigidum* [21], *S. campestre* [22] and *S. tortuosum* [23] as well as from *S. rigidum* fruit [24]. Moreover, in abovementioned EOs monoterpenes were mostly represented with α-pinene (35.9–57.4%). Previous studies also showed that in EOs isolated from aerial parts of other *Seseli* species, terpinolene was not usually the most abundant compound. On the other hand, γ-terpinene was detected in EOs isolated from fruits of *S. petraeum* [25], *S. rigidum* [24], *S. globiferum* [26] and leaves of *S. bocconi* [27] as one of the major components.

Content of terpinolene and γ-terpinene in analyzed EO of *S. gracile* and their potency for various pharmacological

activities, such as antioxidant, antimicrobial and antispasmodic were positively correlated [28–30].

Antioxidant activity (DPPH radical assay)

EOs isolated from the aerial parts of different *Seseli* species did not exhibit significant antioxidant potential [24, 26]. Chemical analysis of *S. gracile* EO showed *γ*-terpinene and terpinolene were the most abundant components, representing 63.89% of total oil [17]. Tepe et al. [31] showed that *γ*-terpinene, as a second most abundant compound of *Clinopodium vulgare* L. (Lamiaceae) EO significantly contributed to its total antioxidant activity with IC_{50} = 122 μg/mL. The study which tested DPPH antiradical activity of twenty-one citrus EOs components demonstrated that *γ*-terpinene and terpinolene had a very similar radical-scavenging effect, but much stronger than that of a Trolox (referent antioxidant substance). Further analysis of structure-activity relation showed that the presence of 1,4-cyclohexadiene moiety and conjugated system enhances antiradical activity [32]. Considering that DPPH radical scavenging activity was not detected for *p*-cymene [31], the antioxidant potential of *S. gracile* EO mainly depends on the content of *γ*-terpinene and terpinolene [31].

Essential oil isolated from aerial parts of *S. gracile* did not exhibit significant antimicrobial activity. Only Gram (-) *E. coli* strain showed a slightly higher sensitivity to the tested sample with MIC value of 500 μg/mL. *Melaleuca alternifolia* (Maiden & Betche) Cheel (Myrtaceae) EO is good source of monoterpenes *γ*-terpinene and terpinolene with a content of 23.0% and 3.1%, respectively [33]. Carson and Riley[30] showed that *γ*-terpinene and terpinolene exhibited significant antimicrobial activity against *Staphylococcus aureus* with MICs 4.0% and 2.0% (expressed as v/v), respectively. Moreover, terpinolene also exhibited significant antimicrobial activity against *E. coli* and *C. albicans* with MICs, 4.0% and 8.0% (v/v), respectively, in the same study. Considering that in the abovementioned study, *γ*-terpinene and terpinolene demonstrated significant individual antimicrobial effects, it was expected that *S. gracile* EO should exhibit strong antimicrobial activity. The lack of expected activity cannot be fully explained unless additional research is conducted. A partial explanation for the absence of activity may be related to the potential antagonistic effect of the major components, the effect already confirmed for the mixture of terpinen-4-ol and *γ*-terpinene. Results of Cox et al.[34] have indicated a decrease in antimicrobial activity of a mixture of these compounds on *S. aureus* versus their individual effects on the same bacterial strain.

Spasmolytic activity

Several EOs rich in monoterpenes showed spasmolytic activity. The most abundant component of *S. gracile* EO, terpinolene showed the ability to inhibit serotonin-induced contraction of the isolated ileum of the rat [35]. The second most abundant component of *S. gracil*e EO *γ*-terpinene exhibited antispasmodic activity on the isolated rabbit jejunum with an IC_{50} = 8.6 μg/mL [36]. EO isolated from *Poliomintha longiflora* A. Gray *(*Lamiaceae*)* exhibits antispasmodic activity based on the presence of *p*-cymene and carvacrol. *p*-Cymene showed good spasmolytic activity on carbachol-induced contractions with an IC_{50} value of 9.85 μg/mL [37]. According to the literature data, more than 70% of *S. gracile* EO possesses the potential for smooth muscle relaxation. However, without further research, it remains unknown whether these components in the mixture have a synergistic effect or, as in the case of *α*- and *β*-pinene, a weaker spasmolytic effect than the collection of individuals [38].

Conclusion

The results from this study suggest moderate DPPH antiradical and good spasmolytic activity of *S. gracile* herb essential oil. Despite expectations based on the data of EO chemical composition analysis, it was not possible to confirm significant antimicrobial activity. These results justify the need for further pharmacological investigations of *S. gracile* EO and its most abundant compounds.

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REFERENCES

1. *Ml Rovira A, Bosch M, Molero J, Blanché C.* Pollination ecology and breeding system of the very narrow coastal endemic *Seseli farrenyi* (Apiaceae). Effects of population fragmentation. Nord J Bot 2008; 22(6): 727−40.
2. *Kljuykov E, Lyskov D, Ukrainskaja U.* An annotated checklist of the endemic Apiaceae of Kazakhstan. Phytotaxa 2018; 360(3): 237−54.
3. *Pimenov MG, Dukhovlinova LI, Sklyar-Yu E, Avramenko LG, Andrianova VB, Sdobnina LI.* Coumarins of some species of the genus Seseli. Rastitel'nye Resursy 1977; 13(4): 647−50.
4. *Matejić J, Džamić A, Mihajilov-Krstev T, Ranđelović VĐ, Krivošej Z, Marin P.* Total phenolic content, flavonoid concentration, antioxidant and antimicrobial activity of methanol extracts from three *Seseli* L. taxa. Cent Eur J Biol 2012; 7(6): 1116−22.
5. *Chen Y, Peng S, Luo Q, Zhang J, Guo Q, Zhang Y,* et al. Chemical and pharmacological progress on polyacetylenes isolated from the family Apiaceae. Chem Biodivers 2015; 12(4): 474−502.
6. *Barrero A, Herrador M, Arteaga P.* Sesquiterpene lactones and other constituents of Seseli vayredanum. Phytochemistry 1994; 37(5): 1351−8.

7. *Bussmann RW*. Ethnobotany of the Caucasus. Switzerland, Cham: Springer International Publishing; 2017.
8. *Seidemann J*. World Spice Plants: Economic Usage, Botany, Taxonomy. New York, NY, U.S.A.: Springer; 2005.
9. *Zhou J*, *Xie G*, *Yan X*. Encyclopedia of Traditional Chinese Medicines - Molecular Structures, Pharmacological Activities, Natural Sources and Applications: Vol. 5: Isolated Compounds T—Z, References. Berlin Heidelberg: Springer-Verlag; 2011.
10. *Carretero Accame ME*, *Gómez-Serranillos M*, *Ortega Hernández-Agero MT*, *Palomino O*. Medicinal Plants used in Folk Medicine for Digestive Diseases in Central Spain. In: *Rai M, Acharya D, José Luis Rios JL*, editors. Ethnomedicinal Plants - Revitalizing of Traditional Knowledge of Herbs. New Hampshire: Enfield; 2011; p. 361−87.
11. *Sahranavard S, Ghafari S, Mosaddegh M*. Medicinal plants used in Iranian traditional medicine to treat epilepsy. Seizure 2014; 23(5): 328−32.
12. *Marčetić M, Božić D, Milenković M, Lakušić B, Kovačević N*. Chemical composition and antimicrobial activity of essential oil of different parts of *Seseli rigidum*. Nat Prod Commun 2012; 7(8): 1091−4.
13. *Stojković S, Petrović S, Kukić J, Džamić A, Ristić M, Milenković M,* et al. Chemical composition and antimicrobial and antioxidant activity of *Seseli rigidum* flower essential oil. Chem Nat Compd 2009; 45(2): 253−6.
14. *Huang L, Kashiwada Y, Cosentino LM, Fan S, Chen CH, McPhail AT,* et al. Anti-AIDS Agents. 15. Synthesis and Anti-HIV Activity of Dihydroseselins and Related Analogs. J Med Chem 1994; 37(23): 3947−55.
15. *Josifović M*. The Flora of the Republic of Serbia. Belgrade: Serbian Academy of Sciences and Arts; 1973. (Serbian)
16. *Pavlović S, Jančić R, Kuznjecova G, Klajn R*. Prilog proučavanju vrste *Seseli gracile*. Waldst. et Kit. sa planine Veliki Štrbac. Arh Farm 1978; 30(1): 9−20. (Serbian)
17. *Marčetić M, Suručić R, Kovačević N, Lakušić D, Lakušić B*. Essential oil composition of different parts of endemic species *Seseli gracile* Waldst. & Kit. (Apiaceae) from natural and cultivated conditions. J Serb Chem Soc 2017; 82(7−8): 815−24.
18. *Adams RP*. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Carol Stream: Allured Pub Corporation; 2001.
19. *Pavlović I, Petrović S, Radenković M, Milenković M, Couladis M, Branković S, et al.* Composition, antimicrobial, antiradical and spasmolytic activity of *Ferula heuffelii* Griseb. ex Heuffel (Apiaceae) essential oil. Food Chem 2012; 130(2): 310−5.
20. *Menezes IA, Moreira IJ, Carvalho AA, Antoniolli AR, Santos MR*. Cardiovascular effects of the aqueous extract from Caesalpinia ferrea: involvement of ATP-sensitive potassium channels. Vascul Pharmacol 2007; 47(1): 41−7.
21. *Marčetić M, Kovačević N, Lakušić D, Lakušić B*. Habitat-related variation in composition of the essential oil of *Seseli rigidum* Waldst. & Kit. (Apiaceae). Phytochemistry 2017; 135: 80−92.
22. *Baser KHC, Oözek T, Kürkçüoglu M, Aytaç Z*. Essential Oil of Seseli campestre Besser. J Essent Oil Res 2000; 12(1): 105−7.
23. *Habibi Z, Masoudi S, Rustaiyan A*. Chemical Composition of the Essential Oil of *Seseli tortuosum L. ssp.* Kiabii Akhani. from Iran. J Essent Oil Res 2003; 15(6): 412−3.
24. *Stankov-Jovanović VP, Ilić MD, Mitić VD, Mihajilov-Krstev TM, Simonović SR, Nikolić Mandić SD,* et al. Secondary metabolites of Seseli rigidum: Chemical composition plus antioxidant, antimicrobial and cholinesterase inhibition activity. J Pharm Biomed Anal 2015; 111: 78−90.
25. *Bulatović VM, Šavikin-Fodulović KP, Zdunić GM, Popović MP*. Essential Oil of Seseli peucedanoides (MB) Kos. Pol J Essent Oil Res 2006; 18(3): 286−7.
26. *Stojković D, Glamočlija J, Soković M, Grubišić D, Petrović S, Kukić J, et al.* Chemical composition, antimicrobial and antiradical properties of the essential oils of *Seseli globiferum* fruits. Nat Prod Commun 2008; 3(11): 1935−8.
27. *Marongiu B, Piras A, Porcedda S, Tuveri E, Maxia A*. Isolation of *Seseli bocconi* Guss., subsp. *praecox* Gamisans (Apiaceae) volatile oil by supercritical carbon dioxide extraction. Nat Prod Res 2006; 20(9): 820−6.
28. *Dorman HJD, Figueiredo AC, Barroso JG, Deans SG*. In vitro evaluation of antioxidant activity of essential oils and their components. Flavour Fragr J 2000; 15(1): 12−6.
29. *Ruberto G, Baratta MT*. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem 2000; 69(2): 167−74.
30. *Carson CF, Riley TV*. Antimicrobial activity of the major components of the essential oil of Melaleuca alternifolia. J Appl Bacteriol 1995; 78(3): 264−9.
31. *Tepe B, Sihoglu-Tepe A, Daferera D, Polissiou M, Sokmen A*. Chemical composition and antioxidant activity of the essential oil of *Clinopodium vulgare* L. Food Chem 2007; 103(3): 766−70.
32. *Sawamura M*. Citrus Essential Oils: Flavor and Fragrance. Hoboken, New Jersey: Wiley; 2011.
33. *Carson CF, Hammer KA, Riley TV*. Melaleuca alternifolia (Tea Tree) oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev 2006; 19(1): 50−62.
34. *Cox SD, Mann CM, Markham JL*. Interactions between components of the essential oil of *Melaleuca alternifolia*. J Appl Microbiol 2001; 91(3): 492−7.
35. *Riyazi A, Hensel A, Bauer K, Geissler N, Schaaf S, Verspohl EJ*. The effect of the volatile oil from ginger rhizomes (*Zingiber officinale*), its fractions and isolated compounds on the 5-HT3 receptor complex and the serotoninergic system of the rat ileum. Planta Med 2007; 73(4): 355−62.
36. *Astudillo A, Hong E, Bye R, Navarrete A*. Antispasmodic activity of extracts and compounds of *Acalypha phleoides* Cav. Phytother Res 2004; 18(2): 102−6.
37. *Rivero-Cruz I, Duarte G, Navarrete A, Bye R, Linares E, Mata R*. Chemical composition and antimicrobial and spasmolytic properties of *Poliomintha longiflora* and *Lippia graveolens* essential oils. J Food Sci 2011; 76(2): C309−17.
38. *Sadraei H, Asghari GR, Hajhashemi V, Kolagar A, Ebrahimi M*. Spasmolytic activity of essential oil and various extracts of *Ferula gummosa* Boiss. on ileum contractions. Phytomedicine 2001; 8(5): 370−6.

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Association between dental caries risk indicators and various stages of caries in newly erupted permanent teeth

Povezanost indikatora rizika od pojave karijesa i različitih stadijuma karijesa na mladim stalnim zubima

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Abstract

Background/Aim. Identification of caries risk indicators involved in the disease development is of great importance. The aim of this study was to assess the association between distinctive risk indicators and different stages of caries disease process in children. **Methods.** The cross-sectional study was conducted on a sample (n = 327) of school children ages 7–11. A questionnaire submitted to parents was used to record the data regarding demographic characteristics, children's dietary habits, oral hygiene habits and oral health behaviors. Caries was assessed using the International Caries Detection and Assessment System (ICDAS) II and subjects were stratified according to the highest and most prevalent caries lesion stage. The data obtained were analyzed using the χ^2 or Fisher`s exact test and multinomial logistic regression. **Results.** Caries lesions were detected in 56.6% of examinees observed in the study. Compared to caries-free subjects, appearance of initial caries lesions was statistically significantly influenced by maternal education level ($p = 0.021$), paternal occupational status ($p = 0.023$), toothbrushing frequency ($p < 0.001$), and caries status of deciduous teeth ($p = 0.027$). Maternal educational level ($p = 0.026$), paternal occupational status ($p = 0.003$), sweets and snacks taking frequency ($p = 0.005$), toothbrushing frequency ($p < 0.001$), and fluoridate dentifrice usage ($p = 0.027$) were associated with moderate caries lesions. Maternal educational level ($p < 0.001$), sweets and snacks taking frequency ($p = 0.022$) and toothbrushing frequency ($p < 0.001$) were associated with extensive caries lesions. Maternal educational level ($p = 0.02$) and brushing frequency ($p < 0.001$) were statistically significantly associated with the highest prevalence of initial caries lesions. Maternal educational level ($p = 0.025$), toothbrushing frequency ($p < 0.001$) and frequency of dental check-ups ($p = 0.016$) were statistically significantly associated with the highest prevalence of moderate caries lesions. **Conclusion.** Parental socioeconomic indicators and children's behavior related to oral health were involved in the changes from caries-free status to different caries stages.

Key words:
child; dental caries; habits; oral health; risk factors; socioeconomic factors; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Identifikacija indikatora rizika od pojave karijesa je od velike važnosti. Cilj ove studije je bio da se proceni povezanost između određenih indikatora rizika od karijesa i različitih faza karijesnog procesa kod dece. **Metode.** Studijom preseka obuhvaćen je uzorak od 327 školske dece uzrasta 7–11 godina. Podaci o demografskim karakteristikama, navikama dece u ishrani, oralnoj higijeni i ponašanju povezanim sa oralnim zdravljem prikupljeni su putem upitnika koje su popunjavali roditelji. Detektovanje i procena karijesnih lezija sprovedeni su korišćenjem *International Caries Detection and Assessment System* (ICDAS) II metode. Ispitanici su stratifikovani prema najvećem i najćešćem stepenu karijesne lezije. Dobijeni podaci analizirani su pomoću χ^2 testa, Fišerovog testa i multivarijantne nominalne logističke regresije. **Rezultati.** Karijesne lezije nađene su kod 56,6% ispitanika. Utvrđeno je da su kod ispitanika na pojavu početnih karijesnih lezija statistički značajno uticali nivo obrazovanja majke ($p = 0,021$), radni status oca ($p = 0,023$), učestalost pranja zuba ($p < 0,001$) i karijes status mlečnih zuba ($p = 0,027$). Nivo obrazovanja

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majke (p = 0,026), radni status oca (p = 0,003), učestalost konzumiranja slatkiša i grickalica (p = 0,005), učestalost pranja zuba (p < 0,001) i upotreba zubne paste sa fluorom (p = 0,027) bili su povezani sa pojavom lezija srednjeg stepena. Nivo obrazovanja majke (p < 0,001), učestalost konzumiranja slatkiša i grickalica (p = 0,022) i učestalost pranja zuba (p < 0,001) bili su povezani sa pojavom ekstenzivnih karijesnih lezija. Nivo obrazovanja majke (p = 0,020) i učestalost pranja zuba (p < 0,001) statistički su značajno bili povezani sa najvećom učestalošću početnih karijesnih lezija kod dece. Nivo obrazovanja majke (p = 0,025), učestalost pranja zuba (p < 0,001) i učestalost stomatoloških pregleda (p = 0,016) značajno su bili povezani sa najvećom učestalošću karijesnih lezija srednjeg stepena. **Zaključak.** Socijalnoekonomski indikatori i ponašanje dece u vezi sa oralnim zdravljem povezani su sa pojavom karijesa i različitim stadijumima karijesnog procesa.

Ključne reči:
deca; zub, karijes; navike; usta, zdravlje; faktori rizika; socijalno-ekonomski faktori; ankete i upitnici.

Introduction

Dental caries is a posteruptive, complex multifactorial disease process. Although preventable, it is the most common childhood disease and noncommunicable disease worldwide [1]. Between 60% and 90% of children are affected by dental caries [1].

In view of the above considerations, children need special attention, particularly in the age when permanent teeth begin to erupt. This period shows the state of the teeth which allows the food retentions to occur and makes it difficult to be cleaned [2]. In the months and years following the tooth eruption, enamel posteruptive maturation, which is thought to reduce vulnerability to caries [2], has not finished yet. This is the period with the highest risk of caries incidence [3]. Even low levels of dental caries in children, especially when observed in the permanent dentition, are of concern since dental caries is a lifelong progressive and cumulative disease that tracks to adulthood [4].

The occurrence of dental caries in children is influenced by many factors such as: caries experience in primary dentition, educational level of parents, socioeconomic status [5], oral hygiene habits, fluoride history [6], dietary habits [7], etc.

Since caries etiology is complex, there is a need to identify risk factors that are involved in the development of the disease in order to plan the appropriate public health measures to prevent it. Caries-associated variables detected in the cross-sectional study are considered to be risk indicators which might be the risk factors of the disease [8].

Caries is characterized by a gradual but cumulative dissolution and destruction of the mineralized tissues of the teeth resulting in a large clinical polymorphism, from small white spot lesions to large cavities. Caries lesions evolution requires a long period of time, usually months or years [9]. It could be assumed that different risk indicators might be involved in caries disease process and they manifest their effects in specific caries stages, playing different roles at different times. Confirmation of this assumption would contribute to a better understanding of caries initiation and progression. Furthermore, early identification of potential risk factors and subjects prone to caries would allow planning of targeted specific measures for caries prevention and increase the efficiency of preventive programs [10].

Traditionally used criteria for caries assessment, established by the World Health Organization (WHO) in 1997, does not distinguish among the different stages of the caries lesions [11, 12]. Considering the importance of assessing dental caries in all its stages, the International Caries Detection and Assessment System (ICDAS) II has been developed. The ICDAS II distinguishes 6 different stages of caries lesions (from the first visual change in enamel to an extensive cavity in dentine) [12] and aids in the collection of better-quality information to support diagnosis, prognosis and clinical management of dental caries [13].

The aim of the study was to determine whether or not specific risk indicators might affect different stages of the caries disease process in newly erupted permanent teeth. According to data available to us, no previous study has explored this issue.

Methods

In order to investigate the association between caries risk indicators and various stages of caries in newly erupted permanent teeth, a cross-sectional study was conducted.

This study was approved by the Ethics Committee of the Faculty of Medical Sciences, University of Priština, with the headquarters in Kosovska Mitrovica, Serbia. The study was conducted in accordance with the ethical standards laid down in the Declaration of Helsinki. The informed consent form was handed out to the school authorities so as to obtain parental permissions. Only children with their parents signed consent were enrolled in the study.

The study was carried out between December 2017 and March 2018, involving children of both genders, 7–11 years olds, attending elementary schools located in the northern part of Kosovska Mitrovica, Serbia. Children who were systemically healthy, exclusively in the mixed dentition phase were recruited. The exclusion criteria, chosen because of their potential to influence the expression of caries, included antibiotic use of 30 days prior to the onset of the study, structural anomalies of the teeth, presence of fixed orthodontic appliances and the presence of pit and fissure sealants on permanent teeth.

The data collection method consisted of a structured questionnaire (Anex) and a subsequently implemented clinical oral examination. In order to test the methodology and comprehension of the questionnaire, a pilot study was done with a sample of parents (n = 30) not included in the main sample. The results revealed no misunderstanding regarding the questionnaire. Prior the questionnaires were administered, parents received explanations regarding the purpose of the questionnaires and importance of getting precise answers. The anonymity and confidentiality of their responses were emphasized, along with the important contribution that their responses would provide. Questionnaires were submitted to parents, who agreed to allow their children to participate in the study, in order to collect data on demographic and socioeconomic details including sex and age of the child, as well as the educational level and occupational status of their parents and behavioral variables related to their child such as: dietary habits, oral hygiene practices and visits to a dentist.

Clinical examinations were performed at school dental offices according to the ICDAS examination criteria [14]. To avoid inter-examiner variability, they were conducted by a single examiner, who underwent a training programme proposed by the ICDAS Committe [15]. Prior to the examination, the dentist passed through calibration procedure. Fifty children who were not part of the sample were examined twice, with a 72-hour interval between examinations. Intra-examiner consistency was assessed. Cohen's kappa [16] statistics with a subject and a tooth surface considered as the units analysis was higher than 0.8 for both. Standard infection control measures followed each examination. In the case of more than one lesion present in the same tooth, the most severe lesion was recorded as valid. Trauma related restored teeth were considered as sound. No radiographs were taken. Only caries lesions in permanent teeth were included in the analysis, while the presence of caries lesions in deciduous teeth was considered as a background variable.

All statistical analyses were performed using IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA). Data derived through clinical examination were grouped as follows: No caries (ICDAS 0), Initial stage (ICDAS 1-2), Moderate stage (ICDAS 3-4) and Extensive stage (ICDAS 5-6) [17]. Study subjects were stratified according to the maximum caries lesion stage (subjects were coded according to the highest ICDAS caries score recorded) and according to the most prevalent caries lesion stage (subjects were coded according to the most prevalent ICDAS caries score recorded) [18].The normally distributed continuous data were presented as mean ± standard deviations (SD) and categorical variables were expressed as a number (percentages). In the univariate analysis, the χ^2 test or Fisher's test was used to compare the categorical variables. The background variables with a *p*-value less than 0.05 in the univariate analysis were included in the multinomial logit model in order to assess their associations with the caries lesion stage in subjects stratified as reported above. The first model with the highest caries lesion stage as a dependent variable included the whole sample, using the caries-free children as the base outcome, while the model with the most prevalent caries lesion stage included only caries affected children, using the subjects with the extensive caries lesions as the base outcome. The criterion for statistical significance was $p < 0.05$.

Results

According to data obtained from school authorities, there were 1,003 children aged 7–11 attending these schools. All of them were invited to participate in the study. A total of 761 parents signed informed consent for their children participation in the study and a questionnaire was applied to each of them. Twenty-four subjects who had returned incomplete questionnaires were excluded from the study. Thus, a total of 737 children were eligible for the clinical examination. A total of 387 children failed to meet the study criteria, 17 children were absent from school on the days scheduled for the clinical examinations and 6 children showed lack of cooperation during the examinations. All of them were excluded from the survey. In our final analyses, we used data from a total of 327 children.

The children were 7–11 years old with the mean age of 9.32 ± 1.40 years. Caries lesions were detected in 56.6% of the children, while caries experience (the sum of decayed, filled and missing teeth due to caries) was 75.2%. According to the maximum caries lesion stage stratifying procedure, initial caries lesion stage was present in 16.6%, moderate caries lesion stage was present in 22.6%, and extensive caries lesion stage was present in 17.4% of the children (Table 1). According to the most prevalent caries lesion stage stratifying procedure, a prevalence of initial caries lesion stage, moderate caries lesion stage and extensive caries lesion stage were present in 41.1%, 41.1% and 17.8% of the affected children, respectively (Table 2).

Parental educational level ($p < 0.001$ for both mother and father), paternal occupational status ($p = 0.028$), toothbrushing frequency ($p < 0.001$), sweets and snacks taking frequency ($p = 0.022$), use of fluoride containing toothpaste ($p = 0.033$), frequency of dental check-ups ($p < 0.001$) and caries status of deciduous teeth ($p = 0.043$) were statistically significantly associated with the highest caries score (Table 1).

Table 2 shows variables that were singled out as statistically significant. It was shown that parental education level ($p = 0.048$ for mother and $p = 0.033$ for father), toothbrushing frequency ($p = 0.010$) and the frequency od dental check-ups ($p = 0.021$) were statistically significantly associated with caries lesion stage prevalence (Table 2).

All variables which were statistically significantly associated with the highest caries lesion stage stratifying procedure and with the most prevalent caries stage stratifying procedure in models of univariate logistic regression were included in the model of multinominal logistic regression (Tables 3 and 4).

Table 1

Distribution of the children according to the highest caries lesion stage stratifying procedure (n = 327)

Variables	Highest caries score (ICDAS), n (%)				*p*
	healthy teeth	initial caries lesion	moderate caries lesion	extensive caries lesion	
	142 (43.4)	54 (16.6)	74 (22.6)	57 (17.4)	
Maternal educational level					
primary school	1 (0.7)	3 (5.6)	3 (4.1)	11 (19.3)	
high school	62 (43.7)	27 (50.0)	50 (67.6)	28 (49.1)	< 0.001*
faculty	79 (55.6)	24 (44.4)	21 (28.4)	18 (31.6)	
Paternal educational level					
primary school	1 (0.7)	2 (3.7)	1 (1.4)	2 (3.5)	
high school	58 (40.8)	19 (35.2)	51 (68.9)	38 (66.7)	< 0.001*
faculty	83 (58.5)	33 (61.1)	22 (29.7)	17 (29.8)	
Maternal occupational status					
employed	89 (63.1)	33 (61.1)	46 (63.0)	30 (53.6)	
unemployed	48 (34.0)	17 (31.5)	24 (32.9)	23 (41.1)	0.728
self-employed	4 (2.8)	4 (7.4)	3 (4.1)	3 (5.4)	
Paternal occupational status					
employed	113 (80.7)	34 (63.0)	51 (68.9)	43 (75.4)	
unemployed	19 (13.6)	9 (16.7)	12 (16.2)	11 (19.3)	0.028*
self-employed	8 (5.7)	11 (20.4)	11 (14.9)	3 (5.3)	
Sweet beverages taking frequency					
never or rarely	17 (12.0)	12 (22.2)	14 (18.9)	11 (19.3)	
2–5/ day	97 (68.3)	29 (53.7)	44 (59.5)	26 (45.6)	0.072
> 5/ day	28 (19.7)	13 (24.1)	16 (21.6)	20 (35.1)	
Sweets and snacks taking frequency					
never or rarely	49 (34.5)	11 (20.4)	13 (17.6)	10 (17.5)	
2–5/ day	59 (41.5)	22 (40.7)	32 (43.2)	23 (40.4)	0.022*
> 5/ day	34 (23.9)	21 (38.9)	29 (39.2)	24 (42.1)	
Toothbrushing frequency					
< 1/ day	13 (9.2)	21 (38.9)	27 (36.5)	39 (68.4)	
1–2/ day	63 (44.4)	29 (53.7)	44 (59.5)	18 (31.6)	< 0.001*
> 2/ day	66 (46.5)	4 (7.4)	3 (4.1)	0 (0)	
Brush teeth for at least 3 minutes					
yes	45 (31.7)	20 (37.0)	22 (29.7)	17 (29.8)	0.818
no	97 (68.3)	34 (63.0)	52 (70.3)	40 (70.2)	
Use of fluoridated toothpaste					
yes	115 (81.0)	42 (77.8)	63 (85.1)	37 (64.9)	0.033*
no	27 (19.0)	12 (22.2)	11 (14.9)	20 (35.1)	
Use of fluoridated mouthwash					
yes	9 (6.3)	5 (9.3)	6 (8.1)	2 (3.5)	0.625
no	133 (93.7)	49 (90.7)	68 (91.9)	55 (96.5)	
Dental check-ups frequency					
periodically	74 (52.1)	24 (44.4)	19 (25.7)	13 (22.8)	
occasionally	62 (43.7)	26 (48.1)	47 (63.5)	25 (43.9)	< 0.001*
visit a dentist when in pain	6 (4.2)	4 (7.4)	8 (10.8)	19 (33.3)	
Deciduous teeth caries					
yes	89 (62.7)	43 (79.6)	57 (77.0)	42 (73.7)	0.043*
no	53 (37.3)	11 (20.4)	17 (23.0)	15 (26.3)	

***Statistically significant result.**
ICDAS – International Caries and Assessment System.

Table 2

Distribution of caries affected children according to the the most prevalent caries lesion stage (n = 185)

Variables	Most prevalent caries stage, n (%)			*p*
	initial	moderate	extensive	
	76 (41.1)	76 (41.1)	33 (17.8)	
Maternal educational level				
primary school	7 (9.2)	5 (6.6)	5 (15.2)	
high school	45 (59.2)	49 (64.5)	11 (33.3)	0.048*
faculty	24 (31.6)	22 (28.9)	18 (51.5)	
Paternal educational level				
primary school	3 (3.9)	1 (1.3)	1 (3.0)	
high school	34 (44.7)	53 (69.7)	21 (63.6)	0.033*
faculty	39 (51.3)	22 (28.9)	11 (33.3)	
Toothbrushing frequency				
< 1/ day	33 (43.4)	30 (39.5)	24 (72.7)	
1–2/ day	38 (50.0)	44 (57.9)	9 (27.3)	0.010*
> 2/ day	5 (6.6)	2 (2.6)	0 (0)	
Dental check-ups frequency				
periodically	28 (36.8)	19 (25.0)	9 (27.3)	
occasionally	37 (48.7)	48 (63.2)	13 (39.4)	0.021*
visit a dentist when in pain	11 (14.5)	9 (11.8)	11 (33.3)	

***Statistically significant result.**

Table 3

Multivariate nominal logistic regression with the highest caries lesion stage as dependent variable (n = 327)

Variable	B (SE)	OR (95% CI)	*p*
Caries-free (ICDAS = 0) – Base outcome			
Initial lesions (ICDAS = 1/2)			
maternal educational level (Primary school)	3.2 (1.4)	25.5 (1.6–397.0)	0.021*
paternal educational level (High school)	-0.5 (0.5)	0.6 (0.3–1.4)	0.266
paternal occupational status (Employed)	-1.6 (0.6)	0.2 (0.06–0.7)	0.009*
sweets and snacks taking frequency (Never or rarely)	-1.2 (0.5)	0.3 (0.1–0.9)	0.023*
toothbrushing frequency (<1/day)	3.8 (0.7)	45.9 (11.6–182.3)	<0.001*
use of fluoridated toothpaste (Yes)	0.5 (0.5)	1.6 (0.6–4.0)	0.350
dental check-ups frequency (Periodically)	-0.3 (0.8)	0.7 (0.1–3.4)	0.668
deciduous teeth caries (Yes)	1.0 (0.5)	2.7 (1.1–6.5)	0.027*
Moderate lesions (ICDAS=3/4)			
maternal educational level (Primary school)	3.2 (1.4)	24.4 (1.5–402.4)	0.026*
paternal educational level (High school)	1.2 (0.4)	3.2 (1.5–6.7)	0.003*
paternal occupational status (Employed)	-1.0 (0.6)	0.4 (0.1–1.2)	0.105
sweets and snacks taking frequency (Never or rarely)	-1.4 (0.5)	0.2 (0.09–0.7)	0.005*
toothbrushing frequency (< 1/day)	4.4 (0.8)	84.5 (18.6–384.0)	< 0.001*
use of fluoridated toothpaste (Yes)	-1.0 (0.5)	0.37 (0.11–0.62)	0.046*
dental check-ups frequency (Periodically)	-1.2 (0.7)	0.3 (0.07–1.3)	0.102
deciduous teeth caries (Yes)	0.6 (0.4)	1.8 (0.8–4.2)	0.141
Extensive lesions (ICDAS = 5/6)			
maternal educational level (Primary school)	4.8 (1.5)	120.4 (6.7–2163.4)	0.001*
paternal educational level (High school)	0.8 (0.4)	2.2 (0.9–5.3)	0.072
paternal occupational status (Employed)	-0.1 (0.9)	0.9 (0.2–5.0)	0.920
sweets and snacks taking frequency (Never or rarely)	-1.4 (0.6)	0.3 (0.08–0.8)	0.022*
toothbrushing frequency (< 1/day)	22.9 (0.5)	8×10^9 (3×10^9–2×10^{10})	< 0.001*
use of fluoridated toothpaste (Yes)	-0.03(0.5)	1.0 (0.4–2.6)	0.960
dental check-ups frequency (Periodically)	-1.8 (0.7)	0.3 (0.08–1.3)	0.017*
deciduous teeth caries (Yes)	0.3 (0.5)	1.3 (0.5–3.3)	0.577

***Statistically significant result.**

ICDAS – International Caries and Assessment System; CI – confidence interval; OR – odds ratio.

Table 4

Multivariate nominal logistic regression with the most prevalent caries lesion stage as dependent variable (n = 185)

Variable	B (SE)	OR (95% CI)	*p*
Initial lesions (ICDAS = 1/2)			
Maternal educational level (High school)	1.1 (0.5)	3.1 (1.2–8.2)	0.020*
Paternal educational level (High school)	-0.6 (0.5)	0.6 (0.2–1.5)	0.258
Toothbrushing frequency (< 1/day)	-17.3(1.0)	0.0 (0.0–0.0)	< 0.001*
Dental check-ups frequency (Occasionally)	0.8 (0.6)	0.3 (0.08–0.8)	0.189
Moderate lesions (ICDAS=3/4)			
Maternal educational level (High school)	1.1 (0.5)	3.0 (1.1–7.9)	0.025*
Paternal educational level (High school)	0.7 (0.5)	1.9 (0.7–5.2)	0.194
Toothbrushing frequency (< 1/day)	-16.4 (0.5)	0.0 (0.0–0.0)	< 0.001*
Dental check-ups frequency (Occasionally)	1.5 (0.6)	4.3 (1.3–13.9)	0.016*
Extensive lesions (ICDAS = 5/6) – Base outcome			

***Statistically significant result.**
ICDAS – International Caries and Assessment System; CI – confidence interval; OR – odds ratio.

It was shown that in caries affected children compared to those who were caries free (considered as the base outcome), initial caries lesion stage was statistically significantly associated with maternal educational level (Primary school, $p = 0.021$), paternal occupational status (Employed, $p = 0.009$), sweet or snacks taking frequency (Never or rarely, $p = 0.023$), toothbrushing frequency (<1/day, $p = <0.001$) and deciduous teeth caries (Yes, $p = 0.027$). Maternal educational level (Primary school, $p = 0.026$), paternal educational level (High school, $p = 0.003$), sweets or snacks taking frequency (Never or rarely, $p = 0.005$), toothbrushing frequency (<1/day, $p < 0.001$), use of fluoridated toothpaste (Yes, $p = 0.046$) were associated with moderate caries lesion stage in caries affected children compared with those being caries free. On the other hand, maternal educational level (Primary school, $p = 0.001$), sweets or snacks taking frequency (Never or rarely, $p = 0.022$), toothbrushing frequency (< 1/day, $p < 0.001$) and dental check-ups frequency (Periodical, $p = 0.017$) were associated with extensive caries lesion stage in subjects with caries compared to those with no caries (Table 3).

Table 4 shows results of the multinominal logistic regression when the most prevalent caries lesion stage was used as a dependent variable in caries affected children. The children with the prevalence of extensive caries lesions were used as the base outcome. Comparing the subjects with the prevalence of initial caries lesions and the base outcome, maternal educational level (High school) and toothbrushing frequency (<1/day) were statistically significantly associated with this caries lesion stage ($p = 0.020$ and $p < 0.001$, respectively). In subjects affected by moderate caries lesions as the most prevalent figure, maternal educational level (High school, $p = 0.025$), toothbrushing frequency (<1/day, $p < 0.001$) and dental check-ups frequency (Occasionally, $p = 0.016$) were statistically significantly associated with this caries lesion stage compared to those affected by the highest prevalence of extensive caries lesions.

Discussion

The present cross-sectional study sought to determine whether distinct caries risk indicators were associated with different stages of a caries disease process using the ICDAS II as the diagnostic criteria. According to the obtained results, a large percentage of subjects affected by caries were fairly similarly distributed through stratified groups. Parental socioeconomic and children behavioral indicators were associated with different stages of the caries disease process and interacted with the disease evolution. Considering the entire sample stratified according to the highest caries lesion stage, parental educational level, paternal occupational status, sweets or snacks taking frequency, toothbrushing frequency, dental check-ups frequency and caries status of deciduous teeth were statistically significantly associated with this stratification. A multinomial model with caries-free children being the base outcome was used to evaluate the role of risk factors in the initiation and progression of a caries disease process. In children with initial caries lesions, a low level of maternal education, paternal employed status, sweets and snacks low taking frequency, low toothbrushing frequency, and caries deciduous teeth caries were involved in changing status from caries-free. A low level of parental education, rare consumption of sweets and snacks, a low toothbrushing frequency and usage of fluoridated toothpaste were associated with moderate lesions in caries affected children with regard to the base outcome. In subjects with extensive caries stages, a low level of maternal education, rare consumption of sweets and snacks, a low level of toothbrushing frequency, and periodical dental check-ups were statistically significantly associated with this caries lesion stage. The distribution of caries affected children according to the most prevalent caries lesion stage showed statistically significant association with parental education level, the frequency of toothbrushing and frequency of dental check-ups. In multinomial model, the subjects with prevalence of the highest caries lesions were used as the base

outcome. In children with the highest prevalence of initial caries lesions, maternal educational level and low level of toothbrushing frequency were significantly assocated with this stage. In subjects with the prevalence of moderate lesions, maternal educational level, low level of toothbrushing frequency and occasional dental check-ups were embodied in this stage. All of these findings confirm that socioeconomic and causal factors act in synergy and play their respective roles in different stages of caries.

Our findings highlighted the importance of parental educational level in caries disease evolution. Insufficient parental education contributes to poor dietary habits and unhealthy lifestyles [19]. Low educational level often means lack of various social benefits and skills such as ability to process certain information, interact with health professionals and adapt to health beneficial behaviors [20]. Parental educational level has been shown to be associated with the children dental visits, toothbrushing frequency, and dental caries prevalence [21–23]. A higher educational level could help an individual acquire a better job and a higher income, which guarantees a better socioeconomic position [24] and easier access to dental services and oral hygiene products [25]. It might be possible that children whose parents have a higher level of education and more knowledge about dental health perform more regular dental visits for preventive measures which results in their having more caries-free teeth and lower degree caries lesions.

The occupational status of parents, particularly that of the father, showed significant association with different caries stages in our study. The access to dental services and oral hygiene products is partially conditioned by family income [23] which could affect the state of oral health of all family members including children. According to a French study [26] children whose parents were employed experienced less caries than those whose parents were unemployed.

According to our study, the frequency of consuming sweets and snacks is associated with the caries lesion progression. The intake of dietary sugars is considered the most important risk factor for dental caries [27, 28]. Years ago, an epidemiological study showed that the frequency of sugar intake was an important risk factor for caries development [29]. It is found that sugar consumption frequency and dental caries experience have a positive corelation [30] and that the former increases caries risk [31].

Studies have shown that the effective removal of dental biofilm by toothbrushing improves hygiene levels [32] and significantly reduces the risk of dental caries [33]. It was observed in our study that low brushing frequency increases risk of caries lesion progression regardless of fluoride content in toothpaste. Although the relationship between oral hygiene habits and caries has been widely explored, the effect of toothbrushing frequency on prevention of dental caries is unclear because evidence is inconsistent and conflicting. While David et al. [7] found no association between brushing frequency and caries prevalence and severity, there are studies on dental caries that reported an association between dental caries and toothbrushing habits [31, 34, 35]. People who brush their teeth less than once daily are in higher risk of dental caries compared to those who brush their teeth regularly [35]. Our finding are consistent with all studies but by the David et al. [7].

Fluoride is a caries defensive factor [33] and the daily use of a fluoride-containing toothpaste helps to minimize the risk of developing caries [34–38]. Our results showed that children who used to brush their teeth with fluoridated toothpaste were less likely to have caries lesions of moderate stage.

The present study showed that children who had visited a dentist periodically were less likely to have extensive caries lesions than those who had visited a dentist occasionally or when symptoms of pain existed. Regular dental visits are important as during them caries can be diagnosed, managed and even avoided on time [20, 39].

Our findings suggest that caries experience in the primary dentition seems to be associated with initial caries lesions in newly erupted permanent teeth. Other researchers have found caries experience in deciduous teeth as a risk indicator of the disease in permanent teeth, too [5, 21]. This could be associated with the presence of bad habits acquired in early childhood.

There are some methodological limitations in this study that should be considered. Firstly, the cross-sectional study design measured the cause and effect at the same point in time, thus the directionality of the associations and the time frames of the exposures were not considered. That allows us to discuss only dental caries indicators, leaving risk factors and risk predictors for future longitudinal studies. Secondly, the information on children behavior and habits collected through questionnaires given to parents might be affected by memory recalls or a social desirability bias. Before the questionnaires were administered, parents were informed about the purpose of the questions and the importance of accuracy in their reports. The anonymity and confidentiality of their responses were emphasized, along with the important contribution that their responses would provide, so we do not expect these biases to have a significant impact on the obtained results. Because it was impossible to determine the caries lesion stage that preceded the fillings and extractions of the teeth, the study analyzed only association between caries risk indicators and untreated caries lesions, while other components of caries experience (filled and missing teeth due to caries) were not considered. Regarding the strong points of the study, despite the demanding study-related criteria, this study relied on a large sample size which provided data on the effects of caries indicators on various stages of caries in the study population. The researcher, conducting the clinical examinations, was trained to perform the assessment with calibrated, standardized and sophisticated method such as the ICDAS. This study evaluated the roles of risk indicators considering the disease as a continuous process. That makes our findings novel, important and based on clinically relevant information that can help raise awareness about caries risk indicators that might be responsible for caries occurrence and lesion evolution in the study population. Our findings can also raise awareness of other populations with the same age range, as these factors are highly prevalent and globally relevant. Social-economic, behavioral and nutritional indicators are interconnected and act in synergy. Socioeconomic factors might be modified, but their modification requires time-consuming macro-level

changes. The others could be targeted for modification by directing the limited resources to prevent disease and retard caries lesion evolution as well.

Conclusion

The results of this research, within the limitations described above, provide valuable information on the risk indicators associated with caries lesion stages in newly erupted permanent teeth. Parental educational level, paternal occupational status, sweets and snacks taking frequency, teeth brushing frequency, caries status of deciduous teeth, usage of fluoridated toothpaste, and dental check-ups frequency were involved in caries occurrence and were associated with different caries stages in newly erupted permanent teeth.

REFERENCES

1. *FDI World Dental Federation.* Habib Benzian White paper. Geneve, Switzerland: FDI World Dental Federation; 2015. Available from: https://www.fdiworlddental.org/sites/default/files/media/documents/2015_wohd-whitepaper-oral_health_worldwide.pdf
2. *Lynch RJ.* The primary and mixed dentition, post-eruptive enamel maturation and dental caries: a review. Int Dent J 2013; 63(Suppl 2): 3−13.
3. *Mejare I, Axelsson S, Dahlen G, Espelid I, Norlund A, Tranæus S*, et al. Caries risk assessment. A systematic review. Acta Odontol Scand 2014; 72(2): 81–91.
4. *Bernabé E, Sheiham A.* Age, period and cohort trends in caries of permanent teeth in four developed countries. Am J Public Health 2014; 104(7): e115–21.
5. *Casanova-Rosado AJ, Medina-Solís CE, Casanova-Rosado JF, VallejosSánchez AA, Maupomé G, Ávila-Burgos L.* Dental caries and associated factors in Mexican schoolchildren aged 6-13 years. Acta Odontol Scand 2005; 63(4): 245−51.
6. *Mascarenhas AK.* Oral hygiene as a risk indicator of enamel and dentin caries. Community Dent Oral Epidemiol 1998; 26(5): 331−9.
7. *David J, Wang NJ, Åstrøm AN, Kuriakose S.* Dental caries and associated factors in 12-year-old schoolchildren in Thiruvananthapuram, Kerala, India. Int J Paediatr Dent 2005; 15(6): 420−8.
8. *Beck JD.* Risk revisited. Community Dent Oral Epidemiol 1998; 26(4): 220−5.
9. *Takahashi N, Nyvad B.* Caries ecology revisited: microbial dynamics and the caries process. Caries Res 2008; 42(6): 409–18.
10. *Tagliaferro EPS, Ambrosano GMB, Meneghim MC, Pereira AC.* Risk indicators and risk predictors of dental caries in schoolchildren. J Appl Oral Sci 2008; 16(6): 408–13
11. *Clara J, Bourgeois D, Muller-Bolla M.* DMF from WHO basic methods to ICDAS II advanced methods: a systematic review of literature. Odontol Trop 2012; 35(139): 5–11.
12. *Iranzo-Cortés JE, Montiel-Company JM, Almerich-Silla JM.* Caries diagnosis: agreement between WHO and ICDAS II criteria in epidemiological surveys. Community Dent Health 2013; 30(2): 108–11.
13. *Pitts N.* "ICDAS"–an international system for caries detection and assessment being developed to facilitate caries epidemiology, research and appropriate clinical management. Community Dent Health 2004; 21(3): 193–8.
14. *International Caries Detection and Assessment System Coordinating Committee.* Criteria Manual. International Caries Detection and Assessment System (ICDAS II) Workshop. Baltimore, Md, USA: International Caries Detection and Assessment System Coordinating Committee; 2005.
15. *Topping GVA, Hally JD, Bonner BC, Pitts NB.* Training for the International Caries Detection and Assessment System (ICDAS II): CD-room and web-based educational software. London: Smile-on; 2008.
16. *Landis JR, Koch GG.* The measurement of observer agreement for categorical data. Biometrics 1977; 33(1): 159−74.
17. *Espelid I, Mejàre I, Weerheijm K, EAPD.* EAPD guidelines for use of radiographs in children. Eur J Paediatr Dent 2003; 4(1): 40–8.
18. *Kühnisch J, Ekstrand KR, Pretty I, Twetman S, vanLoveren C, Gizanini S, et al.* Best clinical practice guidance for management of early caries Lesion in children and young adults: an EAPD policy document. Eur Arch Paediatr Dent 2016; 17: 3−12.
19. *Cagetti MG, Congiu G, Cocco F, Meloni G, Sale S, Campus G.* Are distinctive risk indicators associated with different stages of caries in children? A cross-sectional study. BMC Public Health 2016; 16(1): 1213.
20. *Jürgensen N, Petersen PE.* Oral health and the impact of sociobehavioural factors in a cross sectional survey of 12- year old school children in Laos. BMC Oral Health 2009; 9: 29.
21. *Christensen LB,Twetman S,Sundby A.* Oral health in children and adolescents with different socio-cultural and socioeconomic backgrounds. Acta Odontol Scand 2010; 68(1): 34−42.
22. *Edelstein BL.* Disparities in oral health and access to care: Findings of national surveys. Ambul Pediatr 2002; 2(2 Suppl): 141–7.
23. *Raj R, Vaibhav V.* Maternal factors and child oral health. Int J Health Sci Res 2012; 8: 102−6.
24. *Rajab LD, Petersen PE, Bakaeen G, Hamdan MA.* Oral health behaviour of schoolchildren and parents in Jordan. Int J Paediatr Dent 2002; 12(3): 168–76.
25. *Lynch J, Kaplan G.* Socioeconomic position. In: *Berkman LF, Kawachi I,editors.* Social epidemiology. New York: Oxford Press; 2000. p. 13–35.
26. *Costa SM, Martins CC, Bonfim Mde L, Zina LG, Paiva SM, Pordeus IA,* et al. Asystematic review of socioeconomic indicators and dental caries in adults. Int J Environ Res Public Health 2012; 9(10): 3540–74.
27. *Enjary C, Tubert-Jeannin S, Manevy R, Roger-Leroi V, Riordan PJ.* Dental status and measures of deprivation in Clermont-Ferrand, France. Community Dent Oral Epidemiol 2006; 34(5): 363–71.
28. *World Health Organization.* Sugars intake for adults and children. Geneva: World Health Organization; 2015.
29. *Moynihan PJ, Kelly SA.* Effect on caries of restricting sugars intake: systematic review to inform WHO guidelines. J Dent Res 2014; 93(1): 8–18.
30. *Gustafsson BE, Quensel CE, Lanke LS, Lundquist C, Grahnen H, Bonow BE,* et al. The Vipeholm dental caries study; the effect of different levels of carbohydrate intake on caries activity in 436 individuals observed for five years. Acta Odontol Scand 1954; 11(3−4): 232–64.
31. *Iftikhar A, Zafar M, Kalar MU.* The relationship between snacking habits and dental caries in school children. Int J Collab Res Intern Med Public Health 2012; 4(12): 1943–51.
32. *Chankanka O, Marshall TA, Levy SM, Cavanaugh JE, Warren JJ, Broffitt B,* et al. Mixed dentition cavitated caries incidence and dietary intake frequencies. Pediatr Dent 2011; 33(3): 233–40.

33. *Graetz C, Bielfeldt J, Wolff L, Springer C, El-Sayed KM, Sälzer S,* et al. Toothbrushing education via a smart software visualization system. J Periodontol 2013; 84(2): 186–95.
34. *Macpherson LM, Anopa Y, Conway DI, McMahon AD.* National supervised tooth brushing program and dental decay in Scotland. J Dent Res 2013; 92(2): 109–13.
35. *Alhabdan YA, Albeshr AG, Yenugadhati N, Jradi H.* Prevalence of dental caries and associated factors among primary school children: a population-based cross sectional study in Riyadh, Saudi Arabia. Environ Health Prev Med 2018; 23(1): 60.
36. *Kumar S, Tadakamadla J, Johnson NW.* Effect of Toothbrushing Frequency on Incidence and Increment of Dental Caries: A Systematic Review and Meta-Analysis. J Dent Res 2016; 95(11): 1230−6.
37. *Fejerskov O, Cury JA, Tenuta LM, Marinho VC.* Fluorides in caries control. In: *Fejerskov O, Nyvad B, Kidd E* editors. Dental caries: The disease and its clinical management. 3rd ed. Carribean, USA, Canada: Wiley Blackwell; 2015. p. 245−72.
38. *Hellwig E. Lennon AM.* Systemic versus topical fluoride. Caries Res 2004; 38(3): 258−62.
39. *Skeie MS, Raadal M, Strand GV, Esoelid I.* The relationship between caries in the primary dentition at 5 years of age and permanent dentition at 10 years of age – a longitudinal study. Int J Paediatr Dent 2006; 16(3): 152−60.

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Anex

Questionnaire for parents

Name of a child:_____

1. Gender of the child: □ male; □ female

2. How old is the child? .....

3. What level of education did the mother complete?

□ Primary school

□ High school

□ Faculty

4. What level of education did the father complete?

□ Primary school

□ High school

□ Faculty

5. What is mother's work status?

□ Employed

□ Unemployed

□ Self-employed

6. What is father's work status?

□ Employed

□ Unemployed

□ Self-employed

7. How many main meals does the child have during the day?

□ Less than three

□ Three to five

□ More than five

8. Does your child consume sweets, snacks and sweet beverages between main meals?

□ Yes

□ No

9. How often does the child consume sweets and snacks?

□ Never or rarely

□ Two to five times a day

□ More than five times a day

10. How often does the child consume sugary drinks?

□ Never or rarely

□ Two to five times a day

□ More than five times a day

11. Does your child brush his/her teeth?

□ Yes

□ No

12. How often does the child brush his/her teeth?

□ Less than once a day

□ Once to twice a day

□ More than twice a day

13. How long does the child brush their teeth?

□ Less than three minutes

□ Three minutes or longer

14. Does your child use toothpaste?

□ Yes

□ No

15. Does your child use fluoride toothpaste?

□ Yes

□ No

16. Does the child use fluoride mouthwash?

□ Yes

□ No

17. Has the child ever visited a dentist?

□ Yes

□ No

18. How often does your child visit a dentist?

□ He/ she regularly goes to dental examinations

□ Occasionally

□ Only when there is pain

19. Does your child suffer from a systemic disease or does the child have any health problem?

□ Yes

□ No

Thank you for your time to fill in this questionnaire. Please submit the completed questionnaire to a member of the research team who will do the clinical examinations of the children.

If you have any comments, please write below.

SHORT COMMUNICATION

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Complete denture parameters reflecting the satisfaction of dentists and patients

Parametri totalne proteze koji odražavaju zadovoljstvo stomatologa i pacijenata

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Abstract

Background/Aim. Patients' expectations with regard to their complete dentures are usually high. It is quite challenging to meet the patient's criteria for denture satisfaction. Moreover, it also becomes a challenging task when the patient's expectation exceeds the patient's satisfaction regardless of the denture quality. The aim of this study was to determine the correlation in the denture satisfaction levels between the dentist and complete denture wearers. **Methods**. The study included 134 completely edentulous participants, between 48 and 65 years of age. The data were recorded through a validated questionnaire having a Cronbach α validity of 85%. Sociodemographic data, patients' priorities, dentists' and patients' satisfaction with the dentures were recorded and rated on a Likert scale (1 to 5). Spearman's correlation was applied to measure the similarity between the dentist's and patient's satisfaction score. **Results**. The mean dentists' and patients' overall satisfaction scores were calculated as 3.58 ± 0.74 and 3.538 ± 0.521, respectively. Participants ≥ 60 years of age and females showed a higher correlation than younger patients and men, while the employed participants showed a stronger correlation between dentist's and patient's satisfaction, compared to the opposite. In the educated participants a positive, moderate correlation between dentist's and patient's satisfaction was shown. The correlation between dentist's satisfaction with occlusion and patient's satisfaction with comfort was moderately strong ($p < 0.05$); weakly positive correlation ($p < 0.05$) was found between dentist's satisfaction with the quality of denture and extension and patient's satisfaction with aesthetics, mastication, phonetics, and comfort. **Conclusion**. A positive correlation between the dentists' and patients' satisfaction regarding denture quality was shown in older-age patients, females, as well as employed and educated patients. Improving dentist-patient communication is the most useful strategy to improve patients' satisfaction with their dentures.

Key words:
dentists; denture, complete; tooth, aesthetics; mastication; edentulism; patient, satisfaction; speech, production, measurement; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Očekivanja pacijenata u pogledu totalne proteze obično su visoka i ispunjavanje njihovih kriterijuma predstavlja izazov. Kada, bez obzira na kvalitet proteze, očekivanje pacijenta prevazilazi njegovo zadovoljstvo, to postaje i izazovan zadatak. Cilj rada bio je da se utvrdi korelacija u nivou zadovoljstva protezom između stomatologa i korisnika zubnih proteza. **Metode.** Studijom su obuhvaćena 134 kompletno bezuba ispitanika, starosti 48–65 godina. Podaci su prikupljani validiranim upitnikom koji je imao Cronbach-ov α koeficijent pouzdanosti od 85%. Sociodemografski podaci, prioriteti pacijenata, zadovoljstvo stomatologa i zadovoljstvo pacijenata protezama beleženi su i ocenjivani prema Likertovoj skali, od 1 do 5. Za merenje sličnosti između zadovoljstva stomatologa i pacijenata primenjivana je Spirmanova korelacija. **Rezultati.** Prosečni skorovi ukupnog zadovoljstva stomatologa i pacijenata iznosili su 3,58 ± 0,74 i 3,538 ± 0,521, redom. Kod ispitanika starijih od 60 godina i osoba ženskog pola ustanovljena je veća korelacija između zadovoljstva protezom pacijenata i stomatologa u odnosu na mlađe pacijente i ispitanike muškog pola. Jača korelacija između zadovoljstva stomatologa i pacijenata protezom pokazana je i kod zaposlenih ispitanika, u poređenju sa nezaposlenim ispitanicima. Kod obrazovanih ispitanika ustanovljena je umerena, pozitivna korelacija između zadovoljstva protezom stomatologa i pacijenta. Korelacija između zadovoljstva stomatologa okluzijom i komfora pacijenta bila je umereno jaka ($p < 0,05$). Nađena je slabo pozitivna korelacija ($p < 0,05$) između zadovoljstva stomatologa kvalitetom proteze i ekstenzijom i zadovoljstva pacijenta protezom s obzirom na estetiku, žvakanje, fonetiku i komfor. **Zaključak.** Utvrđena je pozitivna povezanost između zadovoljsta protezom pacijenata i stomatologa kod ispitanika starijeg životnog doba, kod

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žena, kao i kod zaposlenih i obrazovanih pacijenata. Poboljšanje komunikacije između stomatologa i pacijenta je najkorisnija strategija za poboljšanje zadovoljstva pacijenata njihovim protezama.

Ključne reči:
stomatolozi; zubna proteza, totalna; zub, estetika; žvakanje; bezubost; pacijent, zadovoljstvo; govor, produkcija, merenje; ankete i upitnici.

Introduction

Edentulism affects oral health in a diverse manner. However, phonetics, mastication, comfort, and aesthetics are the major constraints disturbing the quality of life [1]. Al Hamdan and Fahmy [2] reported around 82% of edentulism in Riyadh, Saudi Arabia. It is highly challenging to meet patient's criteria of denture satisfaction. With older age, the satisfaction level becomes very high, making it difficult to convince the patient to adapt to their artificial dentures [3]. Different parameters affecting a patient's satisfaction with a new complete denture and satisfaction of the dentist have been investigated [4].

It has been evident that implant-supported overdentures gain higher satisfaction compared to complete dentures [5]. However, most patients prefer complete dentures over the costly prosthesis. It is also noted that the satisfaction levels are highly associated with age, and that physiological and neurological factors greatly impact patient's satisfaction [6]. When patients present with resorbed ridges, it becomes difficult to provide retention in a denture and, hence, patient's and dentist's satisfactions are compromised [7]. Moreover, saliva and other systemic conditions also play an important role [8]. Bilhan et al. [9] concluded that 85% of patients lack denture retention, whereas the highest mean satisfaction score for speech was 77.27 ± 29.04.

It becomes a challenging task when the patient's expectation exceeds the patient's satisfaction regardless of the denture quality. Hence, the dissatisfied patient reflects the failure of the treatment plan [10]. Smith [11] and Berg [12] also revealed that prediction of patient's satisfaction does not correlate with the quality of the denture. In a Brazilian study, most patients complained about phonetics and mastication being very challenging to them post edentulism [13].

It is therefore very important to establish open communication with a patient and to become familiar with the patient's expectations regarding dentures. Having one-to-one interaction with patients and highlighting all the possible outcomes for denture fabrication will help patient's expectations become more realistic and practical, reflecting a positive impact on their overall satisfaction [13]. Another study conducted in 2003 in Croatia, measured using Spearman's rank correlation, ascertained the strongest correlation between dentist's satisfaction and patient's satisfaction, as well as with the retention of complete dentures [14].

Literature has revealed that the success of the treatment is a reflection of the patient's satisfaction and should be evaluated by the patient himself/herself. The chief purpose to accomplish successful treatment is to prioritise and fulfil the patient's demands. However, the assessment criteria lack patients' needs and priorities, and are attributed to the existing criteria [15]. Unfortunately, due to the paucity of local data, most studies have found out the association between patient's satisfaction and complete denture therapy with different factors influencing satisfaction levels. Nevertheless, this study has attempted to prioritise patients' needs in terms of four important parameters that are likely required and to discover the correlation between patient's and dentist's satisfaction levels. The aim of this study was to determine the difference in the denture satisfaction levels between the dentists and complete denture wearers in order to draw a paradigm of satisfaction parameters that could be incorporated in future denture fabrications.

Methods

The present study was a cross-sectional study conducted at the Department of Prosthodontics at King Khalid University in the period from 2016 to 2018. The patients were enlisted through a nonprobability consecutive sampling technique. The sample size was estimated using a sample size calculator for the correlation between the quality of the mandibular denture-bearing area and retention as -0.301 [14], power of test as 80% and 99% confidence level. The estimated sample size was 124. After inflating the sample size by 8% for the lost to follow-up, we included 134 participants in the present study. Ethical approval and implied consent were obtained. Edentulous patients aged between 48 and 65 years of either gender participated in the study. The patients had their blood pressure under control. Also, diabetic patients, with no other systemic disease, were included in the study. The patients who were mentally incapacitated were excluded from the study.

The data were collected through a validated questionnaire having a Cronbach α validity of 85%, which were divided into sociodemographic, patient's priority, dentist satisfaction through denture assessment, and patient satisfaction. Dentist's and patient's satisfactions were rated on a Likert scale (1 to 5), with 5 = very satisfied, 4 = satisfied, 3 = neither satisfied nor dissatisfied, 2 = dissatisfied, and 1 = very dissatisfied.

SPSS version 23 was used to analyse the data. Mean and standard deviation (SD) were calculated for quantitative variables, whereas frequencies and percentages were calculated for qualitative variables. The Spearman's correlation was applied to assess the strength of the relationship between dentist's and patient's satisfaction scores. Furthermore, stratification with respect to effect modifiers such as age, gender, employment, and educational status was done. Poststratification Spearman's correlation was applied to assess the strength of the relationship between dentist's and patient's satisfaction score. $P < 0.05$ was taken as statistically significant.

Results

There were 134 participants included in the study. The average age was 58.75 years; most patients were males (56.7%), whereas 43.3% were females. About 98 participants were unemployed and 8 participants were uneducated. According to patient's priority, most of them preferred mastication, followed by aesthetics, phonetics, and comfort prior to fabrication of dentures (Table 1).

The mean dentist's and patient's overall satisfaction scores were calculated as 3.58 ± 0.74 and 3.538 ± 0.521, respectively. Weak, positive correlations were found between dentist's satisfaction with the quality of denture and extension, and patient's satisfaction with denture characteristics such as aesthetics, mastication, phonetics, and comfort ($p < 0.05$). A weak, positive correlation was found between the dentist's satisfaction with denture extension and patient's satisfaction with denture aesthetics, mastication, and phonetics, whereas no meaningful correlation was found between dentist's satisfaction with denture extension and patient's satisfaction with denture comfort (Table 2). A weak, positive correlation was found between vertical relation and patient's aesthetics, mastication, and comfort, whereas no meaningful correlation was found between vertical relation and patient's phonetics. A very weak, positive correlation (r = 0.171), although significant ($p < 0.05$) was found between dentist's satisfaction with denture occlusion and patient's satisfaction with aesthetics, whereas weak, positive correlations were found between dentist's satisfaction with denture occlusion and patient's satisfaction with mastication and phonetics (r = 0.353 and r = 0.269, respectively). The correlation between dentist's satisfaction with denture occlusion and the patient's satisfaction with comfort was moderately strong (r = 0.444, $p < 0.001$) (Table 2).

With respect to age, the participants ≥ 60 years of age showed a higher value of correlation between the dentist's satisfaction and patient's satisfaction with denture, compared to the participants younger than 60 years. With respect to gender, females showed a higher value of correlation between dentist's and patient's satisfaction, compared to males. With respect to the employment status, the employed participants showed a stronger correlation between dentist's and patient's satisfaction, compared to the unemployed patients. Lastly, the educated participants showed a positive, moderate correlation between dentist's and patient's satisfaction, whereas this correlation in the uneducated participants was insignificant (Table 3).

Table 1

Baseline features of participants (n = 134)

Variables	Values
Age (years), mean ± SD	58.57 ± 7.25
Gender, n (%)	
male	76 (56.7)
female	58 (43.3)
Employment status, n (%)	
employed	36 (26.9)
unemployed	98 (73.1)
Educational status, n (%)	
educated	126 (94.0)
uneducated	8 (6.0)
Patient's priority, n (%)	
aesthetics	40 (29.9)
mastication	62 (46.3)
phonetics	18 (13.4)
comfort	14 (10.4)

SD – standard deviation.

Table 2

Correlation analysis between dentist's and patient's satisfaction with denture parameters

Dentist's satisfaction parameters	Patient's satisfaction parameters, r (*p*)			
	Aesthetics	Mastication	Phonetics	Comfort
Quality of denture	0.223 (0.010)	0.245 (0.004)	0.368 (0.000)	0.205 (0.018)
Extension	0.268 (0.002)	0.218 (0.011)	0.301 (0.001)	0.155 (0.074)
Vertical relation	0.277 (0.001)	0.355 (0.001)	0.056 (0.521)	0.260 (0.002)
Occlusion	0.171 (0.049)	0.353 (0.000)	0.269 (0.002)	0.444 (0.001)

r – Spearman's coefficient of correlation (rho).

Table 3
Stratification of patients with respect to age, gender, employment and educational status

Variables	Correlation between dentist's and patient's satisfaction overall score, r (*p*)
Age group	
< 60 years (n = 89)	0.553 (0.001)
≥ 60 years (n = 45)	0.577 (0.001)
Gender	
male (n = 76)	0.551 (0.001)
female (n = 58)	0.586 (0.001)
Employment status	
unemployed (n = 98)	0.563 (0.001)
employed (n = 36)	0.635 (0.001)
Educational status	
uneducated (n = 8)	0.115 (0.393)
educated (n = 126)	0.581 (0.001)

r – Spearman's coefficient of correlation (rho).

Discussion

In the present study, the Spearman's correlation was applied between the dentist's and patient's satisfaction parameters. The patients in this study preferred chewing as the main reason to visit the dentist for complete denture treatment, as old patients are likely to consider that treatment is required if they experience difficulty in chewing, or a social embarrassment, which is also validated by de Souza et al. [16] and Kossioni and Bellou [17].

A study evaluated problems experienced by patients after denture insertion. The most common complaint was inability to chew appropriately, and when the denture was examined, it was short of retention, which is a possible cause of dissatisfaction for both dentist and patients [9]. Hence, the present study showed that, nevertheless, a weak but positive correlation existed between the dentist's satisfaction with denture quality and extension and patient's satisfaction with aesthetics, mastication, and phonetics ($p < 0.05$).

Yoshida et al. [18] evaluated the correlation between the satisfaction with daily life and satisfaction with complete denture. The study showed that patients who were satisfied with their life were also satisfied with their complete dentures (a strong positive correlation, $p < 0.05$). This can be explained in terms of social and cultural factors, which had a great impact on denture satisfaction; a stress-free life led to higher denture satisfaction scores. In a study by Sato et al. [19], the multivariate regression analysis and χ^2 test were used to assess the relation between denture satisfaction and contributing factors. The study showed significant results and the findings were in complete agreement with the results of the present study showing a strong association between satisfaction and retention of upper dentures, followed by a positive correlation with speech and tasting.

The results of the present study also concur with those reported by Epifania et al. [20]. They studied the quality of complete denture and the satisfaction of patients, and concluded that there was a strong relationship between the quality of denture and patient's satisfaction. In short, the adequate extension and retention of the denture was associated with patient's satisfaction. Other researchers also found a positive correlation between the denture quality and patient's satisfaction [21–23]. Conversely, Anastassiadou et al. [24] have found a weak correlation between denture quality and patient satisfaction. Furthermore, the studies carried out by Erić et al. [25] and Fenlon and Sherriff [26] found absolutely no correlation between the denture quality and patient's satisfaction.

In the present study, with respect to gender, females displayed a high value of association between dentist's and patient's satisfaction, compared to males. Similarly, females were more likely to be satisfied with their aesthetics. However, females were less satisfied with mastication, which might be due to their need to seek treatment solely for aesthetic purposes [27–30]. In another study, the results showed that most patients were satisfied with their aesthetics and that there was no difference between men and women. However, that study also assessed the expectation levels and found that men had higher expectations of their dentures, compared to females [3].

Within the limitation of this study, we recommend evaluating more in-depth contributing factors and their correlation regarding patient's and dentist's satisfaction. Furthermore, a large sample size for future studies would also help to obtain more accurate results.

Conclusion

Generally, the patients were satisfied with the quality of their complete dentures. Positive correlations existed between dentist's satisfaction with denture quality, vertical relation and occlusion on the one hand, and patient's satisfaction with aesthetics, mastication, phonetics, and comfort on the other. A positive, higher correlation existed between dentist's and patient's satisfaction in old-age patients, females, employed and educated patients. Thus, improving dentist-patient communication is the most useful strategy to improve patients' satisfaction with their dentures.

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R E F E R E N C E S

1. *Felton DA.* Edentulism and comorbid factors. J Prosthodont 2009; 18(2): 88−96.
2. *Al Hamdan E, Fahmy MM.* Socioeconomic factors and complete edentulism for female patients at King Saud University, Riyadh, Saudi Arabia. Tanta Dent J 2014; 11(3): 169−73.
3. *McCunniff M, Liu W, Dawson D, Marchini L.* Patients' esthetic expectations and satisfaction with complete dentures. J Prosthet Dent 2017; 118(2): 159−65.
4. *Marchini L.* Patients' satisfaction with complete dentures: an update. Braz Dent Sci 2014; 17(4): 5−16.
5. *Gjengedal H, Berg E, Gronningsaeter AG, Dahl L, Malde MK, Boe OE*, et al. The influence of relining or implant retaining existing mandibular dentures on health-related quality of life: a 2-year randomized study of dissatisfied edentulous patients. Int J Prosthodont 2013; 26(1): 68−78.
6. *Hantash RO, AL-Omiri MK, Yunis MA, Dar-Odeh N, Lynch E.* Relationship between impacts of complete denture treatment on daily living, satisfaction and personality profiles. J Contemp Dent Pract 2011; 12(3): 200−7.
7. *Yamaga E, Sato Y, Minakuchi S.* A structural equation model relating oral condition, denture quality, chewing ability, satisfaction, and oral health-related quality of life in complete denture wearers. J Dent 2013; 41(8): 710−7.
8. *Musa I, Knezović-Zlatarić D, Čelebić A, Bošnjak A.* The influence of gender and age on the values of linear radiomorphometric indices measured on the lower border of the mandible. Acta Stomatol Croat 2002; 36(2): 199−202.
9. *Bilhan H, Geckili O, Ergin S, Erdogan O, Ates G.* Evaluation of satisfaction and complications in patients with existing complete dentures. J Oral Sci 2013; 55(1): 29−37.
10. *Marachlioglou CR, Dos Santos JF, Cunha VP, Marchini L.* Expectations and final evaluation of complete dentures by patients, dentist and dental technician. J Oral Rehabil 2010; 37(7): 518−24.
11. *Smith M.* Measurement of personality traits and their relation to patient satisfaction with complete dentures. J Prosthet Dent 1976; 35(5): 492−503.
12. *Berg E.* The influence of some anamnestic, demographic, and clinical variables on patient acceptance of new complete dentures. Acta Odontol Scand 1984; 42(2): 119−27.
13. *Silva JCM, Santos JFF, Marchini L.* Factors influencing patients' satisfaction with complete dentures: a qualitative study. Braz Dent Sci 2014; 17(2): 83−8.
14. *Čelebić A, Knezović-Zlatarić D, Papić M, Carek V, Baučić I, Stipetić J.* Factors related to patient satisfaction with complete denture therapy. J Gerontol A Biol Sci Med Sci 2003; 58(10): M948−53.
15. *Hlatky MA.* Patient preferences and clinical guidelines. JAMA 1995; 273(15): 1219−20.
16. *de Souza FI, de Souza Costa A, Dos Santos Pereira R, Dos Santos PH, de Brito RB Jr, Rocha EP.* Assessment of satisfaction level of edentulous patients rehabilitated with implant-supported prostheses. Int J Oral Maxillofac Implants 2016; 31(4): 884−90.
17. *Kossioni A, Bellou O.* The effect of aging and dental status on the frequency of eating out. Arch Gerontol Geriatr 2012; 54(2): e130−3.
18. *Yoshida M, Sato Y, Akagawa Y, Hiasa K.* Correlation between quality of life and denture satisfaction in elderly complete denture wearers. Int J Prosthodont 2001; 14(1): 77−80.
19. *Sato Y, Hamada S, Akagawa Y, Tsuga K.* A method for quantifying overall satisfaction of complete denture patients. J Oral Rehabil 2000; 27(11): 952−7.
20. *Epifania E, Sanzullo R, Sorrentino R, Ausiello P.* Evaluation of satisfaction perceived by prosthetic patients compared to clinical and technical variables. J Int Soc Prev Community Dent 2018; 8(3): 252−8.
21. Tôrres ACSP, Maciel AQ, de Farias DB, de Medeiros AKB, Vieira FPTV, Carreiro ADFP. Technical quality of complete dentures: Influence on masticatory efficiency and quality of life. J Prosthodont 2019; 28(1): e21−6.
22. *Alfadda SA.* The relationship between various parameters of complete denture quality and patients' satisfaction. J Am Dent Assoc 2014; 145(9): 941−8.
23. *Cerutti-Kopplin D, Emami E, Hilgert JB, Hugo FN, Rivaldo E, Padilha DMP.* Predictors of Satisfaction with Dentures in a Cohort of Individuals Wearing Old Dentures: Functional Quality or Patient-Reported Measures? J Prosthodont 2017; 26(3): 196−200.
24. *Anastassiadou V, Robin Heath M.* The effect of denture quality attributes on satisfaction and eating difficulties. Gerodontology 2006; 23(1): 23−32.
25. *Erić J, Tihaček Šojić L, Bjelović L, Tsakos G.* Changes in Oral Health Related Quality of Life (OHRQoL) and Satisfaction with Conventional Complete Dentures Among Elderly People. Oral Health Prev Dent 2017; 15(3): 237−44.
26. *Fenlon MR, Sherriff M.* Investigation of new complete denture quality and patients' satisfaction with and use of dentures after two years. J Dent 2004; 32(4): 327−33.
27. *Pan S, Awad M, Thomason JM, Dufresne E, Kobayashi T, Kimoto S,* et al. Sex differences in denture satisfaction. J Dent 2008; 36(5): 301−8.
28. *Awad MA, Feine JS.* Measuring patient satisfaction with mandibular prostheses. Community Dent Oral Epidemiol 1998; 26(6): 400−5.
29. *Ishikawa M, Sato Y, Kitagawa N, Shichita T, Isshiki Y, Kawata D*, et al. Effectiveness of a clinical pathway for complete denture adjustment. J Oral Sci 2019; 61(4): 483–90.
30. *Carletti TM, Pinheiro MA, Meira IA, Amaral CF, Rodrigues Garcia RCM.* Prostheses satisfaction and diet of elderly wearing a single implant overdenture: A six-month assessment. Spec Care Dentist 2019; 39(5): 471–7.

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The place of medical treatment of acromegaly in Serbia: current status

Mesto medikamentne terapije akromegalije u Srbiji – aktuelno stanje

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Key words:
acromegaly; combined modality therapy; dopamine agonists; drug therapy; pasireotide; pegvisomant; radiotherapy; somatostatin; treatment outcome.

Ključne reči:
akromegalija; lečenje kombinovanjem lekova; dopamin, agonisti; lečenje lekovima; pasireotid; pegvisomant; radioterapija; somatostatin; lečenje, ishod.

Introduction

Acromegaly is a chronic, multisystemic disease, caused in 98% of cases by a somatotroph pituitary adenoma [1, 2]. Secretory hyperactivity of somatotroph adenoma results in the abnormal serum concentration of growth hormone (GH) which either directly, or more often through its physiological mediator – insulin like growth factor-I (IGF-1), causes the spectrum of complications of this disease. The prevalence of acromegaly in Europe is estimated to 28–137 cases per million, and the estimation of annual incidence varies from 2 to 11 patients per million [3, 4]. Due to its slow onset and insidious progression, acromegaly often remains long unrecognized in spite of presence of signs and symptoms. Time from first symptoms to diagnosis is estimated as 5–10 years [2, 5]. Acromegaly caused by diseases other than somatotropinoma is exceptional. These rare cases include ectopic GH or GH-releasing hormone (GHRH) secretion from lung, pancreas, adrenal or mediastinal tumors [6]. Over the past 20 years, genetic background has been elucidated for some forms of acromegaly, occurring syndromicaly within MEN 1, MEN 4, McCune Albright or Carney complex or as part of isolated familial pituitary adenoma (FIPA) [7, 8].

Along the hallmark signs and symptoms of acromegaly, such as enlargement of hands, feet, nose and ears, facial soft tissue swelling or mandible protrusion, the most frequent complications of the disease are: diabetes mellitus, arterial hypertension, cardiovascular diseases (heart failure, arrhythmias, atherosclerosis, endothelial dysfunction), articular deformities in large joints, vertebral fractures (with or without osteoporosis), respiratory disfunction (obstructive sleep apnea syndrome) and thyroid, colon or prostate neoplasia [1, 9–14]. Patients with active acromegaly are attributed with a 2 to 3-fold increase in mortality rate compared to general population. The average life expectancy in these patients is reduced by an average of 10 years compared to healthy controls. The leading causes of death in this group are malignancies, cardiovascular and respiratory diseases [15]. An adequate control of acromegaly enables prevention or attenuation of the disease complications and converging of the mortality rate of these patients to the one in general population [4, 16].

The goals in acromegaly treatment are: normalization of serum IGF-1 (for the age specific reference range), achieving serum GH < 1 μg/L, reduction of pituitary tumor mass or its GH-secreting remnant, elimination or reduction of disease symptoms and comorbidities [17, 18]. The treatment of acromegaly includes a combination of several modalities: neurosurgical operation, medical treatment and radiotherapy. Over the last two decades a significant advancement was made in the field of acromegaly medications development, promoting a dramatical improvement in the treatment outcomes in acromegaly. Along with the contemporary internationally accepted guidelines for acromegaly treatment [19, 20], all of the aforesaid treatment modalities are in use in the Republic of Serbia. The emerging availability of novel medical options in Serbia, raises the need for generating recommendations for the place and role of each specific treatment option. Treating acromegaly is a multidisciplinary task. The key decisions should be made by an interdisciplinary team including a neuroendocrinologist, neurosurgeon, pathologist, radiologist and geneticist as needed.

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Surgical treatment

Operation of GH secreting pituitary tumor represents the first line of treatment of acromegaly both worldwide and in Serbia. Somatotropinomas are operated by transsphenoidal approach in more than 90% of cases. Operative outcome is primarily dependent on the experience and skill of neurosurgeon, and on size and propagation of the tumor. A neurosurgeon is recognized as an expert in pituitary surgery if performing more than 200 transsphenoidal pituitary surgeries annually [21]. The surgical remission of acromegaly is usually defined by age-related normal IGF-1 and a random serum GH, or OGTT-nadir GH of < 1 μg/L – assessed 3 months postoperatively. In specialized pituitary neurosurgery units, remission is achieved in 75%–90% of microadenomas and 45%–70% of macroadenomas [22]. Repeated pituitary surgery should be considered in consensus of all members of pituitary multidisciplinary expert team involved in the treatment of patient. Decision on reoperation is based on disease activity, size and location of tumor remnant, optic chiasm compromisation, and the response to medical treatment. Remission achievement after the second operation is reported in about one half of cases [23].

Medical treatment

Since overall in about 60% of acromegaly patients biochemical control of disease is not achieved by operation, the care is continued by medical therapy which represent the second line of treatment [7, 20]. Medical treatment of acromegaly encompasses three groups of drugs: 1) somatostatin receptor ligands of 1st generation (SRL-fg): [octreotide long-acting release (LAR) and lanreotide autogel] or 2nd generation (pasireotide LAR); 2) dopamine agonists – DA (bromocriptine, cabergoline) and 3) GH-receptor antagonists (pegvisomant) (Figure 1).

Somatostatin receptor ligands – first generation

Somatostatin receptor ligands of 1st generation, which are in use in Serbia for treatment of acromegaly are octreotide LAR and lanreotide autogel. These drugs consist the first line of medical treatment and are approved for treatment of acromegaly since 2004. [7]. These are synthetic long acting agonists of somatostatin receptor, with high affinity for subtype 2 of somatostatin receptor (SSTR-2) and a lesser affinity for subtype 5 of somatostatin receptor (SSTR-5). Availa-

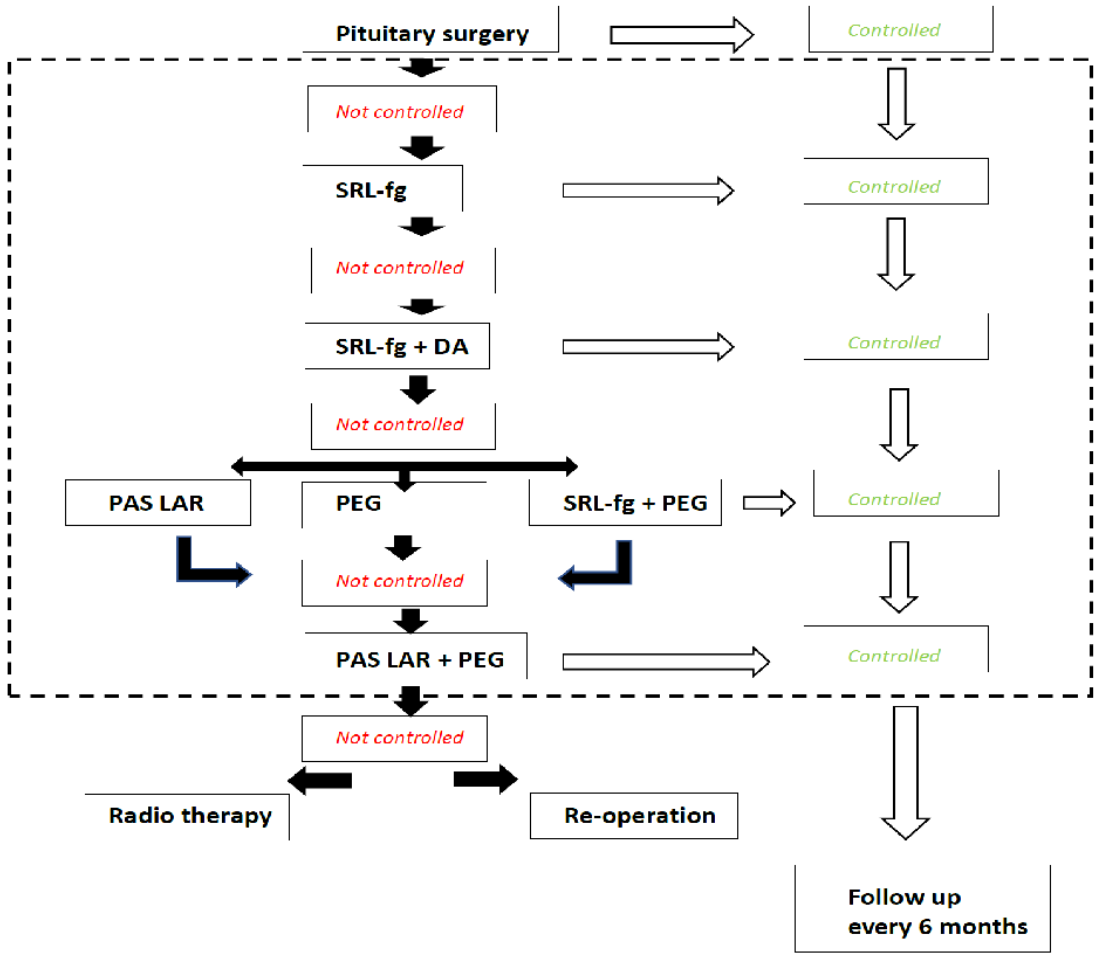

Fig. 1 – Recommended decision sequence in medical treatment of acromegaly and its place within acromegaly multimodal treatment algorithm.
PAS LAR – pasireotide long-acting release; SRL-fg – somatostatin receptor ligands of first generation; DA – dopamine agonists; PEG – pegvisomant.

ble drugs and doses in Serbia include octreotide LAR (20 mg and 30 mg) and lanreotide autogel (90 mg and 120 mg), both applied once in 28 days as an intramuscular injection and subcutaneous injection, respectively. The latest recommendation, also observed in Serbia, advises a maximal initial dose of these medications (octreotide LAR 30 mg or lanreotide LAR 120 mg) over the first 6 months of treatment, followed by optional dose reduction or an increase in dosing intervals (e.g. lanreotide autogel once in 56 days, instead of 28 days) after the achievement of biochemical disease control [24]. The first control of GH and IGF-1 is scheduled for 3 months after the SRL-fg treatment initiation. In the case of inefficiency of one of the drugs from this group, the switch to the other SRL-fg should be tried [25]. Overall, no superiority in efficacy was established for one of SRL-fg over the other [26]. These drugs effectively inhibit GH synthesis and somatotroph cells proliferation, thus inducing reduction in tumor size. A complete biochemical response to SRL-fg is defined by serum GH of < 1 μg/L and IGF1 normalization, and it is achieved in 30% of treated patients. A partial response is defined by a reduction in GH and/or IGF-1 for ≥ 50% from baseline, and it is achieved in 50% of patients. Resistance to SRL-fg is characterized by a decrease in serum GH and IGF1 for < 50% from baseline, and it is observed in 20% of patients [27]. Decrease in tumor remnant size for > 20% is observed in 65% of patients treated with SRL-fg. Reduction of tumor size is expected after 6 months of treatment [28]. SRL-fg treatment is generally well tolerated and these drugs are believed to have a good safety profile. Side effects of these drugs are mostly associated with gastrointestinal impairment (nausea, abdominal pain, diarrhea, gall bladder stones or sludge, constipation, malabsorption, liver function derangements) [29]. Cholelithiasis occurs in about 30% of patients treated with SRL-fg, usually in the first two years of treatment, rarely demanding cholecystectomy. Glucose tolerance impairment is observed in about 30% of patients, while in less than 5% either bradycardia, hypertension or anemia are reported [30]. Dose reduction usually leads to resolution of side effects.

Indications for SRL-fg treatment include conditions when: remission of acromegaly was not achieved by surgery; somatotroph adenoma with extensive propagation into cavernous sinuses makes the expectance of surgical effect unlikely; lack of effect of radiotherapy (as assessed 5 to 10 years after stereotactic treatment).

Pasireotide LAR

SRL of second generation (SRL-sg), pasireotide LAR, represents the second line of medical treatment in acromegaly, reserved for patients which partially respond to SRL-fg (Figure 1). This drug received approval for treatment of acromegaly in 2014 by European Medicine Agency (EMA) and Food and Drug Administration (FDA) [7]. Pasireotide LAR is a multireceptor somatostatin ligand with the highest affinity for somatostatin receptor subtype SSTR-5, followed by the SSTR-1, and with a lesser affinity for SSTR-2 in comparison to the SRL-fg (octreotide and lanreotide). Owing to the greater number of the receptors to which it binds, pasireotide LAR assures a better clinical effect compared to SRL-fg. Results of clinical studies involving this drug demonstrate IGF-1 normalization in 20% of patients resistant to SRL-fg treatment [31]. Pasireotide LAR also exhibits a better antitumor effect compared to SRL-fg, reducing the tumor size by 40% in about 80% of treated patients. All these characteristics make pasireotide LAR the drug of choice for acromegaly patients with a tumor remnant in proximity to optic chiasm [32]. Pasireotide LAR is available world wide in doses of 10 mg, 20 mg, 30 mg, 40 mg and 60 mg, applied as an *im* dose once in 4 weeks. In Serbia, pasireotide LAR is currently available in the 40 mg dose, which is the initial dose for treatment, while 60 mg is the maximal monthly dose of this drug.

The treatment with pasireotide LAR is considered in the following cases: unsuccessful SRL-fg treatment in an acromegaly patient with a tumor remnant of considerable size; in patients younger than 40 years in whom a growth of tumor remnant is observed while they are on SRL-fg treatment, and the tumor is unsuitable for surgical treatment (clinically aggressive tumor); in patients with severe headaches, not controllable by SRL-fg treatment; in patients not responding or not tolerating pegvisomant treatment. The side effects profile of pasireotide LAR is similar to that of SRL-fg, except for glycemic impairment, which is observed in around 70% of treated patients [33]. In about 10% of patients, pasireotide LAR treatment needs to be discontinued due to hyperglycemia. In the first 3 months after treatment initiation, fasting glucose evaluation is advised once weekly, and afterwards once in 6 weeks. In the treatment of diabetes mellitus induced by pasireotide LAR application, metformin is used and additionally if needed, dipeptidyl peptidase-4 inhibitors (DPP4), glucagon-like peptide-1 (GLP-1) receptor agonists, while insulin treatment is reserved for the most severe derangements [34]. In the case of overt diabetes mellitus occurring while on pasireotide LAR treatment, these patients could be considered for further treatment with pegvisomant (Figure 1). Pasireotide LAR may suppress anterior pituitary hormone secretion, and treated patients should be under surveillance for development of hypopituitarism.

Dopamine agonists

Dopamine agonists (DA) currently used in Serbia for acromegaly treatment are bromocriptine and cabergoline. DA are used as a first and second line medical treatment, usually in combination with SRL-fg, pasireotide LAR or pegvisomant [20]. Cabergoline is attributed with a greater efficacy in remission achievement in acromegaly (estimated as 34%) compared to bromocriptine (10%) [35]. Mechanism of action of DA in acromegaly relies on the fact that most somatotroph adenomas exhibit type 2 dopamine receptors (D2R), and about 20% of these tumors, in addition to GH co-secrete prolactin (mixed somatotroph/lactotroph tumors). The efficacy of DA is limited to milder forms of

disease, but oral application and low cost make them nevertheless attractive for treatment in acromegaly [36]. The average dose of cabergoline is 2.5 mg weekly (ranging from 1 to 7 mg) which is 2 to 5-fold greater than the doses used in hyperprolactinemia.

Dopamine agonists are used in treatment of acromegaly in following cases (Figure 1): mild biochemical activity of acromegaly (IGF-1 < 2.5 ULN – upper limit of normal for age) with mild disease symptoms. The optimal effect of DA is achieved in cases with IGF-1 < 1.5 ULN; mixed somatotroph/lactotroph pituitary adenoma, prior to surgery, or if remission is not achieved by surgery; as add-on treatment in patients partially responding to SRL-fg.

Pegvisomant

Pegvisomant is a pegylated recombinant GH analogue, acting as a selective antagonist of GH-receptor (GHR). It is used as a second line medical treatment of acromegaly [7, 20]. Pegvisomant was registered in EU in 2002 (EMA approval) and in USA in 2003 (FDA approval) for treating patients with acromegaly [7]. By competing for the GHR, pegvisomant blocks the binding of GH to its receptor, hence preventing the action of GH. The objective of pegvisomant treatment is normalization of serum IGF-1 level. Considering that pegvisomant does not reduce GH concentration, the effects of treatment can only be followed by analysis of serum IGF-1. Serum IGF-1 normalization is reported in about 65% of patients treated with pegvisomant for 5 years [37]. Pegvisomant is available in doses of 10 mg, 15 mg, 20 mg, 25 mg and 30 mg, for subcutaneous (*sc*) injection once daily. Initial daily dose of the drug is 10 mg, and the maximal dose is 30 mg daily [14]. Treatment dose of pegvisomant needs to be titrated individually. An increase or decrease by a 5 mg in daily dose is advised, until IGF-1 normalization is achieved. Pegvisomant decreases levels of glucose and HbA1c, thus enabling reduction in doses of insulin or oral antidiabetic agents in acromegalic patients with diabetes mellitus [38].

Pegvisomant is recommended in following cases: acromegalic patients treated with SLR-fg with persistent disease but small or undetectable tumor remnant; acromegalic patients uncontrolled on SRL-fg, who also suffer from diabetes mellitus; acromegalic patients developing diabetes mellitus in the course of treatment with pasireotide LAR.

Higher doses of pegvisomant are used in female patients, younger, and those with a higher IGF-1 concentration, as well as in patients with diabetes mellitus, sleep apnea syndrome, and in patients with higher body mass index [39]. Although mainly used once daily, pegvisomant may be used twice weekly or once weekly, alone or in combination with other medical modalities for treatment of acromegaly (e.g. SRL-fg). Pegvisomant acts on extrahepatic (IGF-1 independent) GH effects, thus being useful even in patients in whom IGF-1 normalization was already achieved by other modalities of acromegaly treatment (e.g. SRL-fg). In these patients, small doses of pegvisomant, applied once or twice weekly, may relieve the patient of edema, headaches or fatigue, thus significantly improving the quality of life [40]. Pegvisomant found its place also in patients with familial form of acromegaly (aryl hydrocarbon receptor-interacting protein - AIP mutation positive patients). These are often young patients, with progressive course of disease, invasive pituitary tumors and poor response to SRL [8, 41]. Patients suffering from acromegaly as a manifestation of McCune Albright syndrome exhibit resistance to SRL and a favorable response to pegvisomant [42].

Pegvisomant is generally a safe drug which is well tolerated, in spite of the fact that it is administered daily as *sc* injection. Side effects are not dose dependent, and are usually transient and do not require additional treatment. The largest study of efficacy and safety of pegvisomant treatment, (ACROSTUDY) with 2,090 patients followed for 7.6 years, reported the following side effects: headache (in 4.9%), arthralgia (in 3.7%), erythema or other local skin reactions to drug application (in 3.1%), lipodystrophy or lipo-hypertrophy (in 1.7%), gastrointestinal disturbances (in 1.2%) and elevated liver enzymes (in 3%) [43]. Impairment of liver function tests is reversible upon dose reduction or drug discontinuation. Drug discontinuation is advised if the level of liver enzymes is 5-fold above upper limit of normal (0.5%). Due to the known possible elevation of liver enzymes, follow up of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) is advised every 4–6 weeks in the first 6 months of treatment. To avoid potential local skin reactions to drug application, sites of administration should regularly be altered. Pegvisomant has no effect on pituitary tumor remnant size reduction, and is even attributed with a small potential risk of its increase (in 1–3%) [44]. Treatment with pegvisomant is thus not recommended if the tumor remnant is larger or if its distance from the optic chiasm is less than 3 mm [19]. Follow up sellar region MRI is advised during pegvisomant treatment.

Preoperative medical treatment

Medical treatment in acromegaly could also be considered preoperatively, as the first line of treatment (mainly involving SRL-fg), resulting in an increased rate of biochemical disease control, compared to patients operated without prior medical treatment. However, current literature does not provide sufficient evidence to justify a benefit of preoperative treatment with SRL-fg [45].

Conditions in which medical therapy is the first line of treatment in acromegaly include: contraindications for surgery; need for preoperative reduction in surgical and anesthetic risk in acromegaly complicated by: high output cardiac failure, severe pharyngeal thickness and swelling of soft tissue, or severe sleep apnea syndrome.

Combined medical treatment

Combined medical treatment is advised when monotherapy with SRL-fg is insufficiently effective (Figure 1).

Addition of cabergoline to SRL-fg treatment enables IGF-1 normalization in over 50% of patients uncontrolled by SRL-fg monotherapy [46]. Combination of cabergoline and pegvisomant may be an effective alternative in patients not responding to SRL-fg treatment [47]. IGF-1 normalization is expected in 34% of patients when cabergoline is used as monotherapy, and in up to 68% of patients on combined cabergoline and pegvisomant treatment. If this combination is also ineffective, it is advised to consider the combined treatment of SRL-fg and pegvisomant, which was observed to provide better results in patients uncontrolled by monotherapy with either of the drugs (Figure 1). This combination acts both on normalization of IGF-1 serum level and reduction of tumor remnant size. It was reported to provide IGF-1 serum level normalization in 60–97% of patients while reduction in tumor remnant size is expected in 20% of patients [35, 48]. Combined use of SRL-fg and pegvisomant enables dose reduction for both drugs, possibly decreasing treatment costs. If biochemical control is not achieved with this combination of drugs, or an increase is observed in tumor remnant size, it is advised to consider the combined treatment with pasireotide LAR and pegvisomant [20, 49].

Currently, pasireotide LAR and pegvisomant are available in Serbia under a specific clinical programme.

Other medical treatment

When acromegaly persists despite of all the abovementioned treatment modalities, temozolomide may be considered. This drug is an alkylating agent used in treating advanced aggressive neuroendocrine tumors and pituitary carcinomas [50]. Reports on the efficacy of this drug in treatment of acromegaly are limited, indicating efficacy in about 50% of patients [35].

Radiotherapy

This therapeutic modality represents the third line of treatment in acromegaly, to be considered in patients with aggressive pituitary tumors, resistant to surgical or medical treatment [7, 20]. Stereotactic radiosurgery (SRT) is superior to conventional radiotherapy in efficacy and safety. During SRT treatment, high radiation doses are delivered directly to tumor tissue, largely sparing the neighboring healthy tissue. Radiotherapy is mostly reserved for aggressive tumors. Tumor size control is achieved in over 90% of patients, biochemical control in about 60% of patients, but the full effect of treatment is only expected after 5-10 years from SRT application [51]. Major side effect of radiotherapy is hypopituitarism, observed in 70% of treated patients, while optic nerve lesions, cerebrovascular impairment or secondary tumorigenesis are much less frequent [52]. SRT is not recommended when tumor is in high proximity to optic chiasm. SRT modalities available in Serbia include “gamma knife” and “X knife”. Prior medical treatment of acromegaly (with SRL or DA) is believed to be associated with a possible reduction in radio-sensitivity. A temporary cessation of medical treatment, although not an universally recommended practice, was observed to improve both initial and long-term effect of SRT. SRL are advised to be discontinued 6 to 8 weeks prior of SRT and restarted 4 to 8 weeks after, while DA can be discontinued only 2 weeks before SRT. Pegvisomant, as a drug not targeting the pituitary tumor, should not influence radio-sensitivity and does not need to be discontinued [52].

Radiotherapy should be considered in following cases: tumor remnant and active acromegaly are persistent after somatotroph adenoma surgery followed by multimodal medical treatment (SRL-fg, DA, SRL-sg, pegvisomant); medical treatment is ineffective, unavailable, or needs to be discontinued due to side effects.

Personalized approach to patient and prognostic factors of outcome

Judging by the international registries data, one third of acromegaly patients are undertreated and lack disease control [3]. Considerable cost of some medications used in treatment of acromegaly (pasireotide LAR, pegvisomant), result in their unavailability, particularly in the countries of eastern and central Europe [53]. In the course of treatment of acromegaly, in addition to follow up of GH and IGF-1 serum levels, and tumor remnant size, evaluation of patient`s quality of life is recommended, through the use of AcroQoL questionnaire, as well as disease activity clinical assessment with the use of Acromegaly Disease Activity (ACRODAT) or Signs and Symptoms (SAGIT), Associated Comorbidities, GH levels, IGF1 level, and Tumor Profile questionnaires [54–56].

In more than 50% of acromegaly patients, application of all treatment modalities is necessary. Every patient demands individual approach in selection of optimal treatment modalities or their combinations [3, 57]. Personalized approach to patients with acromegaly is founded on the understanding of prognostic factors, on which the individualized selection of treatment is based upon. The optimal selection of treatment includes not only the wellbeing of the patients, but also the most economical approach to the public funding resources. Predictive factors for treatment outcomes include patient`s clinical characteristics – age, gender, the size of pituitary tumor or tumor remnant, baseline GH and IGF-1 levels, histological and immunohistochemical characteristics of the operated tumor tissue, tumor expression of somatostatin receptors, tumor signal intensity on magnetic resonance imaging (MRI) and possible genetic background of acromegaly including FIPA, MEN-1 syndrome, G-protein-linked receptor mutation – *gsp* oncogene mutation, McCune Albright syndrome, X-linked acrogigantism [58].

Female gender, younger age, larger tumor size and high initial GH levels are general indicators of poor response to treatment, and a poor prognosis of the disease. Patients expected to respond better to SRL-fg are those with: tumor hypointensity in T2w MRI, densely granulated tumor tissue, lower Ki67 proliferative index and higher tumor expression of SSTR2A. A favorable response to pasireotide LAR treat-

ment is expected in patients with higher SSTR5 expression in tumor tissue, although SSTR2A expression is also a predictor of good response [57]. Better prognosis after pegvisomant treatment is anticipated in male patients, prior radiotherapy, and in some of GH receptor gene polymorphisms (lack of d3-RHR). AIP mutation positive patients and patients with McCune Albright syndrome are frequently resistant to SRL, thus making pegvisomant the drug of choice in these groups [58]. On the contrary, *gsp* mutation positive patients are excellent responders to SRL [59] (Table 1).

Conclusion

In spite of significant advancement in discovery of new biomarkers as possible prognostic factors in selection of medical treatment of acromegaly, their value has not been demonstrated in clinical practice. The response to treatment in acromegaly can not be predicted with certainty, despite of the various mentioned prognostic factors. Adequate multimodal treatment of acromegaly enables remission of the disease in almost all patients.

Table 1

Predictors for treatment response to medical therapy in acromegaly

Predictors	SRL-fg	SRL-sg	Pegvisomant
Clinical			
female gender	↓		
male gender			↑
younger age	↓		
larger tumor size	↓		
high initial GH levels	↓		
prior radio-therapy			↑
Radiological			
tumor hypo-intensity in T2w MRI	↑		
Pathological			
densely granulated tumor tissue	↑		
lower Ki67 proliferative index	↑		
higher tumor expression of SSTR2A	↑		
higher tumor expression of SSTR5		↑	
Genetic			
AIP mutation positive	↓		↑
McCune Albright syndrome	↓		↑
gsp mutation	↑		
GH-receptor gene polymorphism			↑

↑ = positive; ↓ = negative; SSTR – somatostatin receptor; SRL-fg – somatostatin receptor ligand-first generation; SRL-sg – somatostatin receptor ligand-second generation; GH – growth hormone; AIP – aryl hydrocarbon receptor-interacting protein; MRI – magnetic resonance imaging.

REFERENCES

1. *Gadelha MR, Kasuki L, Lim DST, Fleseriu M.* Systemic Complications of Acromegaly and the Impact of the Current Treatment Landscape: An Update. Endocr Rev 2019; 40(1): 268−332.
2. *Melmed S.* Medical progress: Acromegaly. N Engl J Med 2006; 355(24): 2558−73.
3. *Bollerslev J, Heck A, Olarescu NC.* MANAGEMENT OF ENDOCRINE DISEASE: Individualised management of acromegaly. Eur J Endocrinol. 2019; 181(2): R57−R71.
4. *Esposito D, Ragnarsson O, Granfeldt D, Marlow T, Johannsson G, Olsson DS.* Decreasing mortality and changes in treatment patterns in patients with acromegaly from a nationwide study. Eur J Endocrinol 2018; 178(5): 459−69.
5. *Hoskuldsdottir GT, Fjalldal SB, Sigurjonsdottir HA.* The incidence and prevalence of acromegaly, a nationwide study from 1955 through 2013. Pituitary 2015; 18(6): 803−7.
6. *Ghazi AA, Amirbaigloo A, Dezfooli AA, Saadat N, Ghazi S, Pourafkari M,* et al. Ectopic acromegaly due to growth hormone releasing hormone. Endocrine 2013; 43(2): 293−302.
7. *Colao A, Grasso LFS, Giustina A, Melmed S, Chanson P, Pereira AM*, et al. Acromegaly. Nat Rev Dis Primers 2019; 5(1): 20.
8. *Stojanovic M, Wu Z, Stiles CE, Miljic D, Soldatovic I, Pekic S,* et al. Circulating aryl hydrocarbon receptor-interacting protein (AIP) is independent of GH secretion. Endocr Connect 2019: 8(4): 326−37.
9. *Pivonello R, Auriemma RS, Grasso LF, Pivonello C, Simeoli C, Patalano R*, et al. Complications of acromegaly: cardiovascular, respiratory and metabolic comorbidities. Pituitary 2017; 20(1): 46−62.
10. *Giustina A, Barkan A, Beckers A, Biermasz N, Biller BMK, Boguszewski C*, et al. A Consensus on the Diagnosis and Treatment of Acromegaly Comorbidities: An Update. J Clin Endocrinol Metab 2019; 105(4): pii: dgz096.
11. *Doknić M, Pekić S, Miljić D, Soldatović I, Popović V, Stojanović M*, et al. Etiology of Hypopituitarism in Adult Patients: The Experience of a Single Center Database in the Serbian Population. Int J Endocrinol. 2017; 2017: 6969286.
12. *Damjanovic SS, Neskovic AN, Petakov MS, Popovic V, Vujisic B, Petrovic M,* et al. High output heart failure in patients with newly diagnosed acromegaly. Am J Med. 2002; 112(8): 610−6.
13. *Damjanović SS, Popović VP, Petakov MS, Nikolic-Durović MM, Doknić MZ, Gligorović MS.* Gonadotrophin and free alpha-subunit secretion in patients with acromegaly and clinically non-functioning pituitary tumors: anterior pituitary function

and the effect of thyrotrophin-releasing hormone. J Endocrinol Invest 1996; 19(10): 663−9.
14. *Popovic V, Damjanovic S, Micic D, Nesovic M, Djurovic M, Petakov M,* et al. Increased incidence of neoplasia in patients with pituitary adenomas. The Pituitary Study Group. Clin Endocrinol (Oxf) 1998; 49(4): 441−5.
15. *Bolfi F, Neves AF, Boguszewski CL, Nunes-Nogueira VS*. Mortality in acromegaly decreased in the last decade: a systematic review and meta-analysis. Eur J Endocrinol 2018; 179(1): 59−71.
16. *Dal J, Feldt-Rasmussen U, Andersen M, Kristensen LØ, Laurberg P, Pedersen L*, et al. Acromegaly incidence, prevalence, complications and long-term prognosis: a nationwide cohort study. Eur J Endocrinol 2016; 175(3): 181−90.
17. *Giustina A, Chanson P, Kleinberg D, Bronstein MD, Clemmons DR, Klibanski A,* et al. Acromegaly Consensus Group. Expert consensus document: A consensus on the medical treatment of acromegaly. Nat Rev Endocrinol 2014; 10(4): 243−8.
18. *Pekic S, Doknic M, Miljic D, Joksimovic M, Glodic J, Djurovic M*, et al. Ghrelin test for the assessment of GH status in successfully treated patients with acromegaly. Eur J Endocrinol 2006; 154(5): 659−66.
19. *Katznelson L, Laws ER Jr, Melmed S, Molitch ME, Murad MH, Utz A*, et al. Endocrine Society. Acromegaly: an endocrine society clinical practice guideline. J Clin Enocrinol Metab 2014; 99(11): 3933−51.
20. *Melmed S, Bronstein MD, Chanson P, Klibanski A, Casanueva FF, Wass JAH,* et al. A Consensus Statement on acromegaly therapeutic outcomes. Nat Rev Endocrinol. 2018; 14(9): 552−61.
21. *Shen M, Tang Y, Shou X, Wang M, Zhang Q, Qiao N*, et al. Surgical results and predictors of initial and delayed remission for growth hormone-secreting pituitary adenomas using the 2010 consensus criteria in 162 patients from a single center. World Neurosurg 2018; pii: S1878-8750(18)32738-4.
22. *Buchfelder M, Schlaffer SM*. The surgical treatment of acromegaly. Pituitary 2017; 20(1): 76–83.
23. *Wilson TJ, McKean EL, Barkan AL, Chandler WF, Sullivan SE*. Repeat endoscopic transsphenoidal surgery for acromegaly: remission and complications. Pituitary 2013; 16(4): 459–64.
24. *Neggers SJ, Pronin V, Balcere I, Lee MK, Rozhinskaya L, Bronstein MD*, et al. LEAD Study Group. Lanreotide Autogel 120 mg at extended dosing intervals in patients with acromegaly biochemically controlled with octreotide LAR: the LEAD study. Eur J Endocrinol 2015; 173(3): 313−23.
25. *Chin SO, Ku CR, Kim BJ, Kim SW, Park KH, Song KH,* et al. Medical Treatment with Somatostatin Analogues in Acromegaly: Position Statement. Endocrinol Metab (Seoul) 2019; 34(1): 53−62.
26. *Jallad RS, Bronstein MD*. The place of medical treatment of acromegaly: current status and perspectives. Expert Opin Pharmacother 2013; 14(8): 1001−15.
27. *Colao A, Auriemma RS, Lombardi G, Pivonello R*. Resistance to somatostatin analogs in acromegaly. Endocr Rev 2011; 32(2): 247−71.
28. *Potorac I, Petrossians P, Daly AF, Schillo F, Ben Slama C, Nagi S,* et al. Pituitary MRI characteristics in 297 acromegaly patients based on T2-weighted sequences. Endocr Relat Cancer 2015; 22(2): 169−77.
29. *Ben-Shlomo A, Melmed S*. Somatostatin agonists for treatment of acromegaly. Mol Cell Endocrinol 2008; 286(1−2): 192−8.
30. *Maffezzoni F, Formenti AM, Mazziotti G, Frara S, Giustina A*. Current and future medical treatments for patients with acromegaly. Expert Opin Pharmacother 2016; 17(12): 1631–42.
31. *Gadelha MR, Bronstein MD, Brue T, Coculescu M, Fleseriu M, Guitelman M*, et al. Pasireotide C2402 Study Group. Pasireotide versus continued treatment with octreotide or lanreotide in patients with inadequately controlled acromegaly (PAOLA): a randomised, phase 3 trial. Lancet Diabetes Endocrinol 2014; 2(11): 875−84.
32. *Colao A, Bronstein MD, Freda P, Gu F, Shen CC, Gadelha M,* et al. Pasireotide C2305 Study Group. Pasireotide versus octreotide in acromegaly: a head-to-head superiority study. J Clin Endocrinol Metab 2014; 99(3): 791−9.
33. *Fleseriu M, Rusch E, Geer EB*. ACCESS Study Investigators. Safety and tolerability of pasireotide long-acting release in acromegaly-results from the acromegaly, open-label, multicenter, safety monitoring program for treating patients who have a need to receive medical therapy (ACCESS) study. Endocrine 2017; 55(1): 247−55.
34. *Coopmans EC, Muhammad A, van der Lely AJ, Janssen JAMJL, Neggers SJCMM*. How to Position Pasireotide LAR Treatment in Acromegaly. J Clin Endocrinol Metab 2019; 104(6): 1978−88.
35. *Lim DS, Fleseriu M*. The role of combination medical therapy in the treatment of acromegaly. Pituitary 2017; 20(1): 136−48.
36. *Sandret L, Maison P, Chanson P*. Place of cabergoline in acromegaly: a meta-analysis. J Clin Endocrinol Metab 2011; 96(5): 1327−35.
37. *Freda PU, Gordon MB, Kelepouris N, Jonsson P, Koltowska-Haggstrom M, van der Lely AJ*. Long-term treatment with pegvisomant as monotherapy in patients with acromegaly: experience from ACROSTUDY. Endocr Pract 2015; 21(3): 264−74.
38. *Brue T, Lindberg A, Jan van der Lely A, Akerblad AC, Koltowska-Häggström M, Gomez R,* et al. Diabetes in patients with acromegaly treated with pegvisomant: observations from acrostudy. Endocrine 2019; 63(3): 563−72.
39. *Neggers SJ, Lely AJV*. Pegvisomant for acromegaly: does it always works? Arch Endocrinol Metab 2019; 63(4): 318−9.
40. *Neggers SJ, van Aken MO, de Herder WW, Feelders RA, Janssen JA, Badia X*, et al. Quality of life in acromegalic patients during long-term somatostatin analog treatment with and without pegvisomant. J Clin Endocrinol Metab 2008; 93(10): 3853–59
41. *Daly A, Cano DA, Venegas-Moreno E, Petrossians P, Dios E, Castermans E*, et al. AIP and MEN1 mutations and AIP immunohistochemistry in pituitary adenomas in a tertiary referral center. Endocr Connect 2019; 8(4): 338−48.
42. *Galland F, Kamenicky P, Affres H, Reznik Y, Pontvert D, Le Bouc Y*, et al. McCune-Albright syndrome and acromegaly: effects of hypothalamopituitary radiotherapy and/or pegvisomant in somatostatin analog-resistant patients. J Clin Endocrinol Metab 2006; 91(12): 4957−61.
43. *Buchfelder M, van der Lely AJ, Biller BMK, Webb SM, Brue T, Strasburger CJ*, et al. Long-term treatment with pegvisomant: observations from 2090 acromegaly patients in ACROSTUDY. Eur J Endocrinol 2018; 179(6): 419−27.
44. *Buchfelder M, Weigel D, Droste M, Mann K, Saller B, Brübach K*, et al. Investigators of German Pegvisomant Observational Study.Pituitary tumor size in acromegaly during pegvisomant treatment: experience from MR re-evaluations of the German Pegvisomant Observational Study. Eur J Endocrinol 2009; 161(1): 27−35.
45. *Fleseriu M, Hoffman AR, Katznelson L*. AACE Neuroendocrine and Pituitary Scientific Committee. American Association of Clinical Endocrinologists and American College of Endocrinology Disease State Clinical Review: Management of Acromegaly Patients: What is the role of pre-operative medical therapy? Endocr Pract 2015; 21(6): 668−73.
46. *Jallad RS, Bronstein MD*. Optimizing medical therapy of acromegaly: beneficial effects of cabergoline in patients uncontrolled with long-acting release octreotide. Neuroendocrinology 2009; 90(1): 82–92.
47. *Higham CE, Atkinson AB, Aylwin S, Bidlingmaier M, Drake WM, Lewis A*, et al. Effective combination treatment with cabergoline and low-dose pegvisomant in active acromegaly: a pro-

spective clinical trial. J Clin Endocrinol Metab 2012; 97(4): 1187–93.
48. *Neggers SJ, Franck SE, de Rooij FW, Dallenga AH, Poublon RM, Feelders RA*, et al. Long-term efficacy and safety of pegvisomant in combination with long-acting somatostatin analogs in acromegaly. J Clin Endocrinol Metab 2014; 99(10): 3644–52.
49. *Muhammad A, Coopmans EC, Delhanty PJD, Dallenga AHG, Haitsma IK, Janssen JAMJL*, et al. Efficacy and Safety of switching to Pasireotide in Acromegaly Patients controlled with Pegvisomant and Somatostatin Analogues: PAPE extension study. Eur J Endocrinol 2018; 179(5): 269−77.
50. *Bengtsson D, Schrøder HD, Andersen M, Maiter D, Berinder K, Feldt Rasmussen U*, et al. Long-term outcome and MGMT as a predictive marker in 24 patients with atypical pituitary adenomas and pituitary carcinomas given treatment with temozolomide. J Clin Endocrinol Metab 2015; 100(4): 1689–98.
51. *Abu Dabrh A, Asi N, Farah WH, Mohammed K, Wang Z, Farah MH*, et al. Radiotherapy versus radiosurgery in treating patients with acromegaly: systematic review and meta-analysis. Endocr Pract 2015; 21(8): 943−56.
52. *Ding D, Mehta GU, Patibandla MR, Lee CC, Liscak R, Kano H*, et al. Stereotactic Radiosurgery for Acromegaly: An International Multicenter Retrospective Cohort Study. Neurosurgery 2019; 84(3): 717−25.
53. *Bolanowski M, Ruchała M, Zgliczyński W, Kos-Kudła B, Hubalewska-Dydejczyk A, Lewiński A*. Diagnostics and treatment of acromegaly - updated recommendations of the Polish Society of Endocrinology. Endokrynol Pol 2019; 70(1): 2−18.
54. *Webb SM, Crespo I, Santos A, Resmini E, Aulinas A, Valassi E*. MANAGEMENT OF ENDOCRINE DISEASE: Quality of life tools for the management of pituitary disease.Eur J Endocrinol. 2017; 177: R13−R26.
55. *Giustina A, Bevan JS, Bronstein MD, Casanueva FF, Chanson P, Petersenn S*, et al. SAGIT Investigator Group. SAGIT®: clinician-reported outcome instrument for managing acromegaly in clinical practice--development and results from a pilot study. Pituitary 2016; 19(1): 39–49.
56. *van der Lely AJ, Gomez R, Pleil A, Badia X, Brue T, Buchfelder M*, et al. Development of ACRODAT, a new software medical device to assess disease activity in patients with acromegaly. Pituitary 2017; 20(6): 692–701.
57. *Kasuki L, Wildemberg LE, Gadelha MR*. MANAGEMENT OF ENDOCRINE DISEASE: Personalized medicine in the treatment of acromegaly. Eur J Endocrinol. 2018; 178(3): R89-R100.
58. *Tritos NA, Biller BM*. Pegvisomant: a growth hormone receptor antagonist used in the treatment of acromegaly. Pituitary 2017; 20(1): 129−35.
59. *Puig Domingo M*. Treatment of acromegaly in the era of personalized and predictive medicine. Clin Endocrinol (Oxf) 2015; 83(1): 3−14.

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CASE REPORTS

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Pulmonary arteriovenous malformation: A case report

Plućna arteriovenska malformacija

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Abstract

Introduction. Pulmonary arteriovenous malformation (PAVM) is pathological communication between pulmonary artery and pulmonary vein, in way that it shunts normal alveolar capillary membrane resulting in inadequate blood oxygenation in this part of the lung parenchyma Modern therapy of PAVMs includes surgical treatment or endovascular embolization. **Case report.** A 30-year-old female patient had signs of parestesia and weakness of the extremities on the left side of her body. On physical examination there was only cyanotic discoloration of her lips and clubbing fingers. On the chest x-ray, in the right hemithorax, in the inferior region of the lung, there was relatively homogeneous and well defined shadow, intensity of the soft tissue, which was about 35 mm. A multislice computed tomography pulmonary angiography was performed and showed, in lung parenchyma on both sides, many PAVMs, of which the largest (35 mm) was in inferior right region of the lung on crossing between apical and posterior basal lung segment with 7 mm diameter feeding artery and 9 mm diameter draining vein. The selective pulmonary angiography was performed by Seldingers technique. Through sheath, we placed a plug with a diameter of 10 mm. The plug was expanded and a complete occlusion of the final part of the feeding branch of this PAVM was achieved (confirmed by control angiography). In that way, the PAVM was fully shut off from the circulation. In 3 months follow-up, the patient was feeling well, without any recorded complication. **Conclusion.** Endovascular embolization is recommended as therapy of the first choice for all of PAVMs that have feeding artery greater than 2 mm. Endovascular embolization has high success rate with minimal complications.

Key words:
arteriovenous malformations; computed tomography angiography; embolization, therapeutic; endovascular procedures; multidetector computed tomography.

Apstrakt

Uvod. Plućna arteriovenska malformacija (PAVM) je patološka komunikacija između plućne arterije i plućne vene kojom se zaobilazi normalna alveo-kapilarna membrana, usled čega se krv u tom delu plućnog parenhima ne oksigeniše. Savremeni pristup lečenju PAVM uključuje hirurški tretman ili embolizaciju endovaskularnim putem. **Prikaz bolesnika.** Bolesnica stara 30 godina javila se zbog trnjenja i slabosti ekstremiteta leve strane. Objektivno, imala je cijanozu usana i batičaste prste. Na radiografiji srca i pluća, u desnom hemitoraksu, u donjem plućnom polju u projekciji medioklavikularne linije, nwwađena je relativno homogena i relativno jasno ograničena senka promera oko 35 mm, intenziteta mekih tkiva. Urađena je multislajsna kompjuterizovana tomografija sa pulmoangiografijom i u plućnom parenhimu, obostrano, utvrđen je veći broj PAVM, od kojih je najveća (promera oko 35 mm) bila u donjem desnom plućnom režnju na prelazu između apikalnog i posterobazalnog segmenta, sa uočljivom dovodnom arterijom prečnika oko 7 mm i odvodnom venom prečnika oko 9 mm. Primenom Seldingerove metode urađena je selektivna pulmoangiografija. Kroz uvodnik, uveden je „čepić" (eng. *plug*) prečnika 10 mm, „čepić" je ekspandiran, čime je indukovana potpuna okluzija završnog dela dovodne grane te PAVM (potvrđeno kontrolnom angiografijom). Na taj način, PAVM je u potpunosti bila isključena iz cirkulacije. Tokom tri meseca praćenja, bolesnica se osećala dobro i nisu zabeležene nikakve komplikacije. **Zaključak.**

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Endovaskularna embolizacija se preporučuje kao terapija prvog izbora za sve PAVM čija arterija hranilica ima dijametar veći od 2 mm. Endovaskularna embolizacija pokazuje visoku stopu uspešnosti sa minimalnom stopom komplikacija.

Ključne reči:
arteriovenske malformacije; angiografija, tomografska, kompjuterizovana; embolizacija, terapijska; endovaskularne procedure; tomografija, kompjuterizovana, multidetektorska.

Introduction

Pulmonary arteriovenous malformation (PAVM) is pathological communication between pulmonary artery and pulmonary vein, in way that it shunts normal alveolar capillary membrane. Therefore, the blood in this part of the lung parenchyma is not oxygenized which leads to hypoxia and symptoms including adynamia, tiredness, dyspnea in physical activity, hemoptysis, palpitations, cough, paradoxal systemic embolism and chest pain [1, 2]. Smaller size PAVMs usually are asymptomatic and are incidental findings or can be discovered in case of complications. Modern therapy of PAVMs includes surgical treatment or endovascular embolization [3].

We presented a patient with PAVM successfully treated with endovascular approach.

Case report

A 30-year-old female patient was admitted to the Clinic for Neurology with signs of parestesia and weakness of the extremities on the left side of her body. On physical examination, there was only cyanotic discoloration of her lips and clubbing fingers. Neurological exam was normal. All laboratory results were in referent ranges.

Chest x-ray was performed in standing and posterior-anterior (PA) position. In the right hemithorax, in the inferior region of the lung on medioclavicular line projection, there was relatively homogeneous, relatively well defined shadow, intensity of the soft tissue, which was about 35 mm. Also, on the same side, there was voluminous hilus and prominent hilo-basal pulmonary vascularity (Figure 1). Based on the localization and the appearance of the shadow, and with anamnestic data that before 15 years the patient had been subjected to the surgery for PAVM, we suspected appearance of the new PAVM. We conducted multislice computed tomography (MSCT) pulmonary angiography which revealed many PAVMs in lung parenchyma on both sides, of which the largest one with diameter of 35 mm was in inferior right region of the lung on crossing between apical and posterior basal lung segment with 7 mm diameter feeding artery and 9 mm diameter draining vein (Figures 2−4).

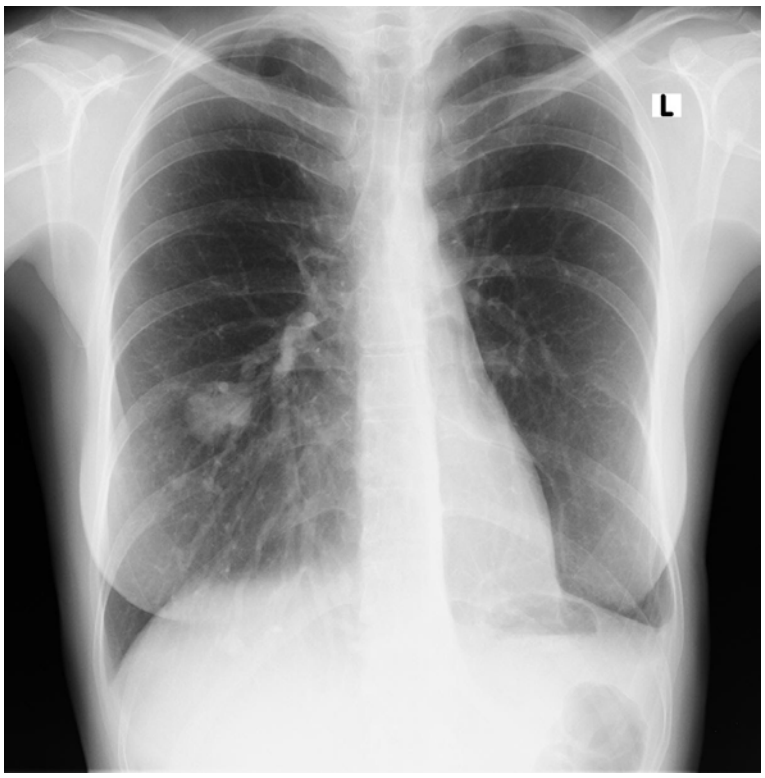


Fig. 1 - Chest x-ray in standing and posterior anterior (PA) position shows relatively homogeneous, relatively well defined shadow, intensity of the soft tissue, size about 35 mm in the right hemithorax, in the inferior region of the lung on medioclavicular line projection. Also, on the same side, there is voluminous hilus and prominent hilobasal pulmonary vascularity.

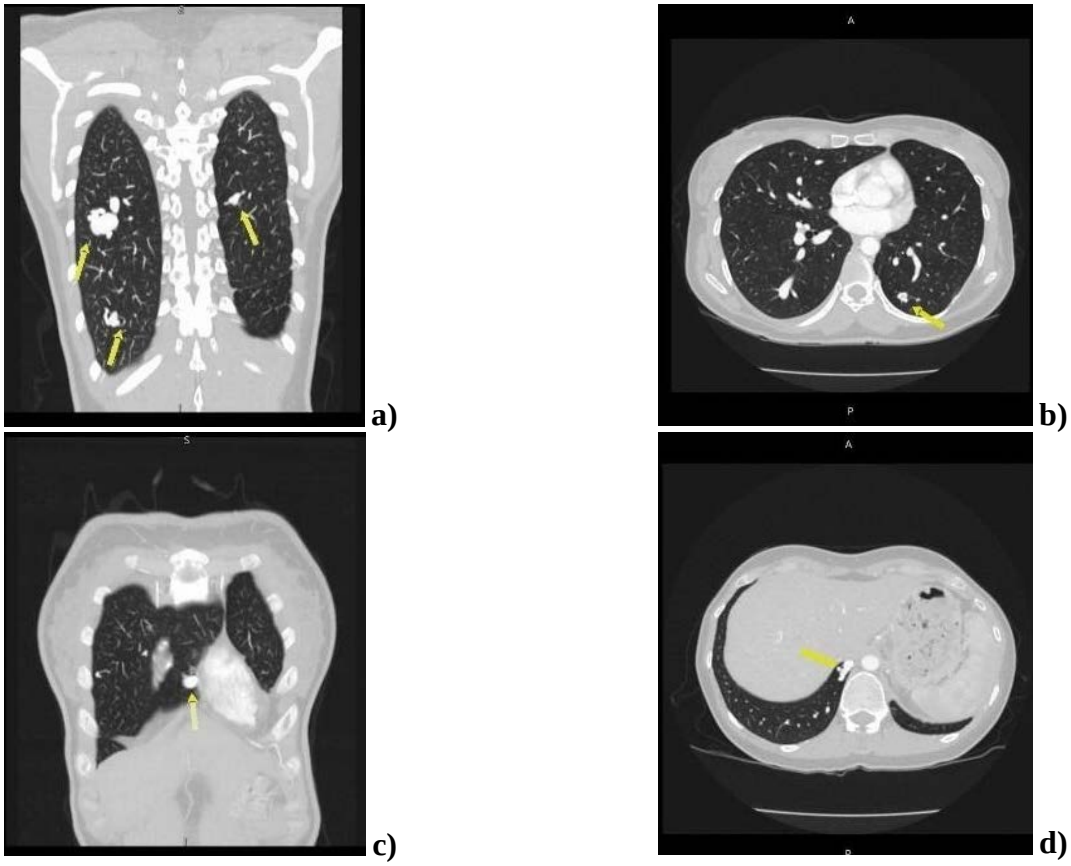


Fig. 2 (a–d) – Multislice computed tomography with pulmonary angiography (MSCT PA) finding shows many pulmonary arteriovenous malformations (PAVMs) in lung parenchyma on both sides.

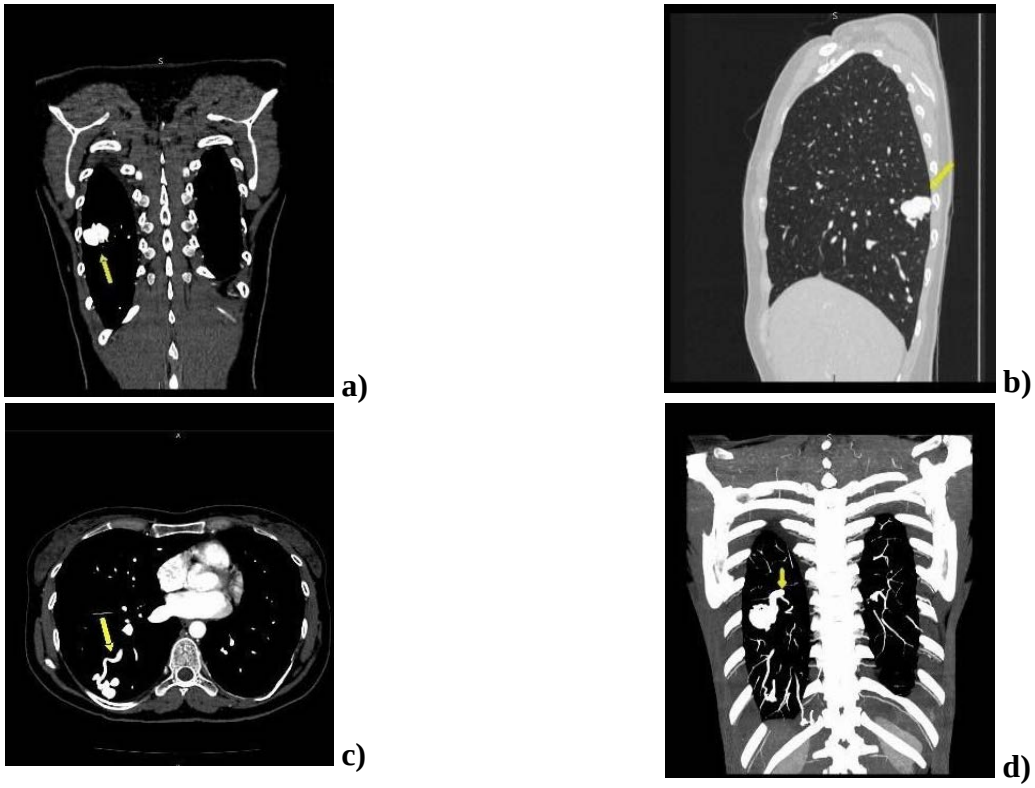
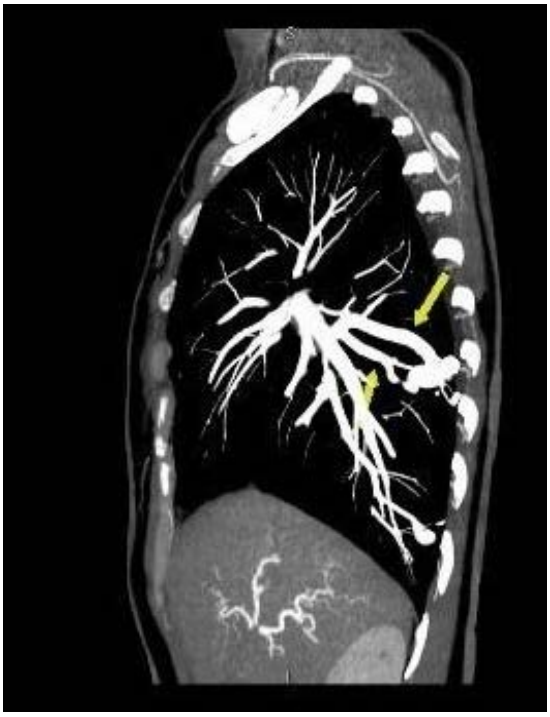
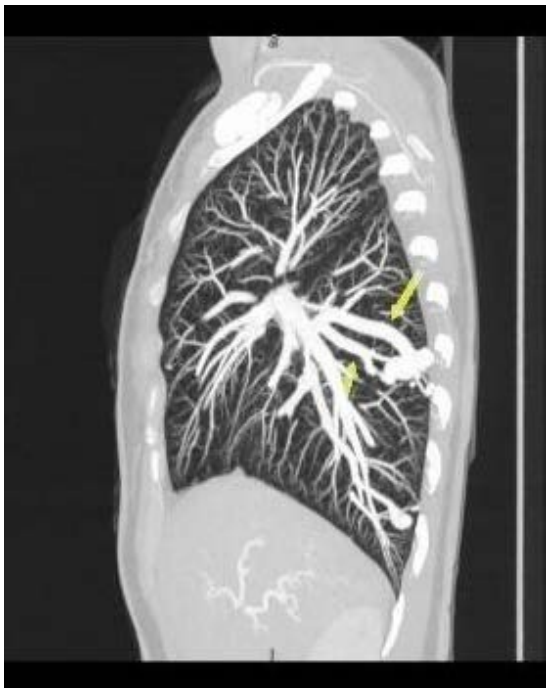


Fig. 3 (a–d) – Multislice computed tomography with pulmonary angiography (MSCT PA) shows the largest pulmonary arteriovenous malformation (PAVM) with diameter of 35 mm in inferior right region of the lung on crossing between apical and posterior basal lung segment.



a)



b)

Fig. 4 (a, b) – Multislice computed tomography pulmonary angiography (MSCT PA) findings: the largest pulmonary arteriovenous malformation (PAVM) with 7 mm diameter feeding artery and 9 mm diameter draining vein.

Medical team, made of a neurologist, thoracic surgeon and radiologist, decided that the largest among PAVMs should be taken care of by endovascular approach. Intervention was conducted in the Department for Interventional Vascular Radiology, Institute of Radiology, Military Medical Academy in Belgrade in collaboration with colleagues from "Dedinje" Cardiovascular Institute, Belgrade.

The right transfemoral access was obtained by Seldingers technique, and a 6F introducer sheath (Merit Medical) was placed. A guide wire (150 mm/ 0.035 In; Merit Medical) and 6F Pigtail catheter (Cordis) were then advanced into the right femoral vein towards the inferior vena cava to the right atrium and ventricle, with electrocardiography (ECG) monitoring, further in *truncus pulmonis*, from where we entered in the main right branch of the *truncus pulmonis*. Then, the selective pulmonary angiography was performed (Figure 5). The PAVM was noticed with feeding and draining blood vessels, as it was shown on MSCT pulmonary angiography.

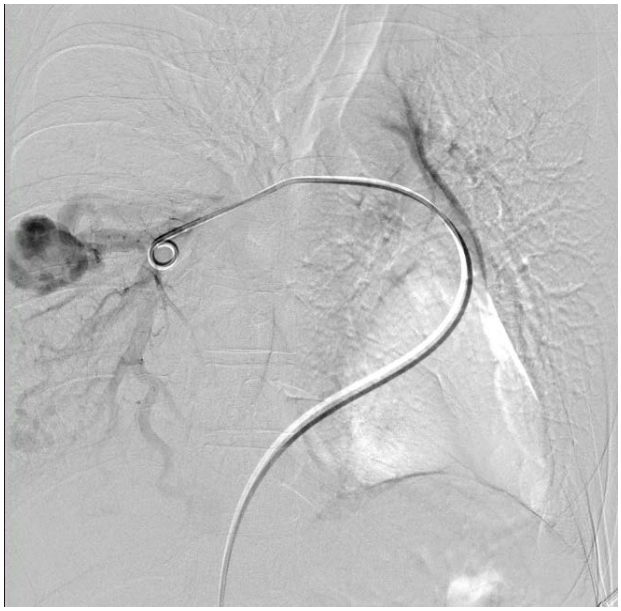


Fig. 5 – Selective pulmonary angiography finding: the pulmonary arteriovenous malformation (PAVM) was noticed with feeding and draining blood vessels.

We entered the feeding branches of the PAVM by using 7F multipurpose (MP) catheter (Cordis) and performed suprase-lective angiography (Figure 6). 7F long peripheral sheath, (Shapeless, Arrow, Terumo) was placed over 260 cm Hi-drostiff wire (Merit Medical), with tip in feeding branch of the PAVM. We placed the 10 mm diameter plug (Amplatzer Vascular Plug II, AGA Medical Corporation) through this sheath. The plug was expanded causing a complete occlusion of the final part of the feeding branch of this PAVM. Control angiography (Figure 7) showed that plug was in correct place with total occlusion of feeding branch of the PAVM, which was now fully shut off from the circulation.

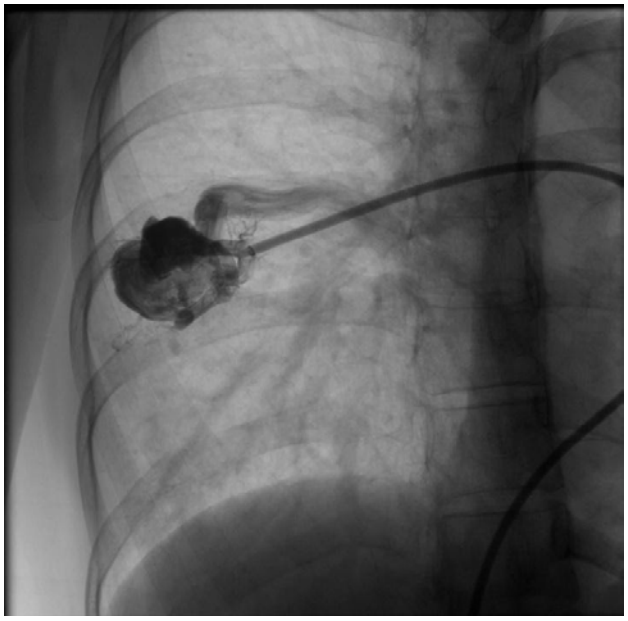


Fig. 6 – Superselective pulmonary angiography after entering the feeding branches of the pulmonary arteriovenous malformation (PAVM).

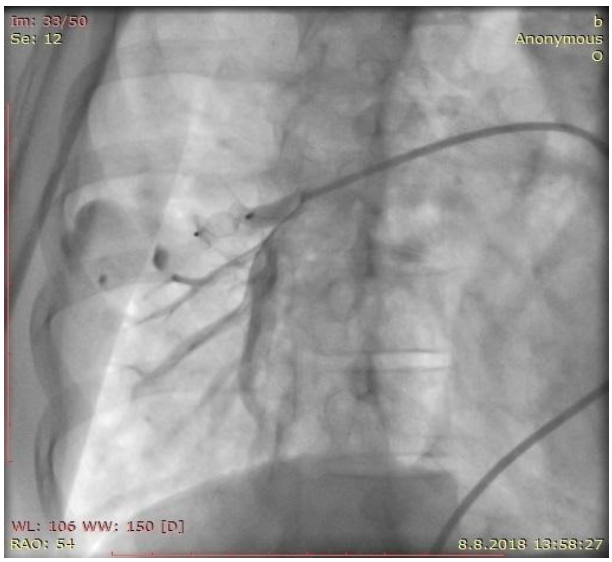


Fig. 7 – Control angiography after expanding the 10 mm diameter plug (Amplatzer Vascular Plug II, AGA Medical Corporation) in final part of the feeding branch of the pulmonary arteriovenous malformation (PAVM) shows that there is total occlusion of feeding branch of the PAVM.

In 3 months follow-up, the patient was feeling well without any recorded complication.

Discussion

Pathologic pulmonary arteriovenous malformation is the direct communication between the branches of pulmonary artery and pulmonary vein in way that there is a shunt of normal lung capillaries which leads to chronic hypoxia [1, 2, 4]. The incidence of PAVM is 2–3 cases in 100,000 people [1, 5, 6]. In more than 80% of cases, it is congenital anomaly (together with hereditary hemorrhagic telangiectasia or Osler-Weber-Rendu syndrome), and in rare cases, it was caused by trauma of the thoracic cavity, thoracic surgery, long-term hepatic cirrhosis, metastatic disease, stenosis of the mitral valve, infections and systemic amyloidosis [1-5, 7, 8]. Also, it can appear in pregnancy. Based on literature, 33% of patients with PAVM had earlier stroke, 18% had transitory ischemic attack, 23% had cerebral abscess, 3% had haemothorax, and 59% of patients had symptoms of dyspnea or intolerance on physical activity [9].

PAVMs are stabile in 75% of the cases or slow growth, and only in small number of cases, most often because of no treatment, PAVMs can induce high rate of morbidity and mortality [10]. Complications of PAVMs are brain abscess, stroke, hemoptysis and haemothorax, hypoxia, polycythemia, endocarditis, transitory ischemic attack, migraine and congestive hearth weakness [1, 4, 6]. Risk of neurological complications is higher in diffuse type of PAVM, large shunt and feeding branch diameter more than 3 mm [11] and in untreated forms of PAVM in comparison to treated forms [4].

Standard thoracic surgical techniques were previously available as the only treatment method (for example: ligation, local excision, segmentectomy, lobectomy, or pneumonectomy) [12]. In some cases, staged bilateral thoracotomies or

video-assisted thoracoscopic resection are performed [13]. Surgical resection is rare method of PAVM treatment and is reserved for the cases with lesions that are resistant on endovascular therapy or when the endovascular treatment is not available. Every time, when it is possible, endovascular embolization is the gold standard in treating PAVM and is conducted since 1980s [9]. Nevertheless, for large, centrally localized lesions, lobectomy is still required. Surgery is a safe method of treatment of PAVMs in selected cases, i.e. when PAVM is solitary and large (> 2 cm diameter), and the risks of embolotherapy are high. Surgery remains choice in cases where treatment of the embolization cannot be performed or has not been successful, in symptomatic and complicated patients with PAVM, and/or cases where the PAVM diagnosis cannot be established [14].

Typically, patients with hereditary telangiectasia are suspected for PAVM and they undergo screening radiographic procedures. In those patients, PAVM is discovered in about 15% of cases [4]. There is 90% of chances of discovering hereditary telangiectasia in patients that have been initially diagnosed with PAVM and they are send on further investigation. Because of that, it is important that every patient with suspected PAVM undergoes detailed and targeted diagnostic. It means that before visiting the interventional radiologist, some other investigations have to be done: multidisciplinary clinical evaluation, imaging for endovascular embolization (number of lesions, localization and type of PAVM, as well as measured diameter of feeding/s arteries), anesthetic assessment for the type of anesthesia, ECG (searching for the block of the left branch and hypertrophy of the right ventricle), complete blood count, coagulation status and other laboratory analyses. It is also important to have patient's informed consent [4]. Gold standard in diagnosing the PAVM is MSCT pulmonary angiography. It is important to identify localization and the type of PAVM, and the diameter of the feeding artery or the feeding branch of pulmonary artery. There is simple PAVMs which have only one feeding artery (80–90% of all PAVMs), complex ones with two or more feeding branches (10–20%) and, rarely, diffuse PAVMs (5%).

Some of the tests which can indicate the PAVM presence are lower oxygen saturation, conventional chest x-ray and transthoracic contrast echocardiography (TTCE). High sensitivity of the TTCE (98.6%) is very important in diagnostics. If this test is positive, the MSCT of the thorax is performed, where the PAVM can be seen, and if it is not visible on MSCT, than there is possibility that PAVM has microscopic dimension [2].

Main indications for treating PAVMs with endovascular approach are: PAVM with diameter of feeding artery greater than 2 mm, symptomatic PAVM with no matter what size it has and atypical lesion that is similar to PAVM on MSCT and presence of suggestive symptoms [4].

Different materials for embolization are available but for those purposes usually coils and plugs are used. These materials function by causing total occlusion of the distal part of feeding artery of PAVM, which leads to its complete occlusion and shunt off of PAVM from the circulation [4].

Embolization of PAVM is very successful method of treatment (success of > 99%) [1, 15]. Successful occlusion can be achieved with only one treatment in 85% of patients, and symptomatic relief can be expected immediately after the treatment [16, 17]. In 83% of the cases in patients with PAVM, treated lesion stays occluded, but in 17–20% of cases there is possibility of reperfusion or formation of new PAVM [15, 18, 19].

Conclusion

Malformations, like PAVM, are associated with high morbidity and mortality if not treated. That is why endovascular embolization is recommended as therapy of first choice for all of the lesions that have feeding artery greater than 2 mm. Endovascular embolization has high success rate with minimal complications. Despite, there is significant risk of recanalization of treated PAVM or more lesions developing. Because of that, long term follow-ups are recommended after embolization.

REFERENCES

1. *Khurshid I, Downie GH.* Pulmonary arteriovenous malformation. Postgrad Med J 2002; 78(918): 191−7.
2. *Chamarthy MR, Park H, Sutphin P, Kumar G, Lamus D, Saboo S,* et al. Pulmonary arteriovenous malformations: endovascular therapy. Cardiovasc Diagn Ther 2018; 8(3): 338−49.
3. *Meier NM, Foster ML, Battaile JT.* Hereditary hemorrhagic telangiectasia and pulmonary arteriovenous malformations: clinical aspects. Cardiovasc Diagn Ther 2018; 8(3): 316−24.
4. *Saboo SS, Chamarthy M, Bhalla S, Park H, Sutphin P, Kay F,* et al. Pulmonary arteriovenous malformations: diagnosis. Cardiovasc Diagn Ther 2018; 8(3): 325−37.
5. *Andersen PE, Tørring PM, Duvnjak S, Gerke O, Nissen H, Kjeldsen AD.* Pulmonary arteriovenous malformations: a radiological and clinical investigation of 136 patients with long-term follow-up. Clin Radiol 2018; 73(11): 951−7.
6. *Hsu CC, Kwan GN, Evans-Barns H, van Driel ML.* Embolisation for pulmonary arteriovenous malformation. Cochrane Database Syst Rev 2018; 1: CD008017.
7. *Dokumcu Z, Ozcan C, Alper H, Erdener A.* Pulmonary arteriovenous malformation in children. Pediatr Int 2015; 57(4): 708−11.
8. *Mowers KL, Sekarski L, White AJ, Grady RM.* Pulmonary arteriovenous malformations in children with hereditary hemorrhagic telangiectasia: a longitudinal study. Pulm Circ 2018; 8(3): 2045894018786696.
9. *Faughnan ME, Palda VA, Garcia-Tsao G, Geisthoff UW, McDonald J, Proctor DD,* et al. International guidelines for the diagnosis and management of hereditary haemorrhagic telangiectasia. J Med Genet 2011; 48(2): 73−87.
10. *Shovlin CL, Jackson JE, Bamford KB, Jenkins IH, Benjamin AR, Ramadan H,* et al. Primary determinants of ischaemic stroke/brain abscess risks are independent of severity of pulmonary arteriovenous malformations in hereditary haemorrhagic telangiectasia. Thorax 2008; 63(3): 259−66.
11. *Moussouttas M, Fayad P, Rosenblatt M, Hashimoto M, Pollak J, Henderson K,* et al. Pulmonary arteriovenous malformations:

cerebral ischemia and neurologic manifestations. Neurology 2000; 55(7): 959−64.
12. *Love BA, Weber HS*. Pulmonary Arteriovenous Fistulae Treatment & Management. MedScape. Available from: https://emedicine.medscape.com/article/900681-treatment#d9 [updated: 2015 January 29].
13. *Ishikawa Y, Yamanaka K, Nishii T, Fujii K, Rino Y, Maehara T*. Video-assisted thoracoscopic surgery for pulmonary arteriovenous malformations: report of five cases. Gen Thorac Cardiovasc Surg 2008; 56(4): 187−90.
14. *Biçakçioğlu P, Gülhan SŞ, Sayilir E, Ertürk H, Ağaçkiran Y, Kaya S,* et al. Surgical treatment of pulmonary arteriovenous malformations. Turk J Med Sci 2017; 47(1): 161−6.
15. *Pollak JC, Saluja S, Thabet A, Henderson KJ, Denbow N, White RI Jr*. Clinical and anatomic outcomes after embolotherapy of pulmonary arteriovenous malformations. J Vasc Interv Radiol 2006; 17(1): 35-44; quiz 45.
16. *Trerotola SO, Pyeritz RE, Bernhardt BA*. Outpatient single-session pulmonary arteriovenous malformation embolization. J Vasc Interv Radiol 2009; 20(10):1287−91.
17. *Gupta P, Mordin C, Curtis J, Hughes JM, Shovlin CL, Jackson JE*. Pulmonary arteriovenous malformations: effect of embolization on right-to-left shunt, hypoxemia, and exercise tolerance in 66 patients. AJR Am J Roentgenol 2002; 179(2): 347−55.
18. *Remy-Jardin M, Dumont P, Brillet PY, Dupuis P, Duhamel A, Remy J*. Pulmonary arteriovenous malformations treated with embolotherapy: helical CT evaluation of long-term effectiveness after 2-21-year follow-up. Radiology 2006; 239(2): 576−85.
19. *Mager JJ, Overtoom TT, Blauw H, Lammers JW, Westermann CJ*. Embolotherapy of pulmonary arteriovenous malformations: long-term results in 112 patients. J Vasc Interv Radiol 2004; 15(5): 451−6.

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CASE REPORT

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Migration of the retained intracranial bullet to the spinal canal: A case report

Migracija zaostalog metka iz lobanjske šupljine u spinalni kanal

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Abstract

Introduction. Retained intracranial missiles migration is rarely reported. Most of the time, the missile will migrate and remain intracranially, but in extremely rare cases, it may reach the spinal canal. The aim of the study was to present a patient with this rare clinical entity. **Case report.** The 29-years-old male suffered from the gunshot wound to the head through the left external auditory meatus. The bullet was located in the posterior *fossa*. Initial debridement without bullet removal was performed. Four months after the injury, the patient came back complaining of neck stiffness and progressive weakness of all extremities. Plain radiography revealed the bullet in the spinal canal at the level of C2 vertebra and computed tomography confirmed localization in the posterior aspect. An emergency procedure was performed for bullet removal and spinal cord decompression. **Conclusion.** The bullets tend to migrate. Migration to the spinal canal is rare, but takes a significant risk, due to the potential secondary injury. The removal of a bullet at-all-costs may not be justified. However, the prediction of migration based on the predisposing factors would be of great value to treating these patients adequately.

Key words:
head injuries, penetrating; neurosurgery; spinal cord compression.

Apstrakt

Uvod. Migracija zaostalih metaka u intrakranijalnom prostoru je opisana u malom broju slučajeva. Najčešće, metak migrira i ostaje unutar lobanjske šupljine, ali, u veoma retkim slučajevima može dospeti i u spinalni kanal. Cilj rada je bio da se prikaže bolesnik sa ovim retkim kliničkim entitetom. **Prikaz bolesnika.** Muškarac star 29 godina je zadobio ustrelnu povredu glave kroz levi spoljašnji slušni kanal. Metak je bio lokalizovan u zadnjoj lobanjskoj jami. Učinjen je inicijalni debridman rane bez uklanjanja metka. Četiri meseca nakon povrede, bolesnik je osetio ukočenost vrata i slabost svih ekstremiteta zbog čega se javio lekaru. Radiografijom je pokazano da se metak nalazi u spinalnom kanalu na nivou C2 pršljena, a kompjuterizovanom tomografijom je potvrđena lokalizacija u posteriornom aspektu. Bolesnik je hitno operisan radi evakuacije metka i dekompresije kičmene moždine. **Zaključak.** Meci imaju tendenciju da se pomeraju. Migracija u spinalni kanal je retka i nosi značajan rizik zbog moguće sekundarne povrede. Uklanjanje metka po svaku cenu nije opravdano, ali predviđanje njegove migracije na osnovu predisponirajućih faktora bi bilo od velikog značaja za adekvatnije lečenje ovih bolesnika.

Ključne reči:
povrede glave, penetrirajuće; neurohirurgija; kičmena moždina, kompresija.

Introduction

Gunshot wounds to the head (GSWH) are among the deadliest injuries known, with up to 70% fatality at the place, up to 90% dying before admission, and about 50% of the remaining who die in the emergency room [1]. These appear in both civilian and military circumstances, and the overall incidence is hard to be estimated.

The outcome is worse in those patients with extensive bullet tracts, in those with brainstem involvement and when the deep midline structures of the brain are wounded [2]. Although GSWH are severe, there are survivors, and complications in this group of patients are related to further decrease of favorable outcome. The most common are various kinds of infections, cerebrospinal fluid (CSF) leaks and fistulas and posttraumatic epilepsy [3].

Intracranial missile migration is considered to be very rare, in cases when the metallic foreign body is left over. Rapp et al. [4] have previously reported an incidence of more than 4%, contrary to other studies that have neglected the

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migration [5, 6]. The migration is usually constrained to the cranial vault, but sometimes, the missile might find its way out through the blood vessels to distal vascular structures or through the *foramen magnum* to the spinal canal [7, 8]. We present a case of delayed spinal cord injury due to the migration of the retained intracranial bullet.

Case report

The 29-years-old male suffered from the GSWH through the left external auditory meatus in an armed conflict. He was delivered to the Emergency Department, where the initial surgery, which included wound debridement, was performed. Due to the distal localization, the bullet was not evacuated and was instead left behind. Postoperative computed tomography (CT) revealed the bullet located in the *posterior fossa* near *foramen magnum* (Figure 1). The patient recovered well and was referred to the regional rehabilitation center for further treatment. The left-sided ear deafness persisted.

During his regular activities at the rehabilitation center, after impetuous verticalization, the patient started to develop symptoms of spinal cord compression in the form of neck stiffness and progressive tetraparesis. Plain radiography was performed right away, which revealed the bullet in the spinal canal at the level of C2 vertebra (Figure 2).

The patient was transferred to the Emergency Department of the Military Medical Academy in Belgrade for further diagnostic assessment and surgical treatment. CT revealed the bullet in the posterior aspect of the C2 vertebra (Figure 3). Emergency surgery was performed for bullet removal, which included extended C2 laminectomy end evacuation of the foreign body.

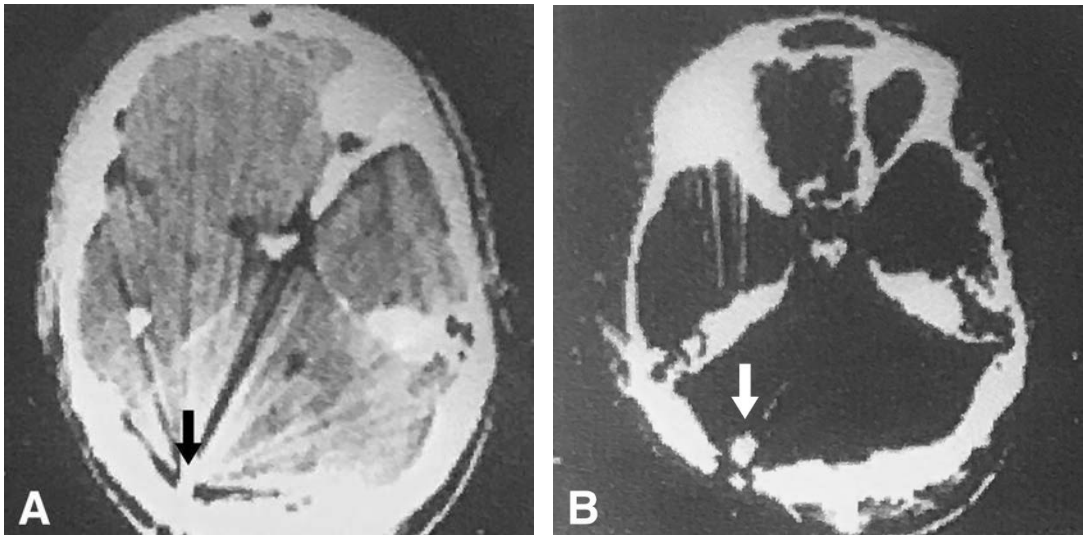

Fig. 1 – Initial postoperative computed tomography showing the retained bullet located in the *posterior fossa* near *foramen magnum*: A) Axial brain scan with many artefacts (arrow pointing to the bullet); B) Axial hard structures scan (arrow pointing to the bullet).

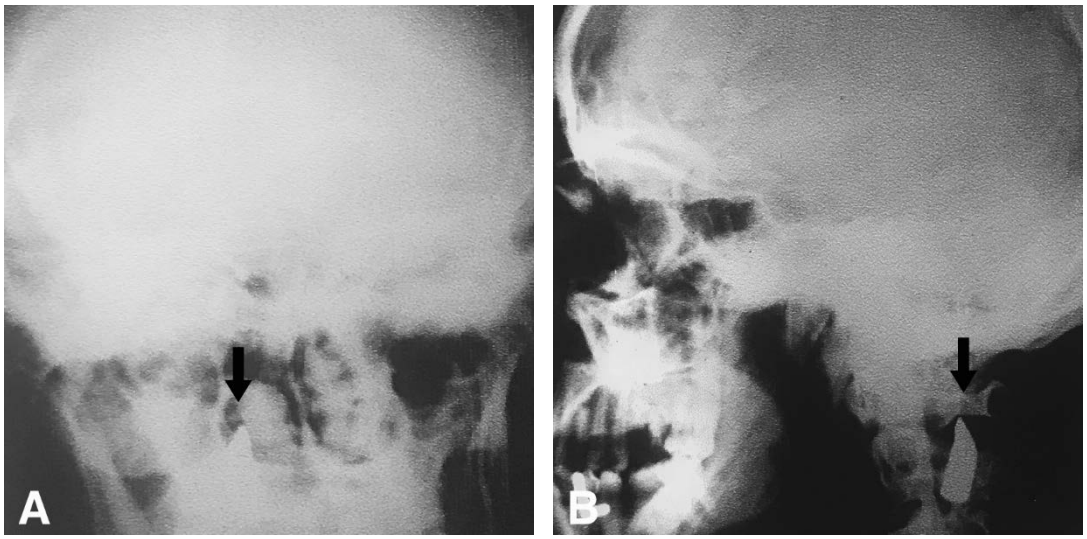

Fig. 2 – Plain radiography performed after the spinal cord related symptoms have occurred. The bullet had migrated to the spinal canal extending from the lower edge of C1 to the C3 vertebral body: A) Anterior view; B) Lateral view.

Postoperative clinical examination confirmed symptoms relief, but with the persistence of spinal cord compression injury signs. No signs of bullet fragments were found on follow-up plain radiography (Figure 4). The patient remained in the Intesive Care Unit for a few days to exclude late complications. He was discharged without any newly developed neurological symptoms.

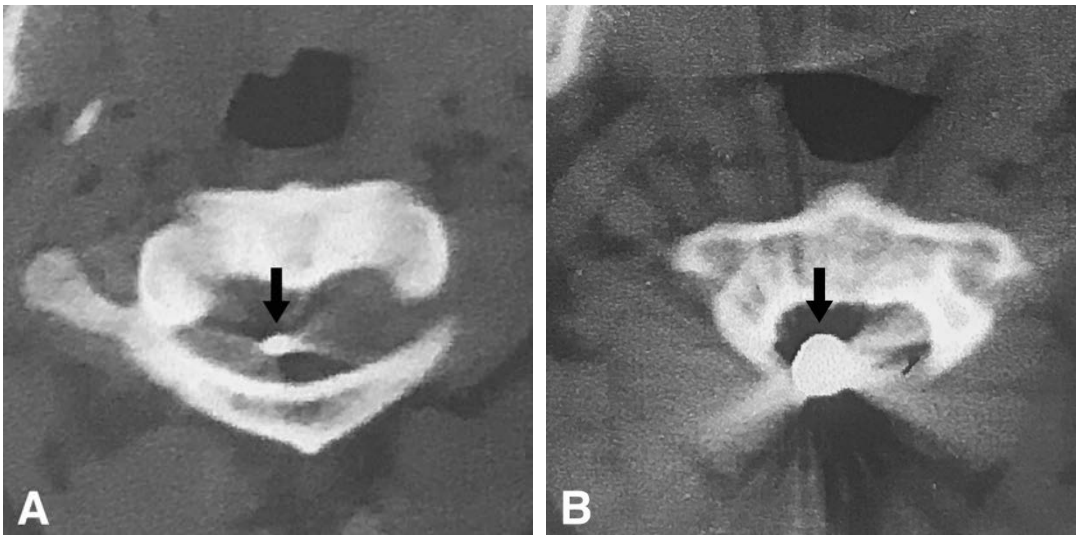

Fig. 3 – Complimentary computed tomography revealed the bullet positioning in the posterior aspect with the narrow tip at the lower edge of C1 vertebra (A) and the main bullet calibre at the C2 level (B).

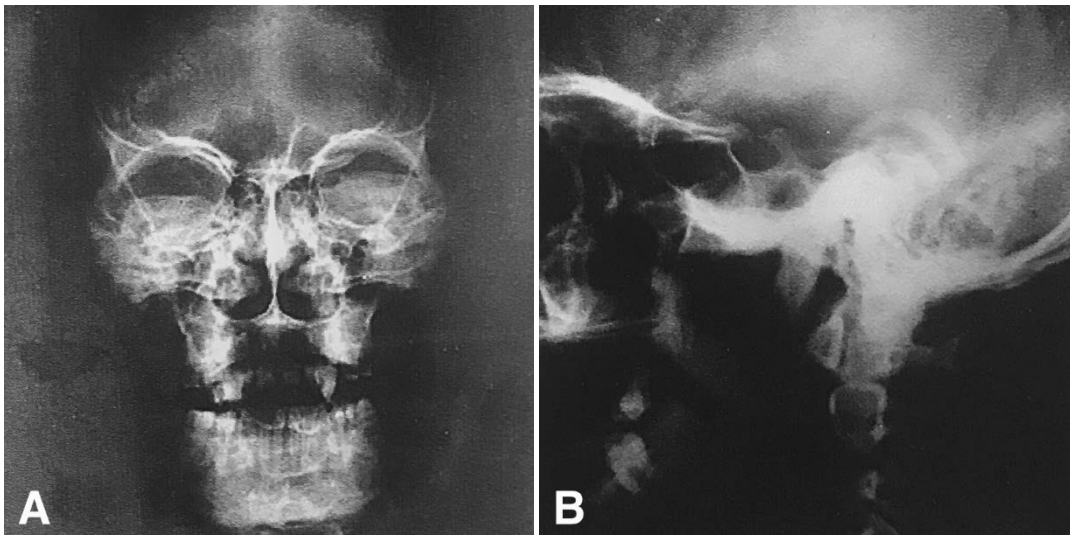

Fig. 4 – Plain radiography performed after bullet evacuation through a laminectomy. No signs of bullet fragments were found on the: A) anterior view; B) Lateral view.

Discussion

The first migration of intracranial missile was reported in 1916 [9], however, not many migrations to the spinal canal were reported to time. The first case was reported in 1939 by Kellhammer [10]. As it is the case with any kind of gunshot wounds and its complications, the patients were male in most cases, with age ranging from 19 to 36 years. Table 1 presents all of the previously reported cases of missile migration into the spinal canal (including ours).

The spontaneous migration of a missile or a its fragment within the cranial cavity may occur from a few days after the injury to a few years later [11–18]. In our case, the migration occurred four months after discharge the patient who recovered well and probably provoked migration with a sudden movement (verticalization). This contributes to the conclusion that every missile will eventually migrate if there are no limiting factors (close proximity of bone or other structures in the vicinity of the missile).

Initially, missiles were located in different brain parts and migrated into the spinal canal from C2 to T6, most often to the C2 level [7]. When smaller shrapnel migrated, even the most distal parts were reached [10, 17]. In our case, the bullet migrated from *posterior fossa* into the

Table 1

Reported cases of missile migration into the spinal canal

Reference	Gender	Age (yrs)	Missile (type/size)	Missile location			Neurological finding			Treatment
				initial	intermit.	final	initial	intermit.	final	
10	-	-	bullet	loose bullet in the ventricle	-	*cauda equina*				
11	female	22	7.65 mm - bullet	right suboccipital region	-	C3-4, dorsal to the cord	apprehensive but well oriented	-	electric-like shocks radiating from neck to hands and feet	delayed removal
12	male	15	air gun pellet	right parietal lobe	right parietal paraventricular region	C2, dorsal to the cord	arousable to painful stimuli, fairly cooperative, mild right sided hemiparesis	right-sided parietal motor seizures	severe headache, vomiting, neck pain triggered by movement	delayed removal
13	female	19	bullet	near torcular herophili on the tentorium	quadrigeminal plate cistern in posterior *fossa*.	C5	not fulfilling commands language and memory deficit, right hemiparesis	same	same	initial debridement
14	male	15	baby bullet, 4.6 mm	prepontine cistern	-	lumbar subarachnoid space L5/S1	burning in left eye and a generalized headache	intact	intact	conservative treatment
15	-	-	-	brain	-	spinal canal	-	-	-	-
16	female	36	9 mm - bullet	between hemispheres	?	T4	Intact	flaccid quadriplegia paresthesias and alterations in vibration sense	T4 dermatomal sensory changes, myelopathy signs	delayed removal
7	male	21	9 mm - bullet	right lateral medullary cistern	-	C2	GCS12	spinal cord injury	-	conservative treatment & delayed removal
18	/	19-24	air gun pellet	right lateral ventricle	-	T6	comatose	-	-	conservative treatment
this case	male	29	bullet	posterior *fossa* near *foramen magnum* on the right	-	C1-2	left sided ear deafness	-	neck pain & teraparesis	initial debridement & delayed removal

spinal canal to the C2 level (which is the most common level). Neurological symptoms were dependant on the level of spinal cord migration/compression [7].

The clinical presentation in previously reported cases is different and conditioned directly by the missile size: the small pellets may be asymptomatic and confirmed on follow-up radiography or CT scan, while the larger will present the compression symptoms at the level they reach. Sometimes a bullet may fall from the *posterior fossa* to the spinal canal with sufficient momentum to cause an acute spinal cord injury [7]. However, compression related symptoms are much more common [16]. In our case, the symptoms of spinal cord compression in the form of neck stiffness and progressive tetraparesis were present, although no signs of spinal cord injury were found. The symptoms mitigated to some extent early after surgery. However, due to the compression injury to the spinal cord, mild numbness and mild lower extremities paraparesis remained. The patient had fully recovered in a two-year follow-up period.

Initial debridement was performed in most of the cases with the delayed procedure performed for the missile removal, although sometimes only the follow-up imaging revealed the migration [16]. So far, the prediction of migration is impossible and unreliable, as no reliable influencing factors were identified. The analysis of these could be very helpful to the surgeon, to make the decision-making process on missile evacuation easier and relatively straight forward.

Conclusion

The tendency to migration of intracranial missiles is obvious. Although every intracranial foreign body that is not trapped by the bones or adjacent structures might mi-

grate at some point, it is a matter of patients' survival, time passed, occultness (symptoms absence), and follow-up imaging. Therefore, migration of the retained intracranial bullets or other missiles should be considered as a potential early or delayed complication.

Conflict of interest

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

R E F E R E N C E S

1. *Aarabi B, Tofighi B, Kufera J A, Hadley J, Ahn E S, Cooper C*, et al. Predictors of outcome in civilian gunshot wounds to the head. J Neurosurg 2014; 120(5): 1138−46.
2. *Ecker R D, Mulligan L P, Dirks M, Bell R S, Severson M A, Howard R S,* et al. Outcomes of 33 patients from the wars in Iraq and Afghanistan undergoing bilateral or bicompartmental craniectomy. J Neurosurg 2011; 115(1): 124−9.
3. *Alvis-Miranda HR, M Rubiano A, Agrawal A, Rojas A, Moscote-Salazar LR, Satyarthee GD,* et al. Craniocerebral Gunshot Injuries; A Review of the Current Literature. Bull Emerg Trauma 2016; 4(2): 65−74.
4. *Rapp LG, Arce CA, McKenzie R, Darmody WR, Guyot DR, Michael DB.* Incidence of intracranial bullet fragment migration. Neurol Res 1999; 21(5): 475−80.
5. *Gönül E, Erdogan E, Îzci Y, Baysefer A, Seber N.* Craniocerebral Gunshot Wounds: Analysis of 288 Cases, A Clinical Review. Turk Neurosurg 1999; 9(1−2): 1−7.
6. *Raimondi AJ, Samuelson GH.* Craniocerebral gunshot wounds in civilian practice. J Neurosurg 1970; 32(6): 647−53.
7. *Cheng JS, Richardson RM, Gean AD, Stiver SI.* Delayed acute spinal cord injury following intracranial gunshot trauma: case report. J Neurosurg 2012; 116(4): 921−5.
8. *Pecirep DP, Hopkins HR.* Removal of a bullet from the right heart using controlled embolization to a peripheral vein. Ann Thorac Surg 1994; 58(6): 1748−50.
9. *Vilvandre G, Morgan J.* Movements of foreign bodies in the brain. Arch Radiol Electrother 1916; 21(1): 22−7.
10. *Kellhammer G.* Geschoßwanderung im Ventrikelsystem. Zbl Chir 1939; 66: 1773−6. (German)
11. *Arasil E, Tascioglu AO.* Spontaneous migration of an intracranial bullet to the cervical spinal canal causing Lhermitte's sign. Case report. J Neurosurg 1982; 56(1): 158–9.
12. *Medina M, Melcarne A, Ettorre F, Barrale S, Musso C.* Clinical and neuroradiological correlations in a patient with a wandering retained air gun pellet in the brain. Surg Neurol 1992; 38(6): 441–4.
13. *Young WF, Jr., Katz MR, Rosenwasser RH.* Spontaneous migration of an intracranial bullet into the cervical canal. South Med J 1993; 86(5): 557–9.
14. *Traeger M, Wood BP.* Radiological cases of the month. The migrating BB and the medicine man. Am J Dis Child 1993; 147(8): 901–2.
15. *Rajan DK, Alcantara AL., Michael DB.* Where's the bullet? A migration in two acts. J Trauma 1997; 43(4): 716–8.
16. *Castillo-Rangel C, Reyes-Soto G, Mendizabal-Guerra R.* Craniothoracic bullet migration over a period of 27 years: case report. Neurocirugia (Astur) 2010; 21(4): 326−9.
17. *Karim NO, Nabors MW, Golocovsky M, Cooney FD.* Spontaneous migration of a bullet in the spinal subarachnoid space causing delayed radicular symptoms. Neurosurgery 1986; 18(1): 97−100.
18. *Lough EG, Glover B, Brown AL.* An unusual case of air rifle pellet migration from the brain to the thoracic spine. Am Surg 2013; 79(1): E33−4.

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CASE REPORT

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A male case of dermatofibrosarcoma protuberans in the breast presenting as gynecomastia

Dermatofibrosarkom protuberans muške dojke prezentovan kao ginekomastija

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Abstract

Introduction. Dermatofibrosarcoma protuberans (DFSP) is a very rare mesenchymal tumor that accounts for approximately 0.1% of all malignancies. It is a locally aggressive fibrous tumor, with a high recurrence rate, which sometimes gives rise to distant metastases, usually to the bones and lungs. DFSP usually occurs on the trunk and extremities with only a small number of cases in the breast, especially in men. **Case report.** We presented a rare case of DFSP in the male breast. A 66-year-old man presented with gynecomastia of the left breast. The diagnostic work-up comprised of clinical examination, ultrasonography, core biopsy, and mammography. Immunohistochemistry revealed diffuse and strong positivity for vimentin, CD99, and CD34, while the tumor cells were completely negative for keratin, S100 protein, STAT6, CD31, and factor VIII, highly suggestive for DFSP. Subsequently, a radical mastectomy was performed and preoperative diagnosis of DESP was confirmed by pathological examination and immunohistochemistry. The patient was still disease-free six months after the surgical treatment. **Conclusion.** DFSP is a soft tissue sarcoma that rarely occurs in the breast, especially in men. The most common clinical presentation in the breast is a mass with extensive nodules on the surface, but it can also be presented as gynecomastia, as in our case. The diagnosis of DFPS is based on anatomopathology with immunohistochemistry analysis since there are no specific imaging features for this rare entity. Surgical excision with wide and negative margins is optimal for reducing the risk of recurrence.

Key words:
breast neoplasms, male; dermatofibrosarcoma; diagnosis, differential; immunohistochemistry; rare diseases.

Apstrakt

Uvod. Dermatofibrosarkom protuberans (DFSP) je veoma redak mezenhimalni tumor koji čini otprilike 0,1% svih maligniteta. To je lokalno agresivni fibrozni tumor, sa velikom stopom recidiva, koji ponekad može dati udaljene metastaze, obično u kostima i plućima. DFSP se obično javlja na trupu i ekstremitetima, a samo kod malog broja obolelih u dojci, posebno kod muškaraca. **Prikaz bolesnika.** Prikazan je redak slučaj DFSP u levoj dojci 66-godišnjeg muškarca koji se manifestovao kao ginekomastija. Dijagnostička obrada obuhvatila je klinički pregled, ultrazvučni pregled, "core" biopsiju i mamografiju. Imunohistohemija je pokazala difuznu i jaku pozitivnost za vimentin, CD99 i CD34, dok su tumorske ćelije bile potpuno negativne na keratin, S100 protein, STAT6, CD31 i faktor VIII, što je sugerisalo da se radi o DFSP. Nakon toga, izvršena je radikalna mastektomija i pregledom patologa i imunohistohemijskim metodama potvrđena je preoperativna dijagnoza DFSP. Bolesnik je šest meseci posle hirurškog lečenja i dalje bio bez bolesti. **Zaključak.** DFSP je sarkom mekog tkiva koji se izuzetno retko razvija u dojkama muškaraca. Uobičajena klinička prezentacija na dojci jeste masa sa širokim krvžicama na površini, mada se može prezentovati i samo kao ginekomastija, kao u opisanom slučaju. Dijagnoza DFPS se zasniva na patoanatomskoj i imunohistohemijskoj analizi jer ne postoje specifični znaci za ovaj retki entitet, vidljivi primenom različitih tehnika snimanja. Hirurška ekscizija sa širokim i negativnim marginama je optimalna za smanjenje rizika od recidiva.

Ključne reči:
dojka, neoplazme, muškarci; dermatofibrosarkom; dijagnoza, diferencijalna; imunohistohemija; bolesti, retke.

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Introduction

Dermatofibrosarcoma protuberans (DFSP) is an uncommon mesenchymal tumor that accounts for approximately 0.1% of all malignancies [1] and represents less than 5% of all soft tissue sarcomas occurring in adults aged 30 to 40 years [2]. The overall incidence is five cases in every 1 million persons annually [3].

This sarcoma usually arises in the dermis and can also extend to the deeper subcutaneous tissues [3]. Rare cases of deep-seated DFSP have been reported [4]. It is a locally aggressive fibrous tumor, with a high recurrence rate [3]. Therefore, it is important to achieve negative margins to minimize disease recurrence [5]. DFSP can give rise to distant metastases, usually to the bones and lungs (incidence is less than 5%) [6].

The most common presentation in a male's breast is a mass with extensive nodules on the surface [7]. The tumor tends to spare adnexal structures and is commonly superficially located, but in recurrent cases and untreated tumors, it can spread to more deeply situated structures [8, 9].

The early symptoms are often non-specific. Consequently, diagnosis is challenging, with a high incidence of misdiagnoses [10]. Due to the lack of pathognomonic clinical and imaging findings, DFSP can be mistaken for a keloid, hypertrophic scar, sebaceous cyst or lipoma. In cases with prior trauma, suspicion of DFSP must be raised in the differential diagnosis [11].

DFSP usually occurs on the trunk and extremities with only a small number of cases in the breast, especially in men [1, 2, 5, 7, 12–14]. We reported a rare case of DFSP in the male breast that clinically presented as gynecomastia.

Case report

A 66-year-old man presented with gynecomastia of the left breast that was slowly growing over the past year. In his personal history, there were no risk factors for breast cancer. There was no information about recent trauma, or scars in the breast area. Family history was unremarkable for breast cancer. Physical examination was delayed due to the patient's psychological discomfort. It showed an enlarged breast with a palpable nodule beneath the skin without any skin changes on the surface. One year after the breast enlargement was observed, the patient finally underwent ultrasound (US) investigation that showed a well-defined hypoechoic lesion with sharp and smooth edges, located in the superior medial quadrant, between 10 and 12 o'clock, measuring 45 x 22 mm in diameter (Figure 1). The distance from the skin was 9 mm. Ipsilateral axillary lymph nodes were inconspicuous.

Additional digital mammography was performed, showing a hyperdense mass without calcifications or fat (Figure 2).

Based on US and mammography findings, the lesion was graded according to the Breast Imaging Reporting and Data System (BI-RADS) as IV lesion, and malignancy could not be excluded. The US-guided core biopsy under local anesthesia was performed. Histological examination revealed

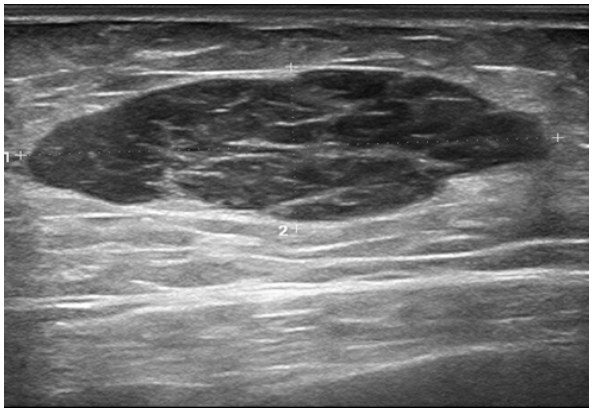

Fig. 1 – Dermatofibrosarcoma protuberans on the ultrasound examination shows hypoechoic well defined oval lesion in subcutaneous fat.

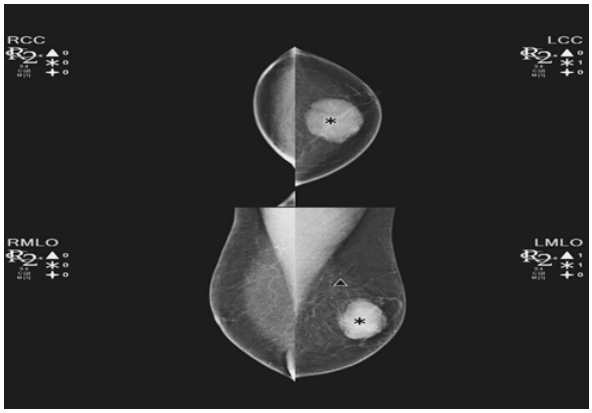

Fig. 2 – Mammogram of the left breast shows a circumscribed, hyperdense mass without calcifications or identifiable fat, while the right breast is normal.

a tumor composed of relatively uniform spindle cells with moderately hyperchromatic nuclei and low mitotic activity. The cells were arranged haphazardly and in short fascicles (Figure 3). No tumor necrosis was found. Immunohistochemistry revealed diffuse and strong positivity for vimentin, CD99 and CD34 molecules (Figure 4) and

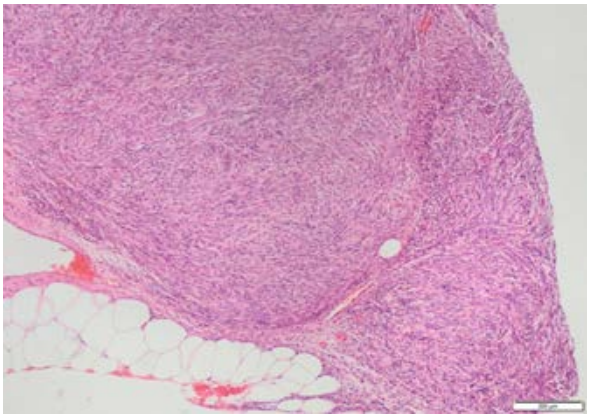

Fig. 3 – Dermatofibrosarcoma protuberans at low magnification. The tumor border is sharp, but there is an entrapped adipocyte in the lower right quadrant (hematoxylin-eosin staining, 40x).

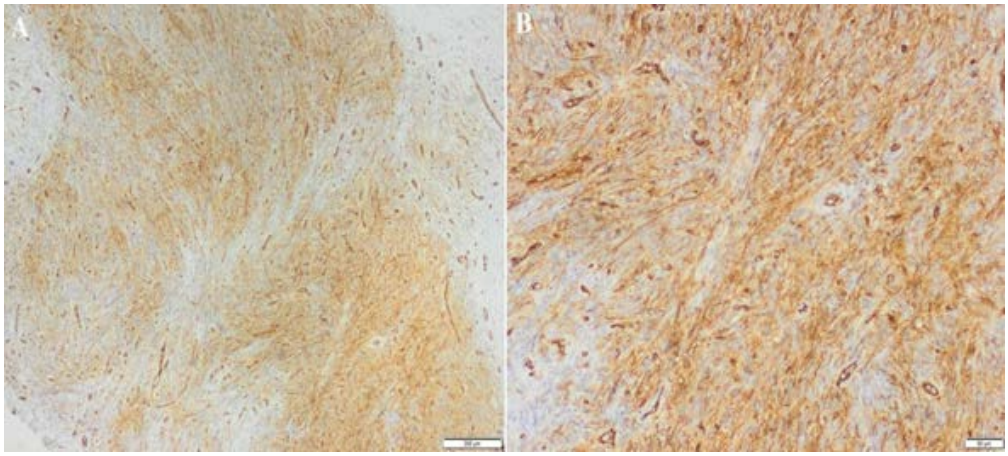

Fig. 4 – Diffuse tumor cells immunoreactivity for CD34.
Immunoperoxidase with hematoxylin counterstain: A) 40x; B) 100x.

focal and weak reaction for smooth muscle actin. The tumor cells were completely negative for keratin, S100 protein (Figure 5), STAT6 (Figure 6), CD31 and factor VIII. Such a combination of morphology and immunophenotype was highly suggestive for DFSP regardless of its subcutaneous localization. Subsequently, a radical mastectomy was performed without any adjuvant treatment. Pathologic examination revealed a firm, tan, well-circumscribed oval tumor just beneath the skin, measuring 40 x 30 mm (Figure 7). Histological and immunohistochemical analyses confirmed the biopsy findings and the diagnosis remained the same. The postoperative care was uneventful without any additional treatment. The patient was disease-free six months after the surgical treatment.

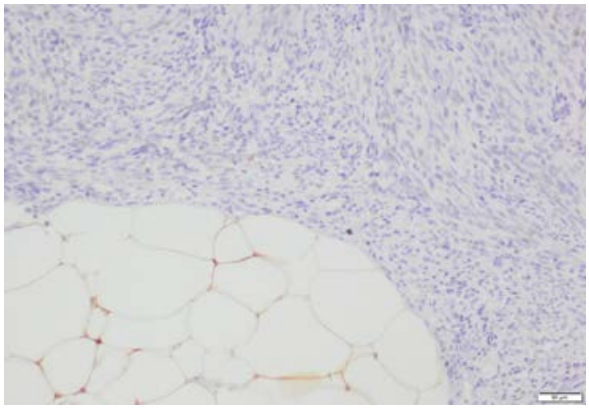

Fig. 5 – No positivity for S100 protein with a few immunoreactive adipocytes as an internal positive control.
Immunoperoxidase with hematoxylin counterstain (100x).

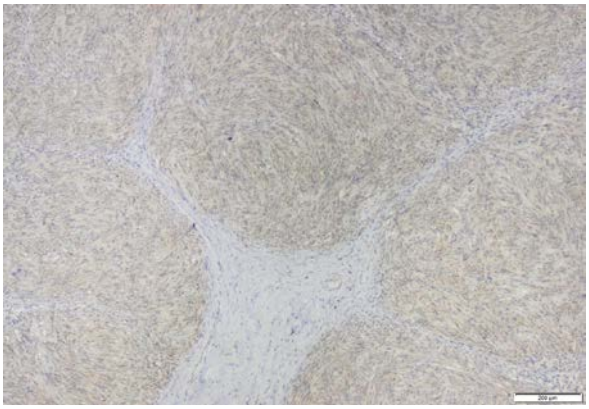

Fig. 6 – Nonspecific cytoplasmic positivity for STAT-6. No visible nuclear staining.
Immunoperoxidase with hematoxylin counterstain (40x).

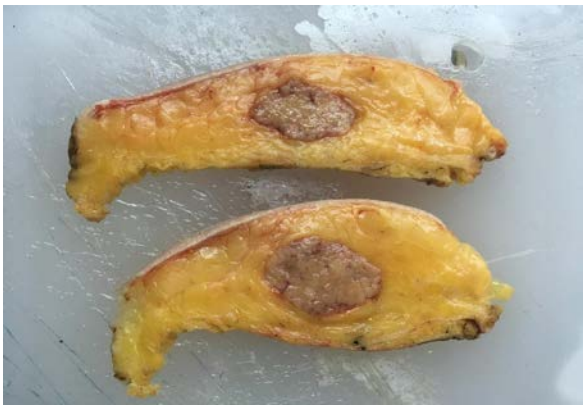

Fig. 7 – Macroscopic examination shows a firm, tan, well-circumscribed oval tumor just beneath the skin.

Discussion

DFSP represents a low-grade malignant soft tissue tumor that arises from the dermis and extends to the deeper structures. There are several histopathological variants of DFSP that have been described, including pigmented DFSP or Bednar tumor, myxoid, juvenile DFSP or giant cell fibroblastoma, atrophic, sclerosing and myoid, occurring in pure form or admixed with one of the others creating hybrid lesions. A small subset of DFSP patients presents with fibrosarcomatous progression that is more aggressive and has higher rates of recurrence and metastasis [11].

The results of some studies concerning the distribution of DFSP between genders showed that women have higher incidence rates than men, except among the elderly [15], but other authors reported that men are slightly more commonly

affected than women [9]. In our case, the tumor on the male breast appeared much later, at the age of 66, compared to the recently published review of Bouhani et al. [7] who state that the mean age of DFSP in the male breast is 32.6 years. The tumor is usually less than 5 cm in size, similarly to our case. DFSPs are superficial in 77% of patients and, according to the report of Bowne et al. [16], invade deeper structures in only 22% of patients.

The pathogenesis of DFSP is poorly explained. DFSP was observed to occur in pre-traumatic areas, including vaccination sites, burn scars, tattoos, surgical scars, and radiotherapy [11]. Almost all molecularly characterized cases have been found to have a COL1A1-PDGFB fusion gene. It was found that DFSP with the new COL6A3-PDGFD fusion variant has a predilection for breast and also has typical histologic and immunohistochemical features [8]. Several case reports and epidemiologic studies suggest that hormones may also be involved in the pathogenesis of DFSP. Kreicher et al. [17] proved no significant association between hormone receptor expression and demographics, but the loss of receptor expression was observed in all recurrent tumors. The presentation of the tumor in our case as gynecomastia suggests that there may be a connection between primary reasons of gynecomastia and occurrence of DFSP in the male breast. It remains unclear if higher estrogen levels or disbalance with testosterone levels can be a potential risk factor for developing this kind of malignancy. Regarding the recently published literature review [7], this case represents the 12th case of the breast DFSP in men, which shows the rarity of this entity.

Most DFSPs are typically small and superficial and diagnosis may be suspected based on the tumor's clinical appearance and pathologic examination. When found in the breast region, patients usually undergo only breast US and mammography without the need for magnetic resonance (MR) imaging (MRI) [11]. Nevertheless, there is a possibility of the *in vivo* usage of MR spectroscopy (MRS) that gives additional valuable information of a normal cholin resonance peak which, combined with other imaging and pathohistological characteristics, can be suggestive of the diagnosis of DFSP [18]. Pathological and immunohistochemical examinations are currently the gold standard for diagnosing DFSP [11].

Immunohistochemically, DFSP usually shows strong positivity for CD34 (in 97% of the patients) [1], vimentin and negative staining for cytokeratin, S-100, epithelial membrane antigen and variable staining for smooth muscle actin (SMA) [12]. The present case showed positivity for CD34 along with negativity for S-100 and only focal positivity for SMA, similar to the most recent reported DFSP case in the male breast [7].

The main differential diagnosis of DFSP of the breast includes primary breast tumors with spindle cell differentiation like benign fibrous histiocytoma, phyllodes tumor, cellular fibroadenoma, dermatofibrosarcoma, neurofibroma, nodular fasciitis, fibrosarcoma, and inflammatory myofibroblastic tumor [12].

It was found that older age and male sex were significant predictors of mortality of patients with DFSP[19]. Factors like histologic subtype, high mitotic index, cellularity, size, location of the tumor, and recurrent lesions were found to be associated with higher recurrence rates [14]. The treatment of DFSP is primarily surgical. In our case, the more radical approach was made, and the patient underwent a mastectomy, like in the similar recently published case [7]. In our case, following the patient's desire, a left mastectomy was performed.

In lesions with positive margins after surgery, or in cases where resection is limited due to anatomical location, adjuvant radiotherapy is suggested [2]. Prognostic factors that are shown to be significant are tumor location, surgical margins, and the presence of a high-grade component [20]. However, these factors are identified for DFSP in locations other than breast. No impact on survival was found in patients undergoing radiation therapy [21]. Imatinib mesylate, a protein tyrosine kinase inhibitor, is used for the treatment of unresectable, recurrent and/or metastatic DFSP in adult patients because it inhibits the overactivity of platelet-derived growth factor (PDGF) receptor in these tumor cells [11]. A response rate of approximately 65% has been achieved among DFSP patients treated with imatinib. A small subset of DFSP lacking the classic translocation t(17:22) seems to have no response to imatinib [14].

Long-term follow-up requires strict US monitoring every 6 to 12 months with biopsy in cases of suspected recurrence. The 5-year survival rate of patients with DFSP is higher than 99% [2].

Conclusion

DFSP is a soft tissue sarcoma that rarely develops in the breast of male patients. The clinical presentation includes a firm, erythematous, subcutaneous lump that has an indolent growth pattern. The diagnosis of DFPS is based on anatomopathology with immunohistochemistry because there are no specific signs for this rare entity. Surgical excision with wide and negative margins is optimal for reducing the risk of recurrence.

REFERENCES

1. *Murase Y, Takeichi T, Matsumoto T, Sakakibara A, Akiyama M.* A juvenile male case of dermatofibrosarcoma protuberans on the breast. Clin Exp Dermatol 2020; 45(1): 111−3.
2. *Al Tarakji M, Toro A, Di Carlo I, Junejo K.* Unusual presentation of dermatofibrosarcoma protuberans in a male patient's breast: a case report and review of the literature. World J Surg Oncol 2015; 13: 158.
3. *Lattin GE Jr, Jesinger RA, Mattu R, Glassman LM.* From the Radiologic Pathology Archives: Diseases of the Male Breast: Radiologic-Pathologic Correlation. Radiographics 2013; 33(2): 461−89.

4. *Llombart B, Serra-Guillén C, Rubio L, Nagore E, Requena C, Traves V* et al. Subcutaneous dermatofibrosarcoma protuberans, a rare subtype with predilection for the head: A retrospective series of 18 cases. J Am Acad Dermatol 2017; 77(3): 503−11.e1.
5. *Iyengar R, Kiwanuka E, Mangray S, Cruz AP, Katarincic J, Bhatt R.* Diagnosis and Management of Dermatofibrosarcoma Protuberans in a 3-Year-Old Patient. J Hand Surg Am 2017; 42(9): 756.e1−756.e5.
6. *Achouri L, Triki A, Bouzaiene H, Chemleli M, Laamouri B, Slimen M*, et al. Transformed dermatofibrosarcoma protuberans: A series of nine cases and literature review. J Dermatol Dermatol Surg 2016; 20(1): 1−4.
7. *Bouhani M, Fertani Y, Zemni I, Adouni O, Bouida A, Chargui* R, et al. Dermatofibrosarcoma Protuberans of the Breast in Man: An Extremely Rare Entity With a Review of the Literature. J Investig Med High Impact Case Rep 2019; 7: 2324709619875634.
8. *Dickson BC, Hornick JL, Fletcher CDM, Demicco EG, Howarth DJ, Swanson D*, et al. Dermatofibrosarcoma protuberans with a novel COL6A3-PDGFD fusion gene and apparent predilection for breast. Genes Chromosomes Cancer 2018; 57(9): 437−45.
9. *Chen X, Chen YH, Zhang YL, Guo YM, Bai ZL, Zhao X.* Magnetic resonance imaging and mammographic appearance of dermatofibrosarcoma protuberans in a male breast: a case report and literature review. J Med Case Rep 2009; 3: 8246.
10. *Lyu A, Wang Q.* Dermatofibrosarcoma protuberans: a clinical analysis. Oncol Lett 2018; 16(2): 1855−62.
11. *Diwakar DK, Wadhwani N, Paruthi S.* Recurrent dermatofibrosarcoma protuberans: challenging a surgeon's dexterity for the 'tricky' margins. Ecancermedicalscience 2018; 12: 858.
12. *Akhtar K, Sherwani RK, Ray PS.* Dermatofibrosarcoma Protuberans of Male Breast: An Unusual Presentation. Oman Med J 2012; 27(4): e038.
13. *Mirza TI, Akhtar K, Abbas HB, Sameena M, Tahir F, Khan S,* et al. Dermatofibrosarcoma protuberans male breast: a case report. Oman Med J 2011; 26(5): e023.
14. *Saikia BK, Das I, Mandal GK.* Fibrosarcomatous change in the background of dermatofibrosarcoma protuberans in male breast: Study of a rare case and review of the entity. J Midlife Health 2016; 7(1): 45−8.
15. *Criscione VD, Weinstock MA.* Descriptive epidemiology of dermatofibrosarcoma protuberans in the United States, 1973 to 2002. J Am Acad Dermatol. 2007; 56(6): 968−73.
16. *Bowne WB, Antonescu CR, Leung DH, Katz SC, Hawkins WG, Woodruff JM*, et al. Dermatofibrosarcoma protuberans: A clinicopathologic analysis of patients treated and followed at a single institution. Cancer 2000; 88(12): 2711−20.
17. *Kreicher KL, Honda KS, Kurlander DE, Bordeaux JS.* Hormone receptor expression in patients with dermatofibrosarcoma protuberans. J Am Acad Dermatol 2016; 75(6): 1205−9.
18. *Djilas-Ivanovic D, Prvulovic N, Bogdanovic-Stojanovic D, Vicko F, Sveljo O, Ivkovic-Kapicl T.* Dermatofibrosarcoma protuberans of the breast: mammographic, ultrasound, MRI and MRS features. Arch Gynecol Obstet 2009; 280(5): 827−30.
19. *Trofymenko O, Bordeaux J, Zeitouni N.* Survival in patients with primary dermatofibrosarcoma protuberans: National Cancer Database analysis. J Am Acad Dermatol 2018; 78(6): 1125−34.
20. *Williams N, Morris CG, Kirwan JM, Dagan R, Mendenhall WM.* Radiotherapy for dermatofibrosarcoma protuberans. Am J Clin Oncol 2014; 37(5): 430−2.
21. *Criscito MC, Martires KJ, Stein JA.* Prognostic Factors of Survival in Dermatofibrosarcoma Protuberans-Reply. JAMA Dermatol 2016; 152(12): 1398−9.

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Dr. Draginja Draga Ljočić – the first female doctor in Serbia

Dr Draginja Draga Ljočić – prva žena lekar u Srbiji

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Key words:
history of medicine; human rights; physicians; sex factors; war; women.

Ključne reči:
istorija medicine; ljudska prava; lekari; pol, faktori; rat; žene.

Introduction

Draginja Draga Ljočić was born on February 22, 1855 in Šabac, Serbia and comes from a wealthy merchant family which experienced a great injustice [1].

Civic life of Serbia at that time "was coloured" by Aromanians, capable merchants and craftsmen, who were distinguished by their wealth, language skills and ambition, so they differed from natives because they educated children and did not make traditional differences between male and female children [2].

Draga's father, Dima Ljočić, fought for their family property, leading a multi-year lawsuit with a prominent family from Šabac and then he was left without money for life. Since he had suffered the great injustice in life, he borrowed five thalers and paid the clerk to ring the bell. Answering the question asked by a citizen from Šabac: "Who died?", Dima said: "Justice died and I am burying it today". He was punished for this act and sentenced to 25 beatings. Once being a wealthy merchant, Dima Ljočić now became a poor "bunner" with the income of only seven thalers. In spite of having two sons, he decided to educate a daughter [2].

This injustice done to her father determined future life of Draginja Draga Ljočić (Figure 1), who would persistently fight for the rights of women in medicine.

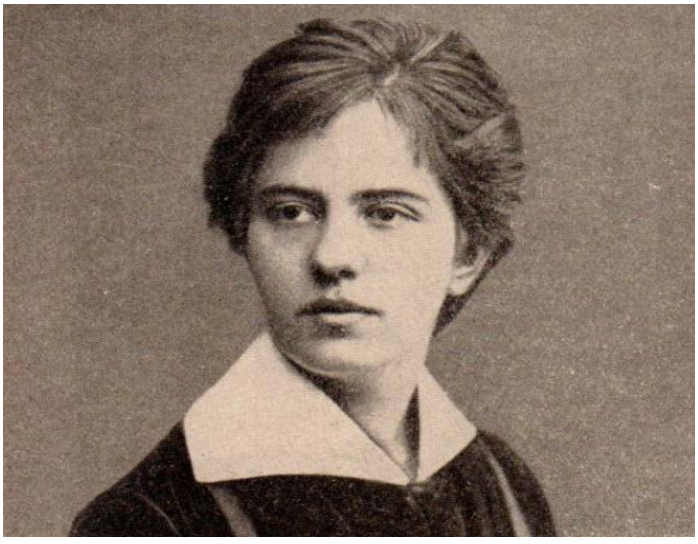

Fig. 1 – Dr. Draginja Draga Ljočić – The Heroine of Medicine
(Photo: Šabacturizam.org Source: www.svetplus.com)

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Life, education, careers

Draga finished elementary school in her hometown. Her teacher, Persida Pinterović, recognized her as a gifted person, and enabled Draga to live with her in Belgrade, where Draga finished "Great school". She had no doubts about the choice of the University. The only choice was "The University of Zurich", since it was the only place in Europe in 1860s where girls were able to study. As an ambitious and hard-working woman, she was successfully studying, constantly struggling with severe shortages. Her brothers helped her occasionally, and since 1874 she had been using the scholarship of forty dinars per month, which the merchant Jevrem Pantić provided for good students in Šabac. Her diary revealed that later, she returned the money which had been borrowed during the studies [3].

In June, 1876, because of her strong patriotic feelings, she interrupted the studies and as medical assistance joined the Serbian-Turkish war in which she got the rank of medical lieutenant. She was dedicatedly treating the wounded at the Military Academy and at Hospital in Svilajnac. She took part in the battle of Šumatovac [3].

After the truce was declared in 1877, Draga returned to Zurich, continued the studies and by the end of 1878 successfully graduated. She wrote a final paperwork, i.e. a doctoral dissertation entitled "Contribution to the operational therapy of uterus fibromyoma" at the University of Zurich, which was published the same year in Zurich [3], as shown in Figure 2.


der

Fibromyome des Uterus

Inaugural-Dissertation

vorgelegt

der hohen medicinischen Facultät

der

Universität Zürich

von

Draga Ljočić,

von Schabatz (Serbien).

Genehmigt auf Antrag von Herrn Prof. Dr. F.

ZÜRICH

Druck von Orell Füssli & Co.

1878

Докторска дисертација Драге Љочић

Fig. 2 – Draga Ljočić's doctoral dissertation, "Contribution to the operational therapy of uterus fibromyoma", The University of Zurich, 1878.
(www.srpskilegat.rs, riznicasrpska.net)

At the age of only twenty-four she became a doctor of medicine, surgery, obstetrics and eye diseases, and later she opened a private consulting-room as a "doctor of medicine, obstetrics and eye diseases". At that time, she was the first woman doctor in Serbia. Just then, her continuous and excellent fight for the equal rights of men and women in medicine began [3].

On February 26, 1879, Draga Ljočić required a permission from the Ministry to work as a doctor of medicine but got rejected, despite the fact that at that time there were only 79 doctors in Serbia, mostly foreigners. Because of the intermise of Dr. Vladan Djordjević, the Chief of Health Department, amenable minister assented. It was decided that Draga Ljočić should pass the civil service examination. If she showed enough knowledge, medical practice would be allowed to her. The Committee gathered was made up of Doctor Vladan Djordjević (envoy of Duchess Natalia), and Doctors Mladen Janković, and Djordje Klinkovski. Dr. Draga Ljočić was asked theoretical and practical questions in anatomy, physiology, prescribing of medicaments, gynecology, obstetrics and internal medicines. Some of the questions were: "What are you going to do in a crossbirth, when one arm of the baby fell out?", "What opiates do you know?" What are the poisons that people are most often poisoned by, and what are the antidotes?" The Commission went into detail to find a single mistake. She successfully answered all the questions.

According to the conducted protocol, the exam was held on April 6, 1879 in the City and District Belgrade Hospital. Having passed the exam and showed that she obtained the necessary medical knowledge completely the same as male doctors, she got the right to start private medical practice, but not the opportunity to work in the civil service, on the grounds that women did not serve the army [4].

At that time higher education of female children was not usual and desired. Nevertheless, Draga Ljočić refused to live in accordance with the rigid principles of the "Balkan time", rejecting dogmas and prejudices of the surroundings. She acquired the highest education and deservingly became the first woman doctor – a Serbian and Yugoslav the Heroine of Medicine [5].

When she later became a member of the Serbian Medical Society, due to serious health problems, she went to the sanatorium in Crimea, near Yalta, where she was being treated for a while. Only on July 6, 1882, the new minister Milutin Garasanin appointed her at the position of a medical assistant in the General State Hospital, the largest acknowledged treatment centre in Serbia at that time. Almost the entire 1883 was the turning point in the private life of Dr. Draga Ljočić. She married Mr. Raša Milošević, one of the founders of the People's Radical Party, thus breaking another prejudice of her time by keeping her maiden name. Shortly after getting married, she got a daughter. After that, the hardest days of her life followed. Raša Milošević was politically sentenced to six years in prison, while Draga stayed alone with the baby, burdened with many worries and troubles. Working very hard in medical practice in many different ways, she tried to pro-

vide material existence for her family, enduring difficult days of Raša's imprisonment with dignity [2].

During 1885, while the Serbo-Bulgarian war was going on, the discrimination against women doctors was decreasing. Dr. Draga was working then as the only doctor in three Belgrade hospitals: General State Hospital, the Infectious Disease Hospital and the Hospital for the Wounded, located in the Great School in Belgrade, headed by Professor Josif Pančić [5].

The war defeat on Slivnitsa led to the change of the political climate in Serbia, and convicted radicals were pardoned and released. Draga's husband returned to the family home on January 1, 1886. At the end of the same year, they got another daughter. Dr. Draga and Mr. Raša Milošević had four daughters – Radmila, Spomena, Zora and Olga [2, 5].

The fight for equal rights with men

In December, 1886, the minister promoted Dr. Draga to an assistant doctor, but her rights were still not the same as the rights of her colleagues. She was affected by this injustice and discrimination, so she addressed to the minister demanding equal rights with men in the service. She suffered another humiliation afterwards, for she was required to show her transcript of records and diploma, which normally was not the custom in any doctors' promotion. She was asked to show the graduation exam certificate, which was impossible to enclose, because at that time, when Dr. Draga finished Higher School for women, graduation exam did not even exist in Serbia. The requested opinion of the Chief of Health Department about Dr. Draga's request was negative. Even the complaint to King Milan or to the State Council did not help to overcome the injustice. As a response to her request she was fired [2, 6]. The dismissal did not harm her reputation because patients had recognized a good person and a good doctor inside her long time before that. She continued private practice and part-time work in the Department of Monopoly. During 1904, she founded the "mother organization" with Sara Karamarković in order to reduce infant mortality and to take care of abandoned children. Thanks to her perseverance and dedication, the organization moved into a building in Studentska Street in Belgrade, where "Home for the foundlings" was later opened. Without any material compensation, Dr. Draga was treating students of vocational schools. To earn money, she worked in the factory of tobacco and matches for more than thirty years. Her diary entries disclose that among these workers she was the favourite and they called her "our mother" [6]. Dr. Draga was the first president of the Society of Belgrade Women Doctors which was established in 1919/1920 and which was very active in raising funds for the construction of the first hospital for women and children; it was planned that only female doctors would work in that hospital. The Scottish Women's Society provided assistance, but the construction of this hospital was slow, because the resources were financially low. Afterwards, the hospital was named Dr. Elsie Inglis – Memorial Hospital, in honour of the brave doctors of the Scottish Mission [6].

Her zealous efforts to monitor trends and the developments of European medicine and to implant them into Serbian medicine were clearly visible. She translated the book "Raising small children" from the Russian language which was dedicated to mothers, because bringing up children depends on their knowledge, as even society and nations do. Dr. Draga was interested in developments in gynecology and obstetrics, so she translate an article from the French medical journals in the journal "Serbian Archives of Medicine" in 1885.

Dr. Draga was 57 years old and of poor health when Balkan wars broke out. Nevertheless, without sparing herself, she worked day and night at the clinic for the public and the poor, as well as in the hospital of Belgrade benefactor Nikola Spasić. In the diary she kept, she clearly expressed her eternal disagreement with the current views on the status and fostering of women doctors. She struggled with the Balkan non-understanding of women and women doctors in the society all her life. She participated in the creation of the Women Medical Society after the World War I and in the establishment of the Women Hospital on Dedinje. When the World War I began, her family retreated to Nis, where she unselfishly continued working at hospital. As the war progressed, her husband Raša and daughter Radmila crossed Albania, went on Corfu, and then on the Salonica front. Dr. Draga with three daughters went from Niš to Thessaloniki, then to Athens, and from there via Rome and Nice they arrived in Lausanne. Throughout the war, she was engaged in humanitarian work, organizing sending packages to our prisoners in German and Hungarian camps [1]. For the sacrifice and courage in offering health care to soldiers and civilians in Serbian liberation wars during 1876−1878 and 1912−1918, Dr. Draga Ljočić was awarded with the Order of St. Sava, 4th class in 1904, and the Gold Medal for Diligent Service in 1913 [7].

In her diary, Dr. Draga often wrote that the colleagues rarely respected her in terms of professional work. Through her continuous and diligent work, she was trying to show that the expertise and knowledge had nothing to do with gender differences (she was called a feminist, through political struggle for the emancipation of women) [8].

After the Balkan Wars and the First World War, she returned to Serbia and only then she got her title a "real doctor". At the end of 1924 she was retired [9].

A few words about the Diary − it consists of three parts: the description of life in Belgrade and two travel books, one about going to Šabac and Bosanska Gradiška, and the other about the travel to Crimea. The diary of Dr. Draga is clearly understandable and it is a valuable source of information for making the whole picture of the life of this remarkable woman, as she speaks about herself, her world and her time [10].

Why do we mention her today? You will certainly conclude that this is from justifiable, essential and humane reasons for the overall and better medical being of today. Much earlier unjustly neglected and not always professionally respected, Dr. Draga Ljočić succeeded in fighting for her personal rights, but a much greater significance of her historical

way is the fact that she cleared the way to her female colleagues and to all women doctors to be.

Because of all the facts previously mentioned, Europe admired the famous, brave Serbian woman, the first female doctor, a woman officer in the Balkans and the fourth lady in the Old World with a medicine university degree. After Dr. Maria Zibold, who was a major, Draga Ljočić was the second Serbian medical officer, which was very rare at the time [11].

Today, a reminder to us (Forgotten Serbian women), she encountered many obstacles in her life and professional struggle, in war and in peace, but her enterprise, endurance and strength won in the end.

Conclusion

Professional contribution of Dr. Draga Ljočić to, at that time, not very developed Serbian medicine is immense, and for even greater admiration is the contribution of Draga's life fighting and all her life's work for the actualization of women's rights.

She died on November 5, 1926 at the age of 71, in her house in Topčider in Belgrade. She was buried in Belgrade and known as the first woman doctor in Serbia or the Heroine of the Spirit. It is believed even today that she had only one flaw – she was not a man.

Fortunately, Dr. Draga Ljočić has her descendants, who with love and pride cherish the memory of their great and courageous mother and grandmother, still today after 161 years. Her daughter Dr. Radmila Milošević was also a highly regarded physician in Serbia and a participant in the wars of 1912–1918.

Today, Health Care Centre in Šabac proudly bears her name, as she was the first woman doctor in Serbia and the most interesting woman in the modern Serbian history.

On September 29, 2017 the bust of the first female doctor in Serbia, Dr. Draginja Draga Ljočić, was erected by the Serbian Medical Society (Figure 3).

Fig. 3 – The bust (monument) of the first female doctor in Serbia *(wikimedia.org)*

Today, there are so many young female doctors and female students in the Serbian Armed Forces. This paper, as part of the history of medicine, should „enlighten", like a lighthouse, the female medical population and the professions of all women, who have not been familiar with the Heroine of Medicine so far.

R E F E R E N C E S

1. *Ivanović D.* The cradele of health care in Serbia. Ruma: Serbian book Grafampromet; 2003.
2. Archive of Serbia. MUD-S 1879 year, f. II/123. (Serbian)
3. *Marković S.* Collected writings. Belgrade: Prosveta and Serbian Literary Association; 1960. p. 223. (Serbian)
4. *Bokun-Đinić S.* Dr. Draga Ljočić: In-local life. Šabac: Intermunicipal Historical Archive; 1986. (Serbian)
5. *Gavrilović V.* Women doctors in the wars of 1876−1945 on the territory of Yugoslavia. Belgrade: Scientific Society for the History of Health Culture of Yugoslavia; 1976. (Serbian)
6. Scrapbook of the Serbian Medical Society. Belgrade: Serbian Medical Society; 1972. (Serbian)
7. *Krstić M, Mirković Lj, Milićević S, Pantović S, Ravilić U, Pantović D.* Female doctors awarded in Serbian liberation wars during 1876−1878 and 1912−1918. Vojnosanit Pregl 2013; 70(9):891−2.
8. *Grujić V.* Pledging of Draga Ljocić for state scholarship for medical studies in Zurich. Serbian. Srp Arh Celok Lek 1969; 97(3): 380−4. (Serbian)
9. *Djordjević S.* From historical works on medical language and terminology in Yugoslavia. Srp Arh Celok Lek 1981; 109(12): 1675−8. (Serbian)
10. *Divljanović D.* Dr. Draginja-Draga, J. Babić (1886−1915). Srp Arh Celok Lek 1968; 96(5): 557−9. (Serbian)
11. *Pavlova VT.* Historical aspects of the medical culture of Balkan nations (end of 18th century-beginning of 19th century. Sante Publique (Bucur) 1968; 11(4): 441−8. (French)

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I N M E M O R I A M

Dr Maja Marković (1958–2021)

Dana 20. septembra 2021. godine zauvek nas je napustila dr Maja Marković, dugogodišnji član Instituta za naučne informacije (INI) Vojnomedicinske akademije (VMA) u Beogradu (sada Centar za medicinske naučne informacije Medicinskog fakulteta VMA, Univerziteta odbrane u Beogradu) i više od 20 godina stalni član Redakcije časopisa „Vojnosanitetski pregled“ (VSP).

Dr Maja Marković je rođena 1. maja 1958. godine u Sarajevu, u porodici zdravstvenih radnika (otac, prof. dr Bogdan Bošković, čuveni jugoslovenski i srpski farmakolog i toksikolog, redovni profesor na VMA i majka, Vasiljka Bošković, farmaceut), što je sigurno uticalo na njeno kasnije opredeljenje da postane lekar.

Diplomirala je na Medicinskom fakultetu u Sarajevu 1982. godine. Posle obavljenog jednogodišnjeg staža u Kliničko-bolničkom centru „Bežanijska kosa“ započinje specijalizaciju iz anesteziologije i reanimatologije u VMA koju uspešno završava krajem 1987. godine položivši specijalistički ispit sa najvišom ocenom. Po završetku specijalizacije nastavlja da radi u VMA, u Klinici za anesteziologiju i intenzivnu terapiju. Zbog zdravstvenih problema koji su joj onemogućavali aktivno bavljenje kliničkim radom, 7. aprila 1999. godine prelazi u INI na mesto lekara-istraživača i stručnog redaktora VSP-a i na tim poslovima ostaje sve do odlaska u penziju sredinom ove godine. U periodu od 2006. do 2011. godine obavljala je i dužnost načelnika Odeljenja za obradu medicinskih naučnih informacija i dokumentaciju u INI i bila tehnički sekretar Uređivačkog odbora VSP-a. U tom periodu VSP ulazi u sistem indeksiranja najpoznatije baze naučne publicistike *Science Citation Index expanded* (SCIe) i postaje međunarodno priznat časopis. Ovom uspehu svakako da je doprinela i dr Maja Marković svojom stručnošću i izuzetnim zalaganjem.

Pored poslova na stručnom redigovanju radova za VSP, dr Maja Marković bila je angažovana i kao saradnik u nastavi u pripremi predavanja i vežbi iz predmeta Medicinska naučna informatika za polaznike specijalističkih i magistarskih studija na VMA i Škole rezervnih oficira sanitetske službe.

Iako narušenog zdravlja, sve svoje radne obaveze obavljala je s velikom požrtvovanošću i odgovornošću kakva se danas retko sreće. Čak i kada joj je bilo najteže, nastojala je da druge ne opterećuje svojim problemima. Bila je odmerena, skromna, nenametljiva, nesvakidašnje obzirna, jednostavno rečeno bila je dobar čovek! Mnogi autori našeg časopisa, pogotovo oni iz VMA, pamtiće je po ljubaznosti i spremnosti da pomogne kad god je to bilo potrebno.

Za svoj rad više puta je pohvaljivana od strane načelnika INI i načelnika VMA.

Njenim preranim odlaskom izgubili smo svi – njena porodica, prijatelji, saradnici. Pamtićemo je po dobroti, plemenitosti, velikom radnom elenu i milom i toplom osmehu.

Draga naša Majo, počivaj u miru! Neka ti je večna slava i hvala!

prof. dr Silva Dobrić

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DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG*, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

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Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure **– Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi **– Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

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Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa interneta citiraju se uz navođenje datuma pristupa tim podacima.

Primeri referenci:

Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG*, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u levom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

Detaljno uputstvo može se dobiti u redakciji ili na sajtu: www.vma.mod.gov.rs/vsp